ELSEVIER

Spatio-temporal epigenetic gene regulation is essential for the proper development of the heart, and aberrant epigenetic mechanisms contribute to susceptibility to cardiovascular disease.

Epigenetic mechanisms in heart development and disease

Q2 **Shannalee R. Martinez, Maresha S. Gay and Lubo Zhang**

Center for Perinatal Biology, Division of Pharmacology, Department of Basic Sciences, Loma Linda University School of Medicine, Loma Linda, CA 92350, USA

Shannalee Martinez received her BS degree from Southern Adventist University in 2009 and is currently pursuing her MD/PhD at Loma Linda University. Her graduate studies have been facilitated by the Loma Linda University Initiative for Maximizing Student Development Program through the National Institutes of Health. Her current research is supported by the NIH National Heart Lung and Blood Institute Diversity Supplement Program. Her research focuses on epigenetic mechanisms in heart development and developmental origins of health and disease.

Suboptimal intrauterine development has been linked to predisposition to cardiovascular disease in adulthood, a concept termed 'developmental origins of health and disease'. Although the exact mechanisms underlying this developmental programming are unknown, a growing body of evidence supports the involvement of epigenetic regulation. Epigenetic mechanisms such as DNA methylation, histone modifications and micro-RNA confer added levels of gene regulation without altering DNA sequences. These modifications are relatively stable signals, offering possible insight into the mechanisms underlying developmental origins of health and disease. This review will discuss the role of epigenetic mechanisms in heart development as well as aberrant epigenetic regulation contributing to cardiovascular disease. Additionally, we will address recent advances targeting epigenetic mechanisms as potential therapeutic approaches to cardiovascular disease.

Developmental origins of health and disease

Q3 Since the early studies in the 1980s, factors contributing to an adverse intrauterine environment have been linked to increased risk of disease in adulthood. Initial studies on the developmental origins of adult disease, performed by Barker and Osmond, revealed a possible link between intrauterine adversity and an increased risk of cardiovascular disease in adulthood [1]. Subsequent studies confirmed that intrauterine insults such as undernutrition, hypoxia, microbial toxins and chemical agents increased the risk of cardiovascular and metabolic disorders later in life, including metabolic syndrome, type 2 diabetes mellitus, hypertension and ischemic heart disease [2]. As a result of these studies, Hales and Barker developed the 'thrifty phenotype hypothesis' to explain this phenomenon [3]. This hypothesis proposes that, when confronted with a suboptimal intrauterine environment, the developing fetus makes irreversible adaptation to ensure survival in such an environment. Following birth, however, these changes can prove to be maladaptive, rendering the neonate and growing individual susceptible to disease development. Although the exact mechanisms translating unfavorable intrauterine conditions into susceptibility to cardiovascular and metabolic diseases are not yet known, recent studies on the role of epigenetic

Corresponding author: Zhang, L. (lzhang@llu.edu)

1359-6446/© 2015 Published by Elsevier Ltd.
http://dx.doi.org/10.1016/j.drudis.2014.12.018

Please cite this article in press as: Martinez, S.R. et al. Epigenetic mechanisms in heart development and

modifications have provided valuable insights into the developmental origins of health and disease. In this review, we summarize recent progress made in our understanding of major epigenetic mechanisms and their roles in regulating heart development and disease.

Epigenetic mechanisms

Epigenetic regulatory mechanisms are, by definition, capable of modulating gene expression without altering DNA sequences. These mechanisms can act at genomic (DNA methylation) or nucleosomal and chromatin (histone modifications, chromatin remodeling complexes) levels. In addition, a post-transcriptional level of regulation is conferred by small, noncoding RNAs termed micro-RNAs (miRs). Although these regulatory systems have distinct mechanisms, there is considerable functional overlap and crosstalk among them. This review will address the roles of DNA methylation, histone modifications and miRs in heart development and disease. For in-depth reviews of the roles of chromatin remodeling complexes in heart development and disease see [4,5].

DNA methylation

DNA methylation: location and function

DNA methylation is the only known epigenetic regulatory mechanism that affects the DNA molecule directly. It has been implicated in gene imprinting, X-chromosome inactivation, the regulation of tissue-specific gene expression, cell development, genomic stability and modulation of splicing events [6,7]. DNA methylation most often occurs in mammals at the fifth carbon of cytosine nucleotides located within CG dinucleotides, termed CpG sites. These CpG sites are distributed throughout the genome in genic and intergenic regions. Genic regions include the promoter and gene body, whereas intergenic regions include distal regulatory elements as well as repetitive elements [8,9]. Promoter methylation is generally repressive and the extent of promoter methylation is inversely related to the CpG density of the promoter. This observation prompted the classification of promoters into two groups based on CpG density: high CpG density (HCG) and low CpG density (LCG) promoters [10]. HCG promoters make up 72% of promoters in the human genome. These promoters contain clusters of CpG sites termed CpG islands, which are largely devoid of methylation, resulting in overall hypomethylation of HCG promoters. These promoters are associated with genes expressed by cells from all or most tissues (housekeeping genes) [10]. By contrast, LCG promoters do not contain CpG islands and are associated with lineage or tissue-specific gene regulation [10–12]. Furthermore, there is evidence to suggest that methylation of gene bodies could provide fine-tuning of promoter methylation patterns, and the combination of promoter hypomethylation and gene body methylation is positively correlated to gene expression [13]. Methylation of gene bodies, defined as the region of the gene beyond the first exon [14], is associated with a higher level of gene expression in dividing cells [8]. Furthermore, CpG sites are more common in exons than introns [10], and recent studies suggest that gene body methylation might play a part in pre-mRNA splicing [15,16]. CpG methylation in intergenic regions affects distal regulatory elements, such as enhancers [9] and repetitive elements.

Although the exact mechanisms translating the methylation code into gene repression or expression are unknown, two mechanisms have been proposed and well-studied: (i) promoter cytosine methylation prohibits the binding of transcription factors, thus inhibiting transcription of the downstream gene; (ii) cytosine methylation is recognized by DNA methyl-binding proteins (MBPs) that orchestrate chromatin remodeling necessary for gene activation or inhibition. The latter mechanism depends upon the detection of 5-methylcytosine (5mC) by DNA MBPs, of which there are three known families, reviewed in [7]. The methyl-binding domain (MBD) family of MBPs comprises MeCP2 and MBD1–6. Only MBDs1, 2 and 4 have been shown to bind methylated DNA. The set and ring finger associated (SRA) domain family includes ubiquitin PHD ring finger (UHRF)1 and 2. Of these, UHRF1 is notable for its essential role in DNA methyltransferase (DNMT)1-mediated maintenance of DNA methylation. Finally, zinc finger family proteins Kaiso and zinc finger protein (ZFP)57 are MBPs responsible for maintenance of methylation of imprinted genes [7].

DNA methylation: reprogramming phases

In mammalian development, there are two phases of DNA methylation erasure and reestablishment or reprogramming. The first episode of DNA methylation erasure occurs in the blastocyst following fertilization [17]. The second episode of DNA methylation occurs in primordial germ cells (PGCs) traveling to the gonadal ridge for development into gametocytes [18]. These DNA methylation reprogramming phases are thought to prime cells for lineage specification and differentiation processes, as well as prohibit the transgenerational propagation of disadvantageous epimutations [19]. Certain regions of the genome retain their methylation patterns and escape demethylation during these phases. Imprinting control regions (ICRs) escape DNA methylation erasure during the blastocyst stage, but they are fully reprogrammed during the PGC reprogramming episode. By contrast, transposable elements including intracisternal A-particles (IAPs) escape methylation erasure during both reprogramming phases, highlighting the importance of repression of these elements [17,19]. These crucial phases of DNA methylation reprogramming are dependent on cellular machinery responsible for deletion, reintroduction and maintenance of these methylation marks.

DNA methylation: enzymatic regulation

DNMTs are a family of enzymes responsible for maintaining and/or introducing DNA methylation marks. The DNMT family comprises three main members: DNMT1, DNMT3A and DNMT3B. A fourth member, DNMT3L, lacks catalytic activity and is thought to regulate the activities of DNMT3A and DNMT3B. DNMT1, together with the DNA MBP UHRF1, maintains DNA methylation during replication. DNMT3A and DNMT3B are responsible for *de novo* DNA methylation during gamete development and during blastocyst implantation, respectively. In addition, these enzymes have been implicated in maintenance of DNA methylation [19,20].

DNA demethylation can occur by passive or active means. Passive DNA demethylation occurs via exclusion of DNMT1 and UHRF1 from the nucleus, resulting in replication-dependent loss of methylation marks. Although no enzyme is yet known that directly converts 5mC to cytosine via active DNA demethylation, indirect demethylation mechanisms have been described, reviewed in [19]. The ten-eleven-translocation (TET) family

Please cite this article in press as: Martinez, S.R. et al. Epigenetic mechanisms in heart development and