

# The emerging role of epigenetics in cardiovascular disease

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**Abstract:** There is a worldwide epidemic of cardiovascular diseases causing not only a public health issue but also accounting for trillions of dollars of healthcare expenditure. Studies pertaining to epidemiology, pathophysiology, molecular biology, gene identification and genetic linkage maps have been able to lay a strong foundation for both the diagnosis and treatment of cardiovascular medicine. Although the concept of ‘epigenetics’ is not recent, the term in current usage is extended from the initial concept of ‘controlling developmental gene expression and signaling pathways in undifferentiated zygotes’ to include heritable changes to gene expression that are not from differences in the genetic code. The impact of epigenetics in cardiovascular disease is now emerging as an important regulatory key player at different levels from pathophysiology to therapeutics. This review focuses on the emerging role of epigenetics in major cardiovascular medicine specialties such as coronary artery disease, heart failure, cardiac hypertrophy and diabetes.

**Keywords:** cardiac hypertrophy, cardiology, cardiovascular disease, coronary artery disease, diabetes, epigenetics, heart failure

## Introduction

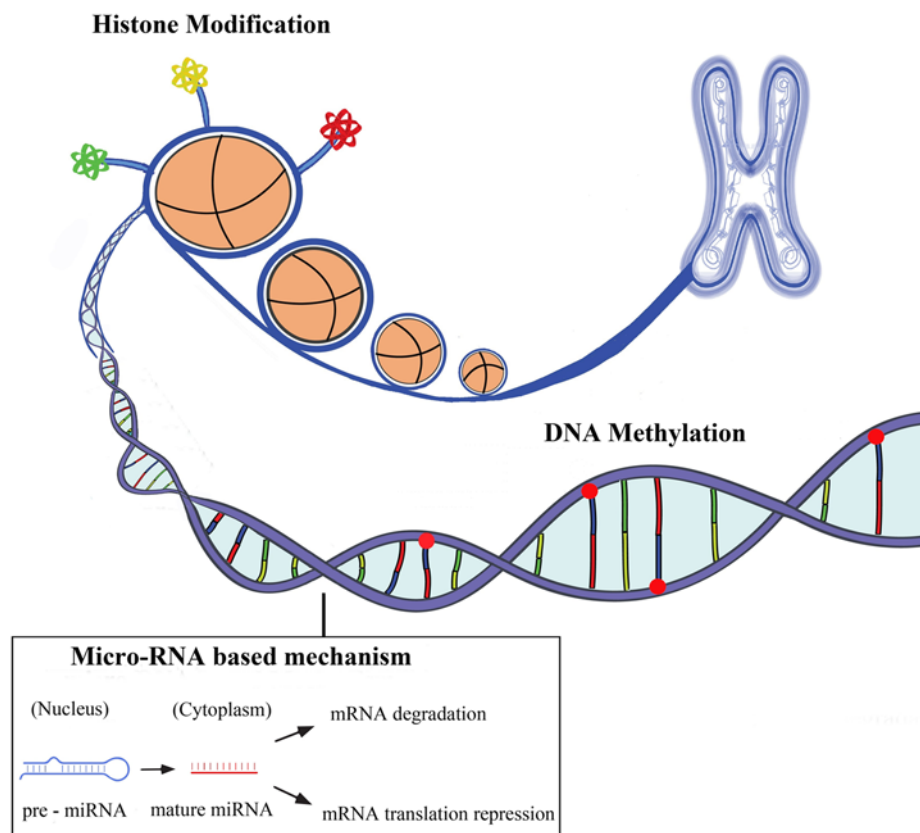
Cardiovascular disease (CVD) is considered to be the leading cause of death in all countries [Murray and Lopez, 1997], despite significant disparities related to socioeconomic strata and gender [The Lancet, 2013]. During the past century, there has been dramatic progress in the diagnosis, prevention and treatment in different fields of cardiovascular medicine [Nabel and Braunwald, 2012; Polonsky, 2012], which in turn reduced global and cause-specific mortality [Abi Khalil *et al.* 2012; Laribi *et al.* 2012; Nichols *et al.* 2013; Yeh *et al.* 2010]. However, current scientific knowledge does not completely explain the complex pathophysiology underlying CVD and other pathways are constantly looked for. Over the past decade, epigenetics has initiated a new era in genetic medicine capable of giving a different approach to human disease [Feinberg, 2010; Portela and Esteller, 2010]. Some international initiatives, including the Human Epigenome Project (HEP) and the International Human Epigenome Consortium (IHEC) have even been launched to catalogue the human epigenome and correlate its relation to pathophysiology [Abbott,

2010; Rakyan *et al.* 2004]. Epigenetics in CVD is the focus of this review.

## Mechanisms of epigenetic imprinting

The term epigenetics refers to all the heritable changes in gene expression regulation other than nucleotide sequence and chromatin organization that depend on the DNA sequences itself [Egger *et al.* 2004; Rodenhiser and Mann, 2006]. Epigenetic inheritance is an essential mechanism that allows the stable propagation of gene activity states from one generation of cells to the next [Kelsey and Feil, 2013]. Epigenetic mechanisms have been involved in the differentiation of many cell types from progenitor or primary cells and with whom they share the same DNA sequence [Ji *et al.* 2010; Miao *et al.* 2008]. They also represent a stable cellular memory for the differentiation state of a cell population. The major epigenetic features of human cells include DNA methylation, post-translational histone modifications and RNA-based mechanisms including those controlled by small noncoding micro-RNAs [Wilson, 2008] (see Figure 1).

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**Figure 1.** The three main areas of epigenetics are DNA methylation, histone modifications (acetylation, methylation, phosphorylation, etc.) and micro-RNA based mechanisms. These three processes are distinct but are interrelated and control gene expression.

### DNA methylation

This primarily happens at specific dinucleotide sites along the genome or at sites where methyl groups attach to cytosine bases followed by guanine (CpG islands). Other chemical modifications to cytosine resulting in hydroxymethylation, formylation and carboxylation also occur but their interrelation with methylation and their impact on epigenetics is not completely understood [Iurlaro *et al.* 2013]. In fact, 40% of genes contain CpG-rich islands and up to 70% of all CpG dinucleotides in the genome are methylated [Bird, 2002; Wilson, 2008]. Methylated CpGs act as docking sites for methyl binding proteins which have the ability to oligomerize through the DNA in order to recruit chromatin remodeling complexes that, in turn, cause chromatin condensation and gene inactivation and silencing [Ng *et al.* 1999; Nikitina *et al.* 2007; Suzuki and Bird, 2008]. Non-CpG island methylation has also been reported to influence protein–DNA interactions, gene expression and chromatin structure and stability [Fouse *et al.* 2010]. Non-CpG

methylation was recently found to be prevalent in human embryonic stem cells [Lister *et al.* 2009; Ramsahoye *et al.* 2000] and in neurons [Guo *et al.* 2013].

Changes in DNA methylation patterns have been observed in association with CVD, inflammation, autoimmune diseases, infections and cancer [Bierne *et al.* 2012; Dang *et al.* 2013; Esteller, 2008].

### Histone modification

Eukaryotic DNA is wrapped around an octamer of the core histones H2-A, H2-B, H3 and H4, thus building the fundamental unit of chromatin, the nucleosome [Berger, 2007]. These chromatin marks are unstable and they change rapidly in response to any external stimuli, and any permanent changes to DNA can lead to the development of defective organs or the development of a disease. Histones undergo a variety of post-translational modifications, mainly targeting amino

acid residues of the *N*-terminal tails that protrude from the chromatin fiber [Natsume-Kitatani *et al.* 2011]. Among the different modifications, histone acetylation, methylation and phosphorylation are the most relevant ones to have been associated with transcription and gene expression [Li *et al.* 2007]. Post-translational modifications of histones also include their binding to specific proteins, called readers, which secondary interfere with chromatin function and mediate processes such as gene expression, apoptosis, and DNA damage repair [Jenuwein and Allis, 2001].

#### Micro-RNAs

Micro-RNAs (miRNAs) have recently emerged as regulators of gene expression that can simultaneously regulate genes in different biological networks. miRNAs have launched a new area in translational biology at a time when processing of a product was thought to be only by translation and transcription. These small (20–40 nucleotides) noncoding RNAs are highly conserved across species, highlighting the evolutionary importance of these molecules, as modulators of genes expression [Friedman *et al.* 2009]. More than 2500 miRNAs are currently identified in the human genome (<http://www.mirbase.org/>) and over 60% of all human genes are predicted to be regulated by these small RNAs (Zhang, 2008). Among miRNAs, many long-coding RNAs had already been identified years ago. Although initially neglected because of a false presumption of a lack of function, recent data show that they also enhance gene expression by modulating chromatin and organization of protein complexes across chromosomes [Geisler and Collier, 2013; Ørom and Shiekhattar, 2013].

#### Epigenetics in CVD

Epigenetics has been initially studied in patients with CVD for its prominent role in inflammation and vascular involvement [Castro *et al.* 2003; Stenvinkel *et al.* 2007]. Furthermore, epigenetic studies in cardiovascular medicine revealed a significant number of modifications affecting the development and progression of CVD. In addition, epigenomics are also involved in cardiovascular risk factors such as smoking [Breitling *et al.* 2011, 2012; Buro-Aurriemma *et al.* 2013], diabetes (see below), hypertension [Rivière *et al.* 2011; Smolarek *et al.* 2010] and age [Fuke *et al.* 2004].

#### Epigenetics in heart disease

*Cardiac hypertrophy.* In experimental models of epigenetics, Cardiac Hypertrophy (CH) has been linked with histone acetylation implicating both histone acetyltransferases (HATs) and histone deacetylases (HDACs). The activity of HATs seems to have a positive role in CH as exemplified by the HAT activity of the transcriptional co-activators CREB binding protein (CBP) and p300. Overexpression of CBP or p300 in cardiomyocytes resulted in hypertrophy, whereas overexpression of mutant CBP and p300 lacking HAT activity did not [Gusterson *et al.* 2003]. However, the activity of HDACs has been reported in both prohypertrophic and antihypertrophic pathways, resulting in conflicting data. Class IIa HDACs have been shown to suppress CH whereas some animals and *in vivo* models showed that HDAC inhibitors have a protective role against hypertrophy [Antos *et al.* 2003; Kee *et al.* 2006; Kook *et al.* 2003]. CH has also been linked with histone methylation, in particular with H3K9 [Haddad *et al.* 2003; Majumdar *et al.* 2008; Zhang *et al.* 2011] and H3K4 methylations [Bingham *et al.* 2007; Stein *et al.* 2011] in animal models. In a different experimental mice model, hypermethylation associated to hydroxymethylation of epidermal growth factor receptor (EGFR) gene was associated with aortic valve calcification and subsequent ventricular hypertrophy [Barrick *et al.* 2009]. Parallel to histone modifications, miRNAs are also involved in CH. In neonatal cardiomyocytes, overexpression of miR-23a, miR-23b, miR-24, miR-195 or miR-214 induced CH whereas overexpression of miR-133 inhibited the phenotype [Carè *et al.* 2007; Latronico and Condorelli, 2009].

*Heart failure.* Heart failure (HF) results from a complex genetic predisposition and multiple environmental factors. In a series of patients with HF, Movassagh and colleagues reported that three angiogenesis-related genes were differentially methylated, irrespective of the etiology: angiomin-2 gene (AMOTL2) was hypomethylated whereas the 5' promoter region in the platelet/endothelial cell adhesion molecule gene (PECAM1) and the gene body of Rho GTPase-activating protein 24 (ARHGAP24) were hypermethylated [Movassagh *et al.* 2010]. The altered epigenomics of the three genes in endstage HF may reflect common epigenetic pathways in heart remodeling and vasculature [Movassagh *et al.* 2011]. Dilated cardiomyopathy represents about one in three cases of HF. Interestingly, Nguyen and colleagues reported in a mouse

model that cardiac specific knockout of Dot1L, the gene encoding for the HKMT Dot1L which catalyzes H3K79 methylation in mammals, caused the appearance of a phenotype similar to dilated cardiomyopathy [Nguyen *et al.* 2011]. Histone modifications are also involved in HF; in a genome-wide histone methylation of heart tissues, Kaneda and colleagues reported that tri-methylated histone H3H4 and H3K9 were altered in HF [Kaneda *et al.* 2009]. Micro-RNAs have a particular importance in epigenetics of HF as shown in experimental models highlighting their role in driving gene expression change during the course of the disease [Ikeda *et al.* 2007; Latronico and Condorelli, 2009].

*Arrhythmias.* The contribution of epigenetics to heart rhythm disorders has recently been highlighted in common pathologies. Atrial fibrillation is the most prevalent arrhythmia in aging populations. In transgenic mice programmed to develop cardiac hypertrophy, Liu and colleagues showed that an injection of a specific HDAC inhibitor reverses atrial fibrosis and diminishes atrial fibrillation vulnerability following an electrical stimulation [Liu *et al.* 2008]. In other models, deletion of both HDAC-1 and HDAC-2 from the myocardium resulted in early rodent death by fetal arrhythmia [Montgomery *et al.* 2007]. Among the complex genetic background of the long QT syndrome, it is thought that a mutation interrupting the CpG island would prevent methylation and silence the paternal allele of the KCNQ1 gene in cardiac tissue [Bokil *et al.* 2010]. Micro-RNAs are also emerging as regulators of the normal electrophysiology of the heart and could also be implicated in arrhythmias [Kim, 2013]. Among the miRNAs that have been widely explored in experimental models, miR-1 was reported to be essential in normal electrophysiological conduction and its deletion was associated with high rate of sudden death [Zhao *et al.* 2007]. Overexpression of miR-208a is also associated with arrhythmia and heart death [Oliveira-Carvalho *et al.* 2013] whereas increase in miR-133a leads to prolonged QT intervals. Several other miRNAs such as the miR-212, miR-17-92, miR-155, miR-181 and miR-181a have been associated with regulation of heart rhythm through regulation of ion channels, transporters and cellular proteins [Kim, 2013].

#### *Epigenetics in vascular diseases*

*Atherosclerosis and artery disease.* Both DNA methylation, miRNAs and epigenetic mechanisms have been described in atherosclerosis. The

era of DNA methylation in atherosclerosis was launched initially by two different groups who showed coexisting hypomethylation with atherosclerotic lesions in mice and rabbits [Hiltunen *et al.* 2002; Laukkanen *et al.* 1999]. Further studies showed that hypomethylation could even be detected at very early stages of atherosclerosis, even before the appearance of the anatomical manifestation of the disease [Lund *et al.* 2004]. In human atherosclerosis, hypermethylation of atheroprotective estrogen receptor  $\alpha$  (ESR1) and estrogen receptor  $\beta$  (ESR2) in vascular smooth muscle cells were described by two independent groups [Kim *et al.* 2007; Post *et al.* 1999]. In cultured tissues of aorta and coronary arteries with varying degrees of atherosclerosis, Zhu and colleagues showed that methylation of the monocarboxylate transporter (MCT3) gene suppresses its transcription and hence allows the passage of smooth muscle cells and the progression of atherosclerosis [Zhu *et al.* 2005].

The contribution of miRNAs to atherosclerosis has been extensively investigated in the past few years. In fact, miRNAs are key players in programming and modulating gene expression of relevant cell types involved in atherosclerosis. They mediate inflammation, cholesterol influx, cellular differentiation and lipid uptake (Table 1).

Clinical studies are aligned to experimental ones. In a follow up of elderly subjects, Baccarelli and colleagues showed that hypomethylation of long interspersed nucleotide elements was associated with higher incidence of ischemic heart disease events [Baccarelli *et al.* 2010]. Friso and colleagues showed that hypomethylation of the promoter of coagulation factor VII was associated with coronary artery disease in patients with angiographically proven coronary lesions [Friso *et al.* 2012].

*Diabetic vascular disease.* Chronic exposure to hyperglycemia induces specific chromatin changes and transcriptional responses, indicating epigenetic events that mediate signaling pathways relevant to diabetic CVD [El-Osta, 2012]. Genome-wide analysis of human vascular cells showed that histone modifications and DNA methylation occur simultaneously during hyperglycemic conditions and predict gene expression [Pirola *et al.* 2011].

Cell culture studies allow a direct comprehension of epigenetics and gene expression. The

**Table 1.** Micro-RNAs involved in atherosclerosis.

miRNA	Gene target	Cell type	Biological process	Reference
miR - 33a/b	ABCA1, ABCG1, CPTA, CROT, HADHB	Monocytes/ macrophages	Cholesterol efflux, fatty acid beta-oxidation	Rayner <i>et al.</i> [2010] Marquart <i>et al.</i> [2010] Najafi-Shoushtari <i>et al.</i> [2010]
miR - 758, 26, 106 and 10b	ABCA1	Monocytes/ macrophages	Cholesterol efflux	Rayner <i>et al.</i> [2010] Horie <i>et al.</i> [2012] Ramirez <i>et al.</i> [2011] Sun <i>et al.</i> [2012a]
miR 155	LOX-1, CD36, CD68 MyD88, BCL-6	Monocytes/ macrophages	Lipid uptake and inflammation	Kim <i>et al.</i> [2012] Huang <i>et al.</i> [2010]
miR 125a-5p	ORP9	Monocytes/ macrophages	Lipid uptake and inflammation	Wang <i>et al.</i> [2012]
miR-26a	SMAD1, SMAD 4	Vascular smooth muscle cells	Vascular differentiation and apoptosis	Chen <i>et al.</i> [2009]
miR-29b	DNMT3b	Vascular smooth muscle cells	Migration	Nazari-Jahantigh <i>et al.</i> [2012]
miR-145	KLF4	Vascular smooth muscle cells	Differentiation	Leeper <i>et al.</i> [2011]
miR-125b	SP7	Vascular smooth muscle cells	Calcification	Chen <i>et al.</i> [2011]
miR-21	PDC4	Vascular smooth muscle cells	Apoptosis	Li <i>et al.</i> [2009]
miR-221/222	P27(kip1), p57(kip2), c-kit	Vascular smooth muscle cells	Proliferation, migration and apoptosis	Qin <i>et al.</i> [2011]
miR-126	RGS16	Endothelial cells	Progenitor cells, apoptosis	Zernecke <i>et al.</i> [2009]
miR143/145	ELK1, KLF4, CAMK2d,SSH2, PHACTR4, CFL1	Endothelial cells	Differentiation	Hergenreider <i>et al.</i> [2012]
miR-10a/miR-181b	HOXA1, M βTRC, AP3K7, KPNA1	Endothelial cells	Inflammation and dysfunction	Fang <i>et al.</i> [2010] Sun <i>et al.</i> [2012b]
miR 155/miR22	eNOS STAT5A	Endothelial cells	Neovascularization	Sun <i>et al.</i> [2012a] Kim <i>et al.</i> [2012]
miRNA, micro-RNA.				

transcriptional co-activator PPARGC1A coordinates gene expression, which in its turn stimulates secretion of mitochondrial reactive oxygen species in different tissues in humans [Puigserver and Spiegelman, 2003]. In pancreatic β-cells of patients with type 2 diabetes (T2D), the PPARGC1A promoter is more methylated compared with nondiabetic individuals [Ling *et al.* 2008]. As a result, its expression is reduced, which confirms the theory that epigenomics have a critical part in regulating gene expression. In addition, Mutskov and colleagues showed that insulin gene in human islet-derived precursor cells displayed high levels of histone modifications (H4 hyperacetylation and dimethylation of H3 lysine 4), which are typical of active genes [Mutskov *et al.* 2007].

It is already known that inflammation is part of the complex biochemical process of initiating and further developing cardiovascular complications of diabetes. Exposure to hyperglycemia could induce epigenomic changes in inflammatory pathways. The nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) is a protein complex that controls DNA transcription and regulation of the activity of genes implicated in inflammation and atherothrombosis [Miao *et al.* 2004]. In individuals with T2D, chronic uncontrolled hyperglycemia enhances NF-κB activity in white blood cells, therefore affecting the activity of cytokines [Shanmugam *et al.* 2003]. HATs that acetylate lysine amino acids by transferring an acetyl group from acetyl CoA to form e-N-acetyl lysine on histone proteins interact with NF-κB, which causes

hyperacetylation of inflammation-related genes promoters such as tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) and cyclooxygenase-2 [Bird, 2007; Hofmann *et al.* 1998]. Other histone transferases that work in a similar way to HATs could also interfere in the regulation of pro-inflammatory genes by recruiting NF- $\kappa$ B to gene promoters. In animal models, such as the ones done on diabetic db/db mice, Reddy and colleagues reported that a hyperglycemic milieu enhances the expression of inflammation-related genes and precipitates pro-atherogenic responses in smooth muscle cells of the vascular system [Reddy *et al.* 2008]. This was also confirmed by Villeneuve and colleagues in a similar model [Villeneuve *et al.* 2008]. They reported that the expression of inflammatory genes in vascular cells cultured in high glucose was increased whereas that of H3K9me3, a histone H3 known to protect against the biochemical state of diabetic inflammation, were decreased at their promoters. Most importantly, El-Osta and colleagues reported that even a transient exposure to high levels of glucose induced sustained epigenomic changes in the p65 subunit promoter of the NF- $\kappa$ B, modifying thereafter the epigenomics of cultured aortic endothelial cells [El-Osta *et al.* 2008]. That might explain, at least partially, the irreversibility of cardiovascular complications of long-standing uncontrolled diabetes often encountered in clinical care. Finally, MicroRNAs are also implicated in the pathophysiology of diabetes and its complications and could even be potential pharmacological targets [Mao *et al.* 2013].

### Conclusion

Epigenetics is likely to be involved in the biology of major disciplines of cardiovascular medicine. To date, there are no significant reports of epigenetics contribution in clinical practice or therapeutics in CVD whereas in cancer, DNA methylation inhibitors, histone methylation inhibitors and histone deacetylase inhibitors have been successful so far [Gal-Yam *et al.* 2008]. Understanding the epigenetic mechanisms, their interactions and alterations using translational research approaches or large human cohorts promises to make a significant contribution to our understanding of CVDs.

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### Conflict of interest statement

The author declares no conflict of interest in preparing this article.

### References

- Abbott, A. (2010) Project set to map marks on genome. *Nature* 463: 596–597.
- Abi Khalil, C., Roussel, R., Mohammedi, K., Danchin, N. and Marre, M. (2012) Cause-specific mortality in diabetes: recent changes in trend mortality. *Eur J Prev Cardiol* 19: 374–381.
- Antos, C., McKinsey, T., Dreitz, M., Hollingsworth, L., Zhang, C., Schreiber, K. *et al.* (2003) Dose-dependent blockade to cardiomyocyte hypertrophy by histone deacetylase inhibitors. *J Biol Chem* 278: 28930–28937.
- Baccarelli, A., Wright, R., Bollati, V., Litonjua, A., Zanobetti, A., Tarantini, L. *et al.* (2010) Ischemic heart disease and stroke in relation to blood DNA methylation. *Epidemiology* 21: 819–828.
- Barrick, C., Roberts, R., Rojas, M., Rajamannan, N., Suitt, C., O'Brien, K. *et al.* (2009) Reduced EGFR causes abnormal valvular differentiation leading to calcific aortic stenosis and left ventricular hypertrophy in C57BL/6J but not 129S1/SvImJ mice. *Am J Physiol Heart Circ Physiol* 297: H65–75.
- Berger, S. (2007) The complex language of chromatin regulation during transcription. *Nature* 447: 407–412.
- Bierne, H., Hamon, M. and Cossart, P. (2012) Epigenetics and bacterial infections. *Cold Spring Harbor Perspect Med* 2: a010272.
- Bingham, A., Ooi, L., Kozera, L., White, E. and Wood, I. (2007) The repressor element 1-silencing transcription factor regulates heart-specific gene expression using multiple chromatin-modifying complexes. *Mol Cell Biol* 27: 4082–4092.
- Bird, A. (2002) DNA methylation patterns and epigenetic memory. *Genes Dev* 16: 6–21.
- Bird, A. (2007) Perceptions of epigenetics. *Nature* 447: 396–398.
- Bokil, N., Baisden, J., Radford, D. and Summers, K. (2010) Molecular genetics of long QT syndrome. *Mol Genet Metab* 101: 1–8.
- Breitling, L., Salzmann, K., Rothenbacher, D., Burwinkel, B. and Brenner, H. (2012) Smoking,

- F2RL3 methylation, and prognosis in stable coronary heart disease. *Eur Heart J* 33: 2841–2848.
- Breitling, L., Yang, R., Korn, B., Burwinkel, B. and Brenner, H. (2011) Tobacco-smoking-related differential DNA methylation: 27K discovery and replication. *Am J Hum Genet* 88: 450–457.
- Buro-Auremma, L., Salit, J., Hackett, N., Walters, M., Strulovici-Barel, Y., Staudt, M. *et al.* (2013) Cigarette smoking induces small airway epithelial epigenetic changes with corresponding modulation of gene expression. *Hum Mol Genet* 22: 4726–4738.
- Carè, A., Catalucci, D., Felicetti, F., Bonci, D., Addario, A., Gallo, P. *et al.* (2007) MicroRNA-133 controls cardiac hypertrophy. *Nat Med* 13: 613–618.
- Castro, R., Rivera, I., Struys, E., Jansen, E., Ravasco, P., Camilo, M. *et al.* (2003) Increased homocysteine and S-adenosylhomocysteine concentrations and DNA hypomethylation in vascular disease. *Clin Chem* 49: 1292–1296.
- Chen, K., Wang, Y., Hu, C., Chang, W., Liao, Y., Dai, C. *et al.* (2011) OxLDL up-regulates microRNA-29b, leading to epigenetic modifications of MMP-2/MMP-9 genes: a novel mechanism for cardiovascular diseases. *FASEB J* 25: 1718–1728.
- Chen, T., Huang, Z., Wang, L., Wang, Y., Wu, F., Meng, S. *et al.* (2009) MicroRNA-125a-5p partly regulates the inflammatory response, lipid uptake, and ORP9 expression in oxLDL-stimulated monocyte/macrophages. *Cardiovasc Res* 83: 131–139.
- Dang, M., Buzzetti, R. and Pozzilli, P. (2013) Epigenetics in autoimmune diseases with focus on type 1 diabetes. *Diabetes Metab Res Rev* 29: 8–18.
- Egger, G., Liang, G., Aparicio, A. and Jones, P. (2004) Epigenetics in human disease and prospects for epigenetic therapy. *Nature* 429, 457–463.
- El-Osta, A. (2012) Glycemic memory. *Curr Opin Lipidol* 23: 24–29.
- El-Osta, A., Brasacchio, D., Yao, D., Poci, A., Jones, P., Roeder, R. *et al.* (2008) Transient high glucose causes persistent epigenetic changes and altered gene expression during subsequent normoglycemia. *J Exp Med* 205: 2409–2417.
- Esteller, M. (2008) Epigenetics in cancer. *N Engl J Med* 358: 1148–1159.
- Fang, Y., Shi, C., Manduchi, E., Civelek, M. and Davies, P. (2010) MicroRNA-10a regulation of proinflammatory phenotype in athero-susceptible endothelium *in vivo* and *in vitro*. *Proc Natl Acad Sci U S A* 107: 13450–13455.
- Feinberg, A. (2010) Epigenomics reveals a functional genome anatomy and a new approach to common disease. *Nat Biotechnol* 28: 1049–1052.
- Fouse, S., Nagarajan, R. and Costello, J. (2010) Genome-scale DNA methylation analysis. *Epigenomics* 2: 105–117.
- Friedman, R., Farh, K., Burge, C. and Bartel, D. (2009) Most mammalian mRNAs are conserved targets of microRNAs. *Genome Res* 19: 92–105.
- Friso, S., Lotto, V., Choi, S., Girelli, D., Pinotti, M., Guarini, P. *et al.* (2012) Promoter methylation in coagulation F7 gene influences plasma FVII concentrations and relates to coronary artery disease. *J Med Genet* 49: 192–199.
- Fuke, C., Shimabukuro, M., Petronis, A., Sugimoto, J., Oda, T., Miura, K. *et al.* (2004) Age related changes in 5-methylcytosine content in human peripheral leukocytes and placentas: an HPLC-based study. *Ann Hum Genet* 68: 196–204.
- Gal-Yam, E., Saito, Y., Egger, G. and Jones, P. (2008) Cancer epigenetics: modifications, screening, and therapy. *Annu Rev Med* 59: 267–280.
- Geisler, S. and Coller, J. (2013) RNA in unexpected places: long non-coding RNA functions in diverse cellular contexts. *Nat Rev Mol Cell Biol* 14: 699–712.
- Guo, J., Su, Y., Shin, J., Shin, J., Li, H., Xie, B. *et al.* (2013) Distribution, recognition and regulation of non-CpG methylation in the adult mammalian brain. *Nat Neurosci* 17: 215–222.
- Gusterson, B., Jazrawi, E., Adcock, I. and Latchman, D. (2003) The transcriptional co-activators CREB-binding protein (CBP) and p300 play a critical role in cardiac hypertrophy that is dependent on their histone acetyltransferase activity. *J Biol Chem* 278: 6838–6847.
- Haddad, F., Bodell, P., Qin, A., Giger, J. and Baldwin, K. (2003) Role of antisense RNA in coordinating cardiac myosin heavy chain gene switching. *J Biol Chem* 278: 37132–37138.
- Hergenreider, E., Heydt, S., Tréguer, K., Boettger, T., Horrevoets, A., Zeiher, A. *et al.* (2012) Atheroprotective communication between endothelial cells and smooth muscle cells through miRNAs. *Nat Cell Biol* 14: 249–256.
- Hiltunen, M., Turunen, M., Häkkinen, T., Rutanen, J., Hedman, M., Mäkinen, K. *et al.* (2002) DNA hypomethylation and methyltransferase expression in atherosclerotic lesions. *Vasc Med* 7: 5–11.
- Hofmann, M., Schiekofer, S., Kanitz, M., Klevesath, M., Joswig, M., Lee, V. *et al.* (1998) Insufficient glycemic control increases nuclear factor-kappa B binding activity in peripheral blood mononuclear cells isolated from patients with type 1 diabetes. *Diabetes Care* 21: 1310–1316.
- Horie, T., Baba, O., Kuwabara, Y., Chujo, Y., Watanabe, S., Kinoshita, M. *et al.* (2012)

- MicroRNA-33 deficiency reduces the progression of atherosclerotic plaque in ApoE<sup>-/-</sup> mice. *J Am Heart Assoc* 1: e003376.
- Huang, R., Hu, G., Lin, B., Lin, Z. and Sun, C. (2010) MicroRNA-155 silencing enhances inflammatory response and lipid uptake in oxidized low-density lipoprotein-stimulated human THP-1 macrophages. *J Invest Med* 58: 961–967.
- Ikeda, S., Kong, S., Lu, J., Bisping, E., Zhang, H., Allen, P. *et al.* (2007) Altered microRNA expression in human heart disease. *Physiol Genomics* 31: 367–373.
- Iurlaro, M., Ficiz, G., Oxley, D., Raiber, E., Bachman, M., Booth, M. *et al.* (2013) A screen for hydroxymethylcytosine and formylcytosine binding proteins suggests functions in transcription and chromatin regulation. *Genome Biol* 14: R119.
- Jenuwein, T. and Allis, C. (2001) Translating the histone code. *Science* 293: 1074–1080.
- Ji, H., Ehrlich, L., Seita, J., Murakami, P., Doi, A., Lindau, P. *et al.* (2010) Comprehensive methylome map of lineage commitment from haematopoietic progenitors. *Nature* 467: 338–342.
- Kaneda, R., Takada, S., Yamashita, Y., Choi, Y., Nonaka-Sarukawa, M., Soda, M. *et al.* (2009) Genome-wide histone methylation profile for heart failure. *Genes Cells* 14: 69–77.
- Kee, H., Sohn, I., Nam, K., Park, J., Qian, Y., Yin, Z. *et al.* (2006) Inhibition of histone deacetylation blocks cardiac hypertrophy induced by angiotensin II infusion and aortic banding. *Circulation* 113: 51–59.
- Kelsey, G. and Feil, R. (2013) New insights into establishment and maintenance of DNA methylation imprints in mammals. *Philos Trans R Soc Lond B* 368: 20110336.
- Kim, G. (2013) MicroRNA regulation of cardiac conduction and arrhythmias. *Transl Res J Lab Clin Med* 161: 381–392.
- Kim, J., Kim, J., Song, K., Lee, Y., Seo, J., Jelinek, J. *et al.* (2007) Epigenetic changes in estrogen receptor beta gene in atherosclerotic cardiovascular tissues and *in-vitro* vascular senescence. *Biochim Biophys Acta* 1772: 72–80.
- Kim, J., Yoon, H., Ramírez, C., Lee, S., Hoe, H., Fernández-Hernando, C. *et al.* (2012) MiR-106b impairs cholesterol efflux and increases A $\beta$  levels by repressing ABCA1 expression. *Exp Neurol* 235: 476–483.
- Kook, H., Lepore, J., Gitler, A., Lu, M., Wing-Man Yung, W., Mackay, J. *et al.* (2003) Cardiac hypertrophy and histone deacetylase-dependent transcriptional repression mediated by the atypical homeodomain protein Hop. *J Clin Invest* 112: 863–871.
- Laribi, S., Aouba, A., Nikolaou, M., Lassus, J., Cohen-Solal, A., Plaisance, P. *et al.* (2012) Trends in death attributed to heart failure over the past two decades in Europe. *Eur J Heart Fail* 14: 234–239.
- Latronico, M. and Condorelli, G. (2009) MicroRNAs and cardiac pathology. *Nat Rev Cardiol* 6: 419–429.
- Laukkanen, M., Mannermaa, S., Hiltunen, M., Aittomäki, S., Airenne, K., Jänne, J. *et al.* (1999) Local hypomethylation in atherosclerosis found in rabbit ec-sod gene. *Arterioscler Thromb Vasc Biol* 19: 2171–2178.
- Leeper, N., Raiesdana, A., Kojima, Y., Chun, H., Azuma, J., Maegdefessel, L. *et al.* (2011) MicroRNA-26a is a novel regulator of vascular smooth muscle cell function. *J Cell Physiol* 226: 1035–1043.
- Li, B., Carey, M. and Workman, J. (2007) The role of chromatin during transcription. *Cell* 128: 707–719.
- Li, Z., Hassan, M., Jafferji, M., Aqeilan, R., Garzon, R., Croce, C. *et al.* (2009) Biological functions of miR-29b contribute to positive regulation of osteoblast differentiation. *J Biol Chem* 284: 15676–15684.
- Ling, C., Del Guerra, S., Lupi, R., Rönn, T., Granhall, C., Luthman, H. *et al.* (2008) Epigenetic regulation of PPARGC1A in human type 2 diabetic islets and effect on insulin secretion. *Diabetologia* 51: 615–622.
- Lister, R., Pelizzola, M., Dowen, R., Hawkins, R., Hon, G., Tonti-Filippini, J. *et al.* (2009) Human DNA methylomes at base resolution show widespread epigenomic differences. *Nature* 462: 315–322.
- Liu, F., Levin, M., Petrenko, N., Lu, M., Wang, T., Yuan, L. *et al.* (2008) Histone-deacetylase inhibition reverses atrial arrhythmia inducibility and fibrosis in cardiac hypertrophy independent of angiotensin. *J Mol Cell Cardiol* 45: 715–723.
- Lund, G., Andersson, L., Lauria, M., Lindholm, M., Fraga, M., Villar-Garea, A. *et al.* (2004) DNA methylation polymorphisms precede any histological sign of atherosclerosis in mice lacking apolipoprotein E. *J Biol Chem* 279: 29147–29154.
- Majumdar, G., Johnson, I., Kale, S. and Raghov, R. (2008) Epigenetic regulation of cardiac muscle-specific genes in H9c2 cells by Interleukin-18 and histone deacetylase inhibitor m-carboxycinnamic acid bis-hydroxamide. *Mol Cell Biochem* 312: 47–60.
- Mao, Y., Mohan, R., Zhang, S. and Tang, X. (2013) MicroRNAs as pharmacological targets in diabetes. *Pharmacol Res* 75: 37–47.
- Marquart, T., Allen, R., Ory, D. and Baldán, A. (2010) miR-33 links SREBP-2 induction to repression of sterol transporters. *Proc Natl Acad Sci U S A* 107: 12228–12232.



- Miao, F., Gonzalo, I., Lanting, L. and Natarajan, R. (2004) *In vivo* chromatin remodeling events leading to inflammatory gene transcription under diabetic conditions. *J Biol Chem* 279: 18091–18097.
- Miao, F., Wu, X., Zhang, L., Riggs, A. and Natarajan, R. (2008) Histone methylation patterns are cell-type specific in human monocytes and lymphocytes and well maintained at core genes. *J Immunol* 180: 2264–2269.
- Montgomery, R., Davis, C., Potthoff, M., Haberland, M., Fielitz, J., Qi, X. *et al.* (2007) Histone deacetylases 1 and 2 redundantly regulate cardiac morphogenesis, growth, and contractility. *Genes Dev* 21: 1790–1802.
- Movassagh, M., Choy, M., Goddard, M., Bennett, M., Down, T. and Foo, R. (2010) Differential DNA methylation correlates with differential expression of angiogenic factors in human heart failure. *PLoS One* 5: e8564.
- Movassagh, M., Choy, M., Knowles, D., Cordeddu, L., Haider, S., Down, T. *et al.* (2011) Distinct epigenomic features in end-stage failing human hearts. *Circulation* 124: 2411–2422.
- Murray, C. and Lopez, A. (1997) Alternative projections of mortality and disability by cause 1990–2020: Global Burden of Disease Study. *Lancet* 349: 1498–1504.
- Mutskov, V., Raaka, B., Felsenfeld, G. and Gershengorn, M. (2007) The human insulin gene displays transcriptionally active epigenetic marks in islet-derived mesenchymal precursor cells in the absence of insulin expression. *Stem Cells* 25: 3223–3233.
- Nabel, E. and Braunwald, E. (2012) A tale of coronary artery disease and myocardial infarction. *N Engl J Med* 366: 54–63.
- Najafi-Shoushtari, S., Kristo, F., Li, Y., Shioda, T., Cohen, D., Gerszten, R. *et al.* (2010) MicroRNA-33 and the SREBP host genes cooperate to control cholesterol homeostasis. *Science* 328: 1566–1569.
- Natsume-Kitatani, Y., Shiga, M. and Mamitsuka, H. (2011) Genome-wide integration on transcription factors, histone acetylation and gene expression reveals genes co-regulated by histone modification patterns. *PLoS One* 6: e22281.
- Nazari-Jahantigh, M., Wei, Y., Noels, H., Akhtar, S., Zhou, Z., Koenen, R. *et al.* (2012) MicroRNA-155 promotes atherosclerosis by repressing Bcl6 in macrophages. *J Clin Invest* 122: 4190–4202.
- Ng, H., Zhang, Y., Hendrich, B., Johnson, C., Turner, B., Erdjument-Bromage, H. *et al.* (1999) MBD2 is a transcriptional repressor belonging to the MeCP1 histone deacetylase complex. *Nat Genet* 23: 58–61.
- Nguyen, A., Xiao, B., Nepl, R., Kallin, E., Li, J., Chen, T. *et al.* (2011) DOT1L regulates dystrophin expression and is critical for cardiac function. *Genes Dev* 25: 263–274.
- Nichols, M., Townsend, N., Scarborough, P. and Rayner, M. (2013) Cardiovascular disease in Europe: epidemiological update. *Eur Heart J* 34: 3028–3034.
- Nikitina, T., Shi, X., Ghosh, R., Horowitz-Scherer, R., Hansen, J. and Woodcock, C. (2007) Multiple modes of interaction between the methylated DNA binding protein MeCP2 and chromatin. *Mol Cell Biol* 27: 864–877.
- Oliveira-Carvalho, V., Carvalho, V. and Bocchi, E. (2013) The emerging role of miR-208a in the heart. *DNA Cell Biol* 32: 8–12.
- Ørom, U. and Shiekhattar, R. (2013) Long noncoding RNAs usher in a new era in the biology of enhancers. *Cell* 154: 1190–1193.
- Pirola, L., Balcerczyk, A., Tothill, R., Haviv, I., Kaspi, A., Lunke, S. *et al.* (2011) Genome-wide analysis distinguishes hyperglycemia regulated epigenetic signatures of primary vascular cells. *Genome Res* 21: 1601–1615.
- Polonsky, K. (2012) The past 200 years in diabetes. *N Engl J Med* 367: 1332–1340.
- Portela, A. and Esteller, M. (2010) Epigenetic modifications and human disease. *Nat Biotechnol* 28: 1057–1068.
- Post, W., Goldschmidt-Clermont, P., Wilhide, C., Heldman, A., Sussman, M., Ouyang, P. *et al.* (1999) Methylation of the estrogen receptor gene is associated with aging and atherosclerosis in the cardiovascular system. *Cardiovasc Res* 43: 985–991.
- Puigserver, P. and Spiegelman, B. (2003) Peroxisome proliferator-activated receptor-gamma coactivator 1 alpha (PGC-1 alpha): transcriptional coactivator and metabolic regulator. *Endocr Rev* 24: 78–90.
- Qin, B., Xiao, B., Liang, D., Xia, J., Li, Y. and Yang, H. (2011) MicroRNAs expression in ox-LDL treated HUVECs: MiR-365 modulates apoptosis and Bcl-2 expression. *Biochem Biophys Res Commun* 410: 127–133.
- Rakyan, V., Hildmann, T., Novik, K., Lewin, J., Tost, J., Cox, A. *et al.* (2004) DNA methylation profiling of the human major histocompatibility complex: a pilot study for the human epigenome project. *PLoS Biol* 2: e405.
- Ramirez, C., Dávalos, A., Goedeke, L., Salerno, A., Warriar, N., Cirera-Salinas, D. *et al.* (2011) MicroRNA-758 regulates cholesterol efflux through posttranscriptional repression of ATP-binding cassette transporter A1. *Arterioscler Thromb Vasc Biol* 31: 2707–2714.

- Ramsahoye, B., Biniszkiwicz, D., Lyko, F., Clark, V., Bird, A. and Jaenisch, R. (2000) Non-CpG methylation is prevalent in embryonic stem cells and may be mediated by DNA methyltransferase 3a. *Proc Natl Acad Sci U S A* 97: 5237–5242.
- Rayner, K., Suárez, Y., Dávalos, A., Parathath, S., Fitzgerald, M., Tamehiro, N. *et al.* (2010) MiR-33 contributes to the regulation of cholesterol homeostasis. *Science* 328: 1570–1573.
- Reddy, M., Villeneuve, L., Wang, M., Lanting, L. and Natarajan, R. (2008) Role of the lysine-specific demethylase 1 in the proinflammatory phenotype of vascular smooth muscle cells of diabetic mice. *Circ Res* 103: 615–623.
- Rivière, G., Lienhard, D., Andrieu, T., Vieau, D., Frey, B. and Frey, F. (2011) Epigenetic regulation of somatic angiotensin-converting enzyme by DNA methylation and histone acetylation. *Epigenetics* 6: 478–489.
- Rodenhiser, D. and Mann, M. (2006) Epigenetics and human disease: translating basic biology into clinical applications. *CMAJ* 174: 341–348.
- Shanmugam, N., Reddy, M., Guha, M. and Natarajan, R. (2003) High glucose-induced expression of proinflammatory cytokine and chemokine genes in monocytic cells. *Diabetes* 52: 1256–1264.
- Smolarek, I., Wyszko, E., Barciszewska, A., Nowak, S., Gawronska, I., Jablecka, A. *et al.* (2010) Global DNA methylation changes in blood of patients with essential hypertension. *Med Sci Monit* 16: CR149–155.
- Stein, A., Jones, T., Herron, T., Patel, S., Day, S., Noujaim, S. *et al.* (2011) Loss of H3K4 methylation destabilizes gene expression patterns and physiological functions in adult murine cardiomyocytes. *J Clin Invest* 121: 2641–2650.
- Stenvinkel, P., Karimi, M., Johansson, S., Axelsson, J., Suliman, M., Lindholm, B. *et al.* (2007) Impact of inflammation on epigenetic DNA methylation - a novel risk factor for cardiovascular disease? *J Intern Med* 261: 488–499.
- Sun, D., Zhang, J., Xie, J., Wei, W., Chen, M. and Zhao, X. (2012a) MiR-26 controls LXR-dependent cholesterol efflux by targeting ABCA1 and ARL7. *FEBS Lett* 586: 1472–1479.
- Sun, X., Icli, B., Wara, A., Belkin, N., He, S., Kobzik, L. *et al.* (2012b) MicroRNA-181b regulates NF- $\kappa$ B-mediated vascular inflammation. *J Clin Invest* 122: 1973–1990.
- Suzuki, M. and Bird, A. (2008) DNA methylation landscapes: provocative insights from epigenomics. *Nat Rev Genet* 9: 465–476.
- The Lancet (2013) Cardiovascular health for all. *Lancet* 382: 572.
- Villeneuve, L., Reddy, M., Lanting, L., Wang, M., Meng, L. and Natarajan, R. (2008) Epigenetic histone H3 lysine 9 methylation in metabolic memory and inflammatory phenotype of vascular smooth muscle cells in diabetes. *Proc Natl Acad Sci U S A* 105: 9047–9052.
- Wang, D., Xia, M., Yan, X., Li, D., Wang, L., Xu, Y. *et al.* (2012) Gut microbiota metabolism of anthocyanin promotes reverse cholesterol transport in mice *via* repressing miRNA-10b. *Circ Res* 111: 967–981.
- Wilson, A. (2008) Epigenetic regulation of gene expression in the inflammatory response and relevance to common diseases. *J Periodontol* 79: 1514–1519.
- Yeh, R., Sidney, S., Chandra, M., Sorel, M., Selby, J. and Go, A. (2010) Population trends in the incidence and outcomes of acute myocardial infarction. *N Engl J Med* 362: 2155–2165.
- Zernecke, A., Bidzhekov, K., Noels, H., Shagdarsuren, E., Gan, L., Denecke, B. *et al.* (2009) Delivery of microRNA-126 by apoptotic bodies induces CXCL12-dependent vascular protection. *Sci Signal* 2: ra81.
- Zhang, C. (2008) MicroRNomics: a newly emerging approach for disease biology. *Physiol Genomics* 33: 139–147.
- Zhang, Q., Chen, H., Wang, L., Liu, D., Hill, J. and Liu, Z. (2011) The histone trimethyllysine demethylase JMJD2A promotes cardiac hypertrophy in response to hypertrophic stimuli in mice. *J Clin Invest* 121: 2447–2456.
- Zhao, Y., Ransom, J., Li, A., Vedantham, V., von Drehle, M., Muth, A. *et al.* (2007) Dysregulation of cardiogenesis, cardiac conduction, and cell cycle in mice lacking miRNA-1–2. *Cell* 129: 303–317.
- Zhu, S., Goldschmidt-Clermont, P. and Dong, C. (2005) Inactivation of monocarboxylate transporter MCT3 by DNA methylation in atherosclerosis. *Circulation* 112: 1353–1361.