Pharmacoepigenetics in Heart Failure

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Abstract Epigenetics studies inheritable changes of genes and gene expression that do not concern DNA nucleotide variation. Such modifications include DNA methylation, several forms of histone modification, and microRNAs. From recent studies, we know not only that genetic changes account for heritable phenotypic variation, but that epigenetic changes also play an important role in the variation of predisposition to disease and to drug response. In this review, we discuss recent evidence of epigenetic changes that play an important role in the development of cardiac hypertrophy and heart failure and may dictate response to therapy.

Keywords DNA methylation · Epigenetics · Heart failure · Histone modification · MicroRNA · Pharmacoepigenetics

Introduction

There is wide variability in an individual's disease predisposition and response to treatment, which is partially ascribed to heritable factors [1]. However, based on recent whole-genome association studies, we have to conclude that variation in nucleotides does not account for all the heritable phenotypic variation [2–4]. In addition to variation in nucleotides, epigenetic variations may play a critical role in

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controlling gene expression and therefore offer another mechanism to explain interindividual variation [5].
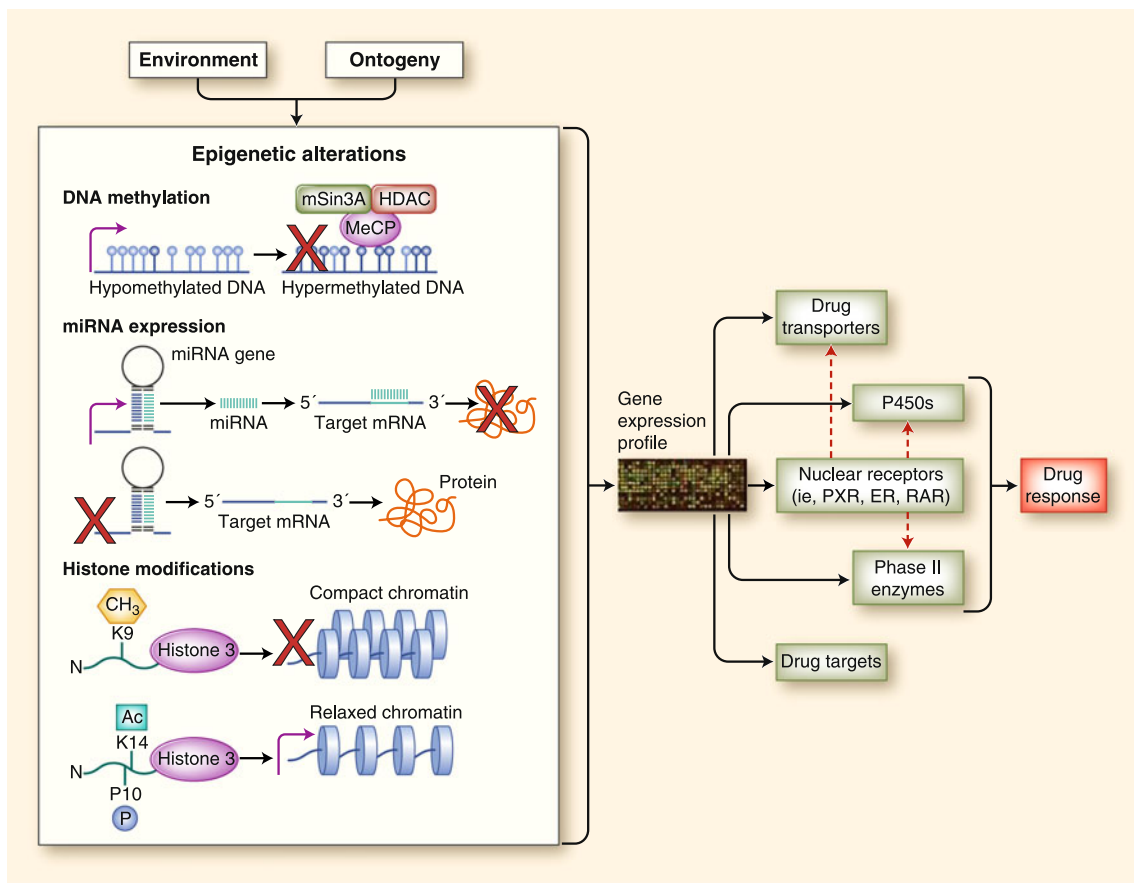
Epigenetics studies inheritable changes of genes and gene expression that do not concern the original DNA nucleotide variations, such as mutations or polymorphisms. Three different types of epigenetic variations are known to alter gene expression control: methylation of genomic DNA, modification of histone proteins, and regulatory noncoding RNAs, such as microRNAs (Fig. 1). All three mechanisms concern extrinsic factors that can modulate the transcription of genes, even though the protein-encoding regions of the genes themselves are unchanged [6]. These epigenetic control mechanisms may differ among tissues and individuals, but they also may change in time during aging or as a result of environmental interactions or diseases. Because the epigenome plays a critical role in programming the expression of the genome, differences in gene expression among individuals that affect the response to drugs may be modified by epigenomic variations on top of nucleotide mutations or polymorphisms. Therefore, epigenetic changes currently are being considered in clinical medicine complementary to nucleotide variations at the drug response level [7]. This rapidly emerging novel discipline, called *pharmacoepigenetics*, involves the study of epigenetic factors in the interpersonal variation to drugs but also the discovery of novel pharmacologic targets [8]. It is expected that pharmacoepigenetics (jointly with pharmacogenetics) will play a crucial role in future pharmacology and clinical medicine [7].

So far, most advances in pharmacoepigenetics have been derived from the oncology field—for example, studies characterizing the interindividual differences of cytochrome P-450 (reviewed by Ingelman-Sundberg et al. [9]). Fortunately, the knowledge of the role of epigenetic modifications is being translated to other complex and heterogeneous con-

Fig. 1 Epigenetic modifications at different genetic levels and their effect on drug response. Environmental factors generate a spectrum of phenotypes in the population through the participation of epigenetic mechanisms such as covalent modification of DNA (ie, methylation) and histones and expression of regulatory noncoding RNA molecules such as microRNAs (miRNAs). Epigenetic factors affect the expression of drug transporters, drug-metabolizing enzymes, nuclear receptors that regulate the expression of various genes, and even drug targets. The dynamic state of epigenetics, as opposed to a rather established nucleotide sequence, provides the basis for an individual's response to a constantly changing environment. Methylation of DNA provides an impediment to transcription factors and the transcription machinery by attracting proteins that affect chromatin configuration. Posttranslational modifications of the N-terminal of the histone proteins (and their various combinations) affect the compaction of the chromatin; that is, methylation of lysine at position 9 of histone 3 or H3K9me is a signature of heterochromatin or compact DNA, whereas acetylation of lysine at position 14 of histone 3 or H3K14ac—and sometimes in combination with phosphorylation of proline at position 10 of histone 3 or H3P10p—creates a more open chromatin configuration (euchromatin) that allows transcription of genes. miRNA molecules arise from miRNA genes, which, when transcribed, can promote posttranscriptional regulation by complementarity with the 3′-untranslated region of target mRNAs and their subsequent degradation. ER—estrogen receptor; HDAC—histone deacetylase inhibitor; PXR—pregnane X receptor; RAR—retinoic acid receptor. (*Adapted from* Gomez and Ingelman-Sundberg [35]; with permission from Macmillan Publishers Ltd: *Clinical Pharmacology and Therapeutics* [85:426–430], © 2009.)

ditions, and the applicability is increasing rapidly. Here, we review the most recent work on the epigenetic modifications that have an effect on heart failure (HF) and cardiovascular disease (CVD) therapy.

Epigenetic Modifications and Heart Failure

Histone Modifications

The large eukaryotic genome is compacted tightly as a result of its association with highly conserved histone

proteins. In the nucleosomes, genomic DNA is folded and compacted around core histone proteins (two copies of each of the core histones H2A, H2B, H3, and H4), forming the basic repeat units of chromatin. The interaction of genomic DNA with these chromosomal proteins has a major influence on the accessibility of transcriptional factors to their target DNA sequences and thereby regulates transcriptional activity (Fig. 1) [10•]. Through this mechanism, nucleosomes carry epigenetically inherited information in the form of covalent modifications of their core histones. Such modifications include acetylation, methylation, phosphorylation, ubiquitination, and sumoylation of histone

proteins [10•]. Core histones have an amino-terminal tail that sticks out from the chromatin fiber and is thought to interact with DNA or other histone or proteins. Lysine and arginine residues within this tail are the main targets for histone modification. Most research was aimed at understanding the role of lysine acetylation and methylation. It turns out that lysine acetylation is associated mainly with chromatin accessibility and transcription, whereas the effect of lysine methylation varies depending on which residue is modified [11].

Interestingly, as reviewed by Mano [10•], the regulation of histone acetylation has been linked to cardiac hypertrophy. The acetylation of histone tails by histone acetyltransferases is required for the induction of hypertrophic changes in cardiac muscle cells by phenylephrine. Consistent with this are the results of studies focused on class II histone deacetylases (HDACs) 5 and 9, which exert antihypertrophic effects by inhibiting the activity of myocyte enhancer factor 2 (MEF2) and further blocking the expression of pro-hypertrophic genes [12]. Contrary to these findings, class I HDACs have rather pro-hypertrophic effects by regulating the expression of phosphatidylinositol (3, 4, 5)-triphosphate phosphatase, which modulates hypertrophy [13]. This means that HDACs control muscle cell size on multiple levels.

DNA Methylation

In eukaryotes, DNA methylation occurs by the addition of a methyl group to the carbon 5′ position of the nucleotide cytosine ring. In mammals, DNA methylation occurs mainly in the sequence 5′-CG-3′, which also is referred to as a CpG dinucleotide; approximately 70% of all CpGs in humans are methylated [14]. On the other hand, unmethylated CpGs are found in the 5′ regulatory regions of many genes as clusters called "CpG islands." This frequency of CpG dinucleotides in CpG islands is higher than that found in other DNA regions. Notably, differential methylation of CpG islands is part of the epigenetic variation found in humans [15].

DNA cytosine methylation alters the accessibility for transcription factor complexes at a local level and, as with histone modifications, affects chromatin structure at regional and genome-wide levels. Thus, a well-characterized functional effect of DNA methylation is control of gene expression [16]. In this respect, hypermethylation of CpG sites may silence a gene, whereas hypomethylation allows gene transcription. One might say that methylation is a stable and heritable modification, but at the same time, it may be affected by the environment. For example, the mouse agouti locus, which affects coat color, is affected by the methylation status of an upstream transposon. Genetically identical parents in whom agouti genes are in different epigenetic states tend to produce offspring with different coat colors [17].

Experimental evidence for a role in transcriptional regulation for HF-specific genes by DNA methylation came from a recent study by Kao et al. [18]. They showed that the proinflammatory gene TNF-α reduces expression of the sarcoplasmic reticulum Ca^{2+}-ATPase (SERCA2A) by enhancing methylation status of the SERCA2A promoter region. Movassagh et al. [19•] recently showed there are methylation status differences between cardiomyopathy and controls in human cardiac tissue. Furthermore, they identified three loci (PECAM1, PECAM1, AMOTL2) whose differential methylation is correlated with altered gene expression in different cardiac samples.

MicroRNAs

MicroRNAs (miRNAs or miR) are short (~20 bp) double-strand RNA molecules that originate from nuclear and cytoplasmic larger precursors and act as important regulators of gene expression at a posttranscriptional level. miRNAs fine-tune the expression of 30% to 50% of the protein-coding genes by binding to partly complementary base pairs in 3′ untranslated regions of mRNAs, thereby interfering with translation; targeted mRNAs are either degraded or temporarily silenced [20]. miRNA regulation of protein expression is very complex because several miRNAs can target the same gene and several genes may be targeted by the same miRNA [20]. The expression of miRNAs is tissue and disease specific. In recent years, a molecular miRNA signature has been identified in many pathologic conditions, ranging from various types of cancer to many aspects of CVD [21•].

Growing evidence indicates that miRNAs are involved in basic cell functions [22]. The link between miRNAs and several aspects of HF are now very clear, and the number of publications in this field has increased rapidly in the past few years (Table 1). Research on CVDs has focused mainly on the two miRNA families specifically expressed in heart tissue (miRNA-1/miRNA-133 and miRNA-208). Several studies investigated miRNA expression in the healthy heart, during pressure overload, and in different etiologies of human, mouse, and rat HF, as reviewed by Divakaran and Mann [23]. van Rooij et al. [24] determined that the cardiac-specific miRNA-208 regulates cardiomyocyte hypertrophy, fibrosis, and expression of β-myosin heavy chain (β-MHC) in response to stress and hypothyroidism. This miRNA is encoded by an intron of the α-MHC gene. Thus, the gene encoding α-MHC, in addition to encoding a major cardiac contractile protein, regulates cardiac growth and gene expression in response to stress and hormonal signaling through miRNA-208, via targets of miRNA-208 such as β-MHC. Furthermore, targeted deletion of the muscle-specific miRNA, miRNA-1-2, revealed numerous functions in the heart, including regulation of cardiac morphogenesis, electri-

Table 1 Cardiac microRNAs, their functions, and validated targets

MiR family	Function	Validated cardiac targets
All (Dicer)	Regulation of cardiogenesis	Not applicable
	Cardiomyocyte-specific deletion in mice is embryonically [25] or neonatally [44] lethal (depending on promotor)	
1	*Embryonic*	
	Modulates cardiogenesis [25, 45–47]	Delta, the Notch ligand, involved in cardiac cell differentiation [45]; Hand2, a cardiac transcription factor involved in cardiomyocyte expansion [25, 46]; and HDAC4, a transcriptional repressor of muscle gene expression [47]
	Essential to maintain muscle gene expression [45]	
	Deletion in mice is partially embryonically lethal (ventricular–septal defect) [25]	
	Regulation of cardiomyocyte cell cycle [25]	
	Adult	
	Conduction [25]; proarrhythmic [48–51]	Irx5, a cardiac TF that represses the potassium channel Kcnd2 [18]; potassium channels KCNJ2 [48] and KCNE1 [51]; pacemaker channels HCN2 and HCN4 [50]; connexin 43 [48]; and B56α subunit of protein phosphatase 2A [49]
	Regulation of cardiomyocyte growth [52, 53]	Pro-hypertrophic genes calmodulin and Mef2a [52]; cell cycle regulators RasGAP and Cdk9 and Rheb [53]; and fibronectin [31]
	Proapoptotic [54]	HSP60 and HSP70 [54]
133	*Embryonic*	
	Modulates cardiogenesis [47, 55]	SRF [47, 55]
	Deletion in mice is partially embryonically lethal (ventricular–septal defect) [55]	
	Regulation of cardiomyocyte cell cycle [55]	Cyclin D2 [55]
	Adult	
	Regulation of collagen synthesis [55, 56]	CTGF [56]
	Conduction [55]; proarrhythmic [50, 57]	Potassium channels KCNQ [51] and HERG [57] and pacemaker channel HCN2 [50]
	Regulation of cardiomyocyte growth [22, 58]	RhoA, a GDP–GTP exchange protein regulating cardiac hypertrophy [22]; Cdc42, a signal transduction kinase implicated in hypertrophy [22]; and Nelf-A/WHSC2, a nuclear factor involved in cardiogenesis [22]
	Antiapoptotic [54, 55]	Caspase-9 [54]
208	*Adult*	
	Regulates cardiomyocyte hypertrophy and fibrosis in response to pressure overload	THRAP1, a thyroid hormone transcription factor [24]

GDP—guanosine diphosphate; GTP—guanosine triphosphate; TF—transcription factor.

(*From* Schroen and Heymans [20]; with permission.)

cal conduction, and cell cycle control [25]. Thum et al. [26] showed that the miRNA signature of failing myocardium closely resembles the miRNA expression pattern of fetal heart, and this underlies the reonset of the fetal gene program in failing hearts. Another interesting study by Thum et al. [27] showed that miRNA-21 regulates the ERK-MAP kinase pathway specifically in cardiac fibroblasts, not in cardiomyocytes, and this affects cardiac structure and function. Increased miRNA-21 levels in fibroblasts activate ERK kinase activity through inhibition-specific genes, and by this mechanism, miRNA-21 regulates interstitial fibrosis and cardiac hypertrophy. These findings revealed an extra level of gene regulation via miRNA-mediated paracrine secretion, in this case via cardiac fibroblasts.

The profound effects of miRNAs on the heart and the hypertrophic response are subject to further study, and miRNAs are emerging as chief regulators of gene control. Thus far, miRNAs have been shown to affect the myocardium but also the electrical properties of the heart, as well as the modulation of angiogenesis [28].

Methods of Epigenetic Screening

Epigenomic profiles vary from cell type to cell type and may change over time and in response to physiologic, pathologic, and pharmacologic triggers. Therefore, mapping the epigenome, as a follow-up to the human genome

sequencing project, is a tremendous task. Although it is possible to determine the epigenetic status of a sequence in the genome, mapping the entire epigenome will require the sequencing of dozens of genomes covering all the different cell types of an organism at different points in life [7].

Bisulfite mapping is the most accurate method for mapping DNA methylation patterns. Genomic DNA is treated with sodium bisulfite, which results in modification and eventual deamination of the unmethylated cytosines to uracil, whereas the methylated cytosines are protected from this conversion. To determine the methylation state of specific genes, gene-specific primers are used to amplify the sequence of interest, which is then subjected to sequencing. Methylated cytosines register as Cs, whereas unmethylated cytosines appear as Ts in the sequence [7].

Several whole-genome approaches to methylation mapping were developed recently that are based on the differential sensitivity of methylated and unmethylated CpGs to restriction enzymes. Restriction length genome scanning uses a double-digestion of DNA with a frequent-cutter methylation-insensitive restriction enzyme and a rare methylation-sensitive enzyme such as *Not1*, which cleaves its recognition site exclusively when it is unmethylated [7]. A different whole-genome approach that takes advantage of DNA chip technology allows rapid profiling of the status of methylation of thousands of CpG islands at once, using the differential methylation hybridization method [29]. This method is used to identify CpG islands, which are methylated in tumor samples relative to their normal controls.

An alternative to bisulfite conversion is the ChIP-seq (chromatin immunoprecipitation combined with sequencing) technology. Protein-DNA interactions are crosslinked in situ followed by immunoprecipitation and sequencing of genome-wide sites associated with a modification of interest. This approach allows the identification of DNA binding sites of any DNA-associated protein. This technique also provides information on histone modifications (acetylation, methylation, phosphorylation, ubiquitination, and sumoylation). Variations on the ChIP technology have been developed, such as the "DCS" method, which couples ChIP with subtraction polymerase chain reaction (PCR) [30, 31]. This technique aims to avoid the nonspecific signals generated by hybridization of genome-derived fragments to microarrays.

In the same manner, to examine the role of miRNAs in human pathology, most studies have used high-throughput methods to analyze global miRNA expression in clinical samples [32]. High-throughput methods are represented by microRNA microarray and quantitative real-time PCR. Although the discrimination between molecules represents a great challenge, the main advantage of miRNA microarray is represented by the high specificity of the reaction, whereas the main disadvantage is represented by low sensitivity [21•].

Drugs Modifying the Epigenetic Status

Epigenetics modulates phenotypic variation in health and disease, and it seems likely that understanding and manipulating the epigenome hold enormous promise for preventing and treating common human illnesses. Epigenetics also offers an important window to understanding the role of the environment's interactions with the genome in causing disease, and in modulating those interactions to improve human health [33].

Antagomirs are single-strand RNAs complementary to the miRNA. Chemical modification of antagomirs might represent a very attractive approach to targeting pathologic miRNAs. However, this strategy is challenging because miRNAs all belong to closely related families and the specificity of antagomirs for one miRNA might be difficult to achieve. Furthermore, a single miRNA targets several genes; among them, some might be beneficial for the myocardium. In that regard, chemically modified oligonucleotides that would specifically disrupt the binding between the miRNA and a single mRNA might be good therapeutic candidates. However, the in vivo final effect of the modulation of miRNAs remains unclear, because each of them can target hundreds of genes with different intensity [28]. Finally, the challenges in bringing miRNA antagonists to the clinical arena are similar to the ones we encountered with gene therapy, namely the mode of delivery, vectors, specificity, and toxicity in vivo [34]. At least theoretically, targeting specific miRNAs implicated in ischemic heart disease, cardiac hypertrophy, HF, angiogenesis, and channelopathies might turn out to be an attractive therapeutic tool in the future, aiming to prevent the development of HF [28].

An alternative approach would be to target DNA methylation. Several chemical compounds that affect the genomic DNA methylation landscape already are being used in the clinic. Drugs such as 5-azacytidine and azacytidine inhibit methyltransferases, causing demethylation of DNA sequences. Other drugs inhibit DNA methylation by blocking the maintenance methyltransferase. For more detailed information, see the article by Gomez and Ingelman-Sundberg [35]. The lack of specificity of the current drugs, however, leads to a global effect, which might affect genomic stability. Drugs that can target a specific methylation site have yet to be designed. In addition to work involving drugs that modify DNA methylation, increasing work is being carried out in developing drugs that affect histone modifications.

HDAC inhibitors have been the focus of attention in anticancer drug development because they are seen as presenting a potential strategy to reverse aberrant epigenetic changes associated with cancer [36]. There also is evidence that HDAC inhibitors may restore gene expression programs in cardiomyocyte hypertrophy. Gallo et al. [37]

demonstrated in vitro inhibition of cardiac hypertrophy using trichostatin A and sodium butyrate.

Epigenetics and Environment

It is known that environmental factors such as toxins and diet may have an effect on DNA methylation and chromatin modification and that these changes are passed on to the next generation [38, 39]. Estrogenic and antiandrogenic toxins that decrease male fertility alter DNA methylation, and these changes are inherited by subsequent generations [39]. The hypothesis that environmental factors alter heritable epigenetic marks and change patterns of gene expression is an exciting possibility in human disease research. Most common diseases are influenced by both genetic and environmental factors; thus, environmentally induced changes in epigenetic structures may provide a mechanistic link between genes and the environment [40].

Age plays an important role in gene-environment interactions. Common diseases increase with aging, which is related to the accumulation of epigenetic changes through the lifetime of an individual. This idea is supported by a study showing higher levels of variation of total DNA methylation and histone H3K9 acetylation in older monozygotic twins than in younger twins, although that study did not measure epigenetic changes over time in the same individual [41].

Conclusions

The field of epigenetics offers a new playground for researchers to study and modify interindividual variation in clinical effects, variation in drug response and toxicity, and new targets for drug therapy. The increasing knowledge regarding epigenetic mechanisms derived from the Human Epigenome Project is leading to a better understanding of human disease and a new range of molecular targets for epigenetic drugs [42]. Although most advances in pharmacogenomics have been applied to its use in the oncology field, this review presents evidence that in the past years, there has been a significant increase in the number of cardiovascular epigenetic studies, and in miRNAs more specifically [8]. The review by Mishra et al. [43] offers a clear picture of the recent progress made in the microR-Nomics of CVD and the future of miRNAs as potential therapeutic targets or drug agents.

As epigenetics has an important role in shaping phenotypic variation in health and disease, it seems likely that understanding and manipulating the epigenome hold enormous promise for preventing and treating common human diseases, including CVD and HF.

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