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Epigenetic mechanisms underlying cardiac degeneration and regeneration*

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Abstract

Epigenetic modifications which are defined by DNA methylation, histone modifications and microRNA mediated gene regulation, have been found to be associated with cardiac dysfunction and cardiac regeneration but the mechanisms are unclear. MicroRNA therapies have been proposed for cardiac regeneration and proliferation of stem cells into cardiomyocytes. Cardiovascular disorders are represented by abnormal methylation of CpG islands and drugs that inhibit DNA methyl transferases such as 5-methyl Aza cytidine are under trials. Histone modifications which include acetylation, methylation, phosphorylation, ADP ribosylation, sumoylation and biotinylation are represented within abnormal phenotypes of cardiac hypertrophy, cardiac development and contractility. MicroRNAs have been used efficiently to epigenetically reprogram fibroblasts into cardiomyocytes. MicroRNAs represent themselves as potential biomarkers for early detection of cardiac disorders which are difficult to diagnose and are captured at later stages. Because microRNAs regulate circadian genes, for example a nocturnin gene of circadian clockwork is regulated by mir122, they have profound role in regulating biological clock and this may explain the high cardiovascular risk during the morning time. This review highlights the role of epigenetics which can be helpful in disease management strategies.

Keywords

Epigenetics; stem cells; cardiomyocytes; microRNA; cardiac disorders; biomarkers

Introduction

Epigenetics has emerged as one of the important phenomena behind cardiac disorders and includes small non coding RNAs like microRNAs, DNA methylation and histone modifications (Fig 1). Epigenetics refers to changes in gene expression which are not due to change in the DNA sequence but attributed to chromatin alteration or packaging which changes the accessibility of DNA [1]. Epigenetic changes often are a result of gene environment interactions or surrounding conditions [2] leading to enhanced/decreased expression or silencing of genes e.g. in the case of diabetes, hypertension and obesity. DNA methylation are the most studied epigenetic modifications and mainly involve methylation

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of CpG islands in the promoter of genes. Diseased states such as cardiovascular disorders are represented by abnormal methylation of the CpG islands consequently leading to modifications in gene expression [3]. Movassagh et al [4] have identified different patterns of DNA methylation in heart failure patients. Recently, homocysteine which is a marker for cardiovascular disease has been shown to play crucial role in epigenetics [5, 6]. Hyoperhomocysteinemia has been reported to be associated with alteration in the DNA mthyltransferase activities involving methionine, folic acid and cystathione B synthase. DNA methylations can be transferred mitoticaly to next generations through cell divisions as they are quite stable. Histone modifications are another mechanisms to altered gene expression via chromatin remodeling by histone acetyltransferases or deacetylases which change the DNA accessibility. The role of histone regulatory proteins in heart disease have been demonstrated by Backs et al [7]. Furthermore, RNA based mechanisms like microRNAs and non-coding RNAs are also involved in altering gene expression of target genes and are studied extensively in cardiac disorders [8–11]. MicroRNAs have also been implicated in stem cell therapies e.g. miR133a has been reported to be involved in differentiation of cardiogenic Mesenchymal stem cells by targeting epidermal growth factors [12]. This review aims to elucidate epigenetic mechanisms underlying cardiac disorders and how these epigenetic mechanisms can be beneficially introduced for cardiac therapies.

Role of epigenetics in Cardiac degeneration

Role of DNA methylation

The pathogenesis of cardiovascular disease is well studied but the role of epigenetics still needs to be explored. The epigenetic modifications in cardimyopathy have been summarized in Table 1. Many studies have demonstrated with help of animal models that DNA methylation plays an important role in atherosclerosis and cardiovascular disease [13]. DNMTs (DNA methyltransferases) and MTHFR (Methylene tetrahydrofolate reductase) represent two important genes in DNA methylation and mice deficient in these genes show hypomethylation of their DNA [13, 14]. Chen et al have shown the formation of aortic fatty streaks in MTHFR deficient mice. In leucocytes of DNMT deficient mice, there is increase in the expression of inflammatory mediators which represent hypomethylation [15]. In peripheral blood leukocytes, there are changes in DNA methylation in ApoE−/− mice, which lead to atherosclerosis promotion and dysregulation of inflammation [14].

The DNA methylation status is also affected by dietary folate and vitamin levels and providing these dietary supplements to female mice before conception causes higher methylation of CpG islands in the offspring [13]. Due to this the offspring has a characterstic phenotype of brown coat color, insulin resistance, cancers, lengthened life span and reduced susceptibility to obesity. The aortas of ApoE knockout mice represent a decrease in DNA methylation which can be detected at 4 weeks and any histological changes associated with atherosclerosis can be determined [16]. The estrogen receptors α and β show increased methylation at the promoter region in atherosclerotic tissues and the methylation of estrogen receptor increases with age. There is hypermethylation of the HSD11B2 gene, and loss of global methylation of genomic content in blood leukocytes of hypertensive patients [17]. In another report it was shown that in patients with atherosclerotic cardiovascular disease, there is lower DNA methylation in blood leukocytes [18]. Baccarelli et al [19] have shown that incidence and mortality from ischemic heart disease and stroke can be predicted by lower LINE-1 (Long Interspersed Nucleotide Elements) methylation in peripheral blood leukocytes. LINE-1 have been evaluated as surrogate markers for global methylation status [20].

Hypertension which is associated with cardiac degeneration is influenced by global genomic methylation content in the peripheral blood leukocytes [17] of hypertensive patients. In

Chinese individuals, elevated *Alu* methylation status in peripheral blood leucocytes have been related to the prevalence of cardiovascular disease and obesity [21]. Friso et al [22] have linked hypermethylation of the HSD11B2 gene with blood pressure control. Impaired lipid and glucose metabolism which leads to increased cardiovascular risk and diabetes, are represented by hypermethylation of MEG3, IL-10, GNASAS, ABCA1 and hypomethylation of IGF2 and INSIGF genes [23].

Role of microRNAs

MicroRNAs have emerged recently as one of the epigenetic mechanisms underlying cardiovascular diseases (Fig 2, Table 2). In patients with atherosclerotic plaque, the elevated levels of mir127 leads to disruption of endothelium and subsequently, vascular senescence via inhibiting SIRT1 [24]. In animal models and patients with myocardial infarction, mir133b and miR499 have been shown to be upregulated and are potential candidates for biomarkers [8, 25]. Additionally, in patients with coronary artery disease, the level of mir126 and mir145 is decreased profoundly [26]. Downregulation of mir126 indicates inflammation of vessel walls during the development of atherosclerosis by promoting the expression of VCAM-1 [27, 28]. In unstable angina patients, the levels of mir134, mir370 and mir198, were found to be significantly increased which showed increased risk of cardiovascular disease [29]. Sondermeijer et al [30] reported that mir340 and mir624 were significantly increased in patients with cardiovascular diseases.

The role of miR21 in early phase MI is evident from an animal study, where acute MI created by left vertricular coronary artery ligation leads to decrease in expression of mir21 in the infracted area as compared to the surrounding area [31]. Wang et al [8] found elevated levels of miR208a in CAD patients as compared to healthy subjects, where this microRNA was undetectable. In another study by Corsten et al [32], the levels of miR208a were increased to 90–100 fold. In CAD, miR1 is the most widely studied and plasma miR1 levels are significantly increased in MI patients as compared to healthy controls. This increase was independent of clinical parameters such as age and sex [33, 34]. After the MI event, in a cohort of patients, miR1, miR21, miR133a and miR208 were found to be significantly elevated [35]. Similarly, D'Allessandra et al [25] also showed increase in miR1, miR133a/b, miR499-5p by upto 140 fold in MI patients as a consequence of cardiac injury. They also confirmed these findings in mouse models of coronary artery ligation. In an attempt to determine diagnostic potential of microRNA, Olivieri et al [10] have found increased levels of miR423-5p in congestive heart failure than in MI, while miR499-5p is upregulated in both MI and HF.

MicroRNAs extracted from whole blood have been assessed for diagnostic and prognostic properties, so that even when troponin T and ischemic heart disease biomarkers are negative, the microRNAs can predict MI [11]. miR30c and miR145 showed good correlation with troponin T levels and their expression increased in MI patients. Additionally, mir1291 and mir663b were able to distinguish case-control patients and were highly sensitive. The correlation between indicators of cardiac necrosis (Troponin and cardiac kinase) and circulating microRNA levels have been investigated to find out early biomarkers e.g. mir1 and mir133a/b were detected before troponin 1 increase in patients affected by ST elebvation MI [25]. Similarly, creatine kinase and troponin T levels were significantly correlated with circulating levels of mir208b and mir499 [9, 32, 36]. In elderly patients, mir499-5p is more sensitive marker than troponin T and electrocardiogram and is able to differentiate MI from acute congestive heart failure [10].

In mouse models of cardiac hypertrophy and congestive heart failure, microRNA profiling with microarray revealed five microRNAs (mir24, mir125b, mir195, mir199a, mir214) to be upregulated (Table 2). These findings were also confirmed in patients with heart failure [37].

The authors also showed that over-expression of mir195, correlated with cardiomyocyte disorganization in transgenic mice model, which leads to heart failure. In HF patients with different aetiologies (ischemic, aortic stenosis and dialated cardiomyopathy), the microRNA expression profiling was performed in left ventricular tissue by Ikeda et al [38] and 43 microRNAs were found to be differentially expressed in cardiomyopathy while thirteen microRNAs were specific to aortic stenosis [38]. In two groups of patients affected by idiopathic dialated cardiomyopathy and ischemic cardiomyopathy, the microRNA expression profile specific to each aetiology was determined by Sucharov et al [39]. Several microRNAs common to all failing hearts were also found e.g. mir92 and mir133b were downregulated, while mir195 and mir100 were upregulated [39], which are speculated to prevent changes in β-adrenergic mediated gene expression- an important pathway in heart failure. Matkovich et al [40] performed microarrays and mRNA profiling of myocardial samples and found 28 microRNAs to be upregulated in heart failure. The study suggested that in myocardial response to stress, microRNA can serve as more specific biomarkers [40].

Role of Histone modifications

Histone modifications together with DNA methylation and microRNAs are dynamic processes that lead to chromatin remodeling and modulate gene expression. Histones (H_2A, H_2B, H_3 and H_4) which form nucleosome- a basic unit of chromatin, are modified by post translational modifications such as acetylation, methylation, phosphorylation, ADP ribosylation, sumoylation and biotinylation [41, 42] (Fig 1, Table 1). Histone acetylation/ deacetylation represents central mechanisms that alter chromatin structure to control gene expression [43–45]. Histone acetylation by histone acetyltransferases (HAT) loosens the histone-DNA interactions, ‘relaxes’ chromatin structure and activates transcription, while deacetylation by histone deacetylases (HDAC) increases histone-DNA interactions, results in chromatin condensation and leads to shut down of transcription machinery. Acetylation of histones by acetyltransferases- p300 or CREBP (cAMP responsive element binding protein), are important in heart development and deletion of either of genes is lethal in the embryo [46]. Ablation of the histone acetyltransferase (HAT) domains in both p300 and CREBP leads to abnormality in the cardiovascular system [47]. P300 acetylates transcription factors such as GATA4 and overexpression of p300 in mouse heart results in depressed cardiac dialation [43, 48, 49].

The expression of eNOS (Nitric Oxide Synthase) in the endothelial cells is important in vascular functioning and is controlled by cell-specific histone modifications [50]. The authors demonstrate that the core promoter of eNOS gene is rich in acetylated H_3K9 and H_4K12 in the endothelial cells and these histone modifications are functionally relevant for the expression of the eNOS gene. Also the expression of the eNOS gene is remarkably decreased in cardiovascular disease [50]. In a study by Hang et al [51] it was demonstrated that cardiac hyopertrophy and failure is represented by chromatin remodeling and transcriptional reprogramming following myocardial stress. In mouse model, the expression of *Brg* 1 (Brahma related gene) is increased in hypertrophic cardiomyopathy and repression of this gene decreases hypertrophy and reverses myosin isoform shifts [51].

Histone methylation takes place at arginine or lysine residues at H_3K4, H_3K9, H_3K27, H_3K36 and H_4K20, with mono-, di- or tri- methylated (H_3Kme3) histones [52]. As compared to acetylation, which leads only to active state of chromatin, methylation can lead to active, repressed or ‘poised’ states of chromatin. Many studies have explored the role of histone methylation in cardiac development as well as heart failure and cardiac hyopertrophy [53–56] (Table 1). Stein et al [57], have shown that H_3K4 methylation levels are critical for physiological functions in adult murine cardiomyocytes and loss of H_3K4 methylation, increases intracellular calcium resulting in increased contractility [57]. The

promoter regions of β-MHC (Myosin Heavy Chain) is hypermethylated at H_3K4me3 in cardiac hypertrophy, while for α-MHC, the H_3K4me3 methylation level is decreased showing both activation and inactivation of genes under pathological stress [57]. Similarly, in heart failure rodents and patients with end stage heart failure, the H_3K4me3 and H_3K9me3 levels are disturbed [58, 59].

The eNOS (Nitric Oxide Synthase) gene which is important in endothelial function and angiogenesis, is controlled by promoter methylation at $H_3K27me3$ and H_3K4me3. To suppress angiogenesis triggered by hypoxia, the ratio of active H_3K4me3 to $H_3K27me3$ increases, which leads to increased expression of histone demethylase JMJD3, which suppresses angiogenesis [60]. UTX gene which is a H_3K27 demethylase, contains Jumonji C domain and interacts with cardiac specific transcription factors such as GATA4, NKX2.5, TBX5 and SRF [61]. The removal of $H_3K29me3$ is important in heart development and mice deficient in UTX gene suffer severe heart malformation [62]. JMJD2A is another demethylase which belongs to Jumonji C domain- containing family and catalyzes demethylation of H_3K9me3 and $H_3K36me3$. The level of methylation of H_3K9 and H_3K36 is increased in JMD2A deficient mice, however they are resistant to cardiac stress, though they represent normal phenotype under normal conditions [63]. On the other hand overexpression of JMD2 in transgenic mice increases cardiac hypertrophy after pressure overload [63]. JMD2 enhances cardiac hypertrophy by binding of myocardin and SRF transcription factors to FHL1 promoter (Full-and-a-half LIM domains) which leads to reduced H_3K9 methylation and thus, proper cardiac functioning after pathological insult [63].

Majority of methylation takes place at the histone tail i.e. H_3K9 position, though additional residues such as H_3K79, which are located in the globular domain are also subject to methylation. The DOTIL (Disruption of Telomeric silencing protein) catalyzes the methylation of H_3K79 and deletion of DOTIL in mice causes dialation of cardiac chambers, reduced contractility and increased lethality [64]. Deletion of DOTIL gene also reduces H_3K79 methylation at the dystrophin promoter and decreases the transcription of dystrophin gene which is important for cardiac muscle functioning [59]. Additionally, in patients with idiopathic dialated cardiomyopathy, DOTIL gene is downregulated and H_3K9 methylation is impaired which leads to reduced contractility [64].

The importance of histone modifications, have triggered exploration of whole genome studies which includes co-immunioprecipitaiton (ChIP) and ChIP with microarray analysis (ChIP-ChIP). The method of choice is ChIP-followed by massive sequencing which gives information about global histone modifications and gene expression on large scale. Hence studies have revealed that different regions in the gene are associated with different histone modification patterns and different load of gene activity. The active genes clearly exhibit histone acetylation, in the promoter regions while methylation can be detected in either active or repressed genes, hence active and repressive states can co-exist within the same inactive promoters [64–67]. Till date, there are two genome wide studies for hostone modifications in failing heart [68, 69]. One study states that during development of heart failure the genes of calcium signaling and cardiac contractility exhibit differential methylation patterns at H_3K4me3 and H_3K9me3. The other study, demonstrates that $H_3K36me3$ is enriched in activity transcribed regions of the genome in patients with end stage heart failure.

Apart from the role of epigenetics in cardiovascular diseases such as hypertension, acute MI and congestive heart failure, the relation between these heart diseases and myocardial degeneration remain elusive. Cardiac degeneration is underlined by cell death which is primarily caused by apoptosis, necrosis or autophagic mechanisms. In models of MI, cell

death takes place within the area of ischemia over the first 6–24 hrs [70]. In the ischemic zone, cardiomyocytes die by both apoptosis and necrosis. Studies have also shown autophagy associated cell death in myocardial infarction [71–74]. Although in failing hearts, the cell death is defined by apoptosis, necrosis and autophagy, the percentage of these three mechanisms in contributing to cell death varies and depends upon the pathology of the disease [75–77]. The knowledge of cell death and its regulation may be helpful in manipulating death pathways to therapeutic advantages.

Role of epigenetics in cardiac regeneration

Role of microRNAs

Heart development during embryonic stages involves increase in the number of cardiomyocytes for heart enlargement, however, after a short time of birth, cardiomyocytes stop proliferating [78–80]. In adults, cardiac proliferation occurs mainly through hypertrophic enlargement of cardiomyocytes, though the proliferation capacity of cardiomyocytes to proliferate is limited [81, 82] (Fig 2). Hence, the ability of heart to regenerate and repair itself after injury such as myocardial infarction or heart failure is very limited [83–87]. Mollova et al [88] have reported that cardiomyocyte proliferation occurs at the rate of 900 million per year upto one year after birth and it decreases to 70 million per year by the age of 20 [88]. Cardiomyocytes have been associated with altered expression of microRNAs and microRNAs involved in cardiomyocyte proliferation have also been identified [89] (Fig 2, Table 3). Eualalilo et al screened a library of 875 microRNA mimics (http://mirbase.org) and identified 204 microRNAs that increased proliferation in neonatal rat cardiomyocytes and 40 microRNAs that proliferated mouse cardiomyocytes (Table 3). The authors found two microRNAs mir199a-3p and mir590-3p that most efficiently promote cardiomyocyte proliferation in both mouse and rat. Porello et al [90] showed that the regenerative capacity of neonatal heart is more than the adult in mammals and the regenerative potential of neonatal mouse heart after resection of left ventricle is lost after first postnatal week. There is upregulation in the expression of mir15 gene family members with the loss of regeneration potential within first postnatal week.

Senyo et al [91] have shown that mammalian heart regenerates by the division of pre-existing cardiomyocytes after myocardial injury. These findings are in agreement with the studies in zebra fish where cardiac regeneration has been shown to occur through pre-existing cardiomyocytes [92, 93]. Jayawardena et al [94] have shown that fibroblasts can be directly converted into cardiomyocyte lineage by using specific microRNAs (mir1, 133a, 208 and 499). The converted cardiomyocytes possess spontaneous Ca^{2+} oscillations, mechanical contractions and proteins specific for cardiomyocytes. Several studies have shown that the proliferation and differentiation of cardiomyocytes from human embryonic stem cells [95] or from human progenitor cardiomyocytes [96] is regulated by microRNAs (mir1, 133a and 499). Additionally, mir17-92 cluster has been identified as an important regulator of cardiomyocyte proliferation by deletion/overexpression of this cluster in adult cardiomyocytes [97]. The authors suggest that mir17-92 cluster can become a therapeutic target for heart regeneration and cardiac repair. The delivery of microRNAs specific to target cells is important in modulating cardiomyocyte proliferation as there may be off target inhibition or activation of microRNAs in non-cardiomyocytes. New techniques are required to deliver microRNAs to specific targets and to address potential safety concerns.

Role of stem cell epigenetics

Stem cells have been used efficiently in cardiac regeneration for the past few years; however, the clinical trials with stem cell therapy have not been far successful and have been modest or short lived. The limitations that need to be addressed in order to improve

efficiency of stem cell therapy are 1) stem cell homing and survival; 2) tumorogenicity of pleuripotent stem cells; 3) stem cell should be derived from appropriate cell source and; 4) differentiation of stem cells towards cardiomyocyte lineage. Some of these problems have been addressed by the use of microRNAs to modify stem cells and proliferate them into cardiomyocytes [12]. The cardiomyocytes derived from embryonic stem cells (ECS) have been found to have elevated expression of mir1, -208, -133 and -499 during differentiation [95, 96, 98]. Micro RNA-1 overexpression induces cardiac differentiation in mouse and human embryonic stem cells while mir133 opposes cardiomyocyte differentiation and represses cardiac gene markers in ESC [96, 98]. Inhibition of mir499 blocks differentiation of cardiomyocytes in vitro [95, 96, 99] and overexpression of mir499 in ECS and cardiac progenitor cells induces cardiac gene markers. Mir499 has been proposed to act through the repression of SOX6 transcription factor [96].

When embryonic stem cells expressing mir1 were transplanted to mouse heart with infarction, it protected from ischemic injury in the infarction zone [100, 101]. Similarly, in c-kit$^+$ cardiac progenitor cells, overexpression of mir499, enhances cardiomyocyte differentiation and restores myocardial zone after injecting it into rat heart with myocardial infarction [102]. These studies demonstrate that stem cells can be differentiated effectively into cardiomyocytes by using microRNAs in vivo and in vitro to improve stem cell therapies. Apart from the exogenous transplantation of modulated stem cells, microRNAs have been efficiently used to induce the endogenous cardiac progenitor stem cells. For example, mir17 has been overexpressed in the adult c-kit$^+$ progenitor cells in vivo and resulted in the proliferation of cardiac progenitor cells [103]. Alternative to stem cell approach, researchers have also used fibroblast reprogramming into cardiomyocytes (Fig 2), which has an advantage that after myocardial infarction, the human heart provides rich endogenous supply of fibroblasts. Leda et al [104] have demonstrated that by using the transcription factors i.e. GATA4, MEF2c and TBX5, mouse fibroblasts can be programmed into cardiomyocytes in vitro. The use of fibroblasts and their programming into cardiomyocytes in the infracted heart have been demonstrated efficiently by Quian et al and Song et al [105, 106]. Apart from this, viral vectors such as lentivirus have also been used to carry microRNAs to the infacrted heart and the conversion of fibroblasts into cardiomyocytes in situ [94].

Mesenchymal stem cells (MSC) derived from bone marrow have been used for transplantation into infracted myocardium and resulted in enhanced myocardial regeneration, reduced infarct size and improved cardiac function [107–111]. In patients with acute MI and chronic ischemia, clinical studies using MSC have documented safety and feasibility [112–116]. MSC mediate myocardial tissue repair by two processes: 1) transdifferentiation into cardiomyocytes and endothelial cells and; 2) release of cardioprotective factors which are biologically active, proangiogenic and termed as paracrine factors [117–122]. The heart regeneration capacity of MSC can be enhanced by modulating the expression of certain genes and microRNA. Yu et al [123] demonstrated that cardiomyocyte protection is enhanced by the expression of GATA-4 gene in MSC, which leads to improved left ventricular function and smaller infarct size. The MSC transfected with GATA-4 released more growth factors and increased mir221 expression, which is delivered to adjacent cells through microvesicles and helps in cardioprotection. In another study by Cai et al [124] it was demonstrated that mir124 regulated cardiomyocyte differentiation of bone marrow derived MSC by targeting STAT3 mRNA. Similarly, mir16 has been reported to enhance the differentiation of bone marrow derived mesenchymal stem cells into cardiac phenotype [125]. Zhang et al [126] have reported the cardiac differentiation of Bone marrow derived mesenchymal stem cells (BMSC) in rat by overexpressing mir499 which increased the expression of cardiac specific genes such as NKx2.5, GATA-4, MEF2C and cTnl, hence activating the wnt/β-catenin signaling pathway.

Wen et al [127] have summarized the role of microRNAs in regulating BMSC for post-MI cardiac repair.

The regeneration potential of BMSC decreases with increasing age due to changes in cellular functions. Ayala-Lugo et al [128] demonstrated that the availability and functionality of bone marrow stem cells depends upon age, by using experimental model of acute and chronic myocardial infarction. The study explained that the age and the time of treatment post-MI, act synergistically to determine the efficacy of bone marrow stem cell treatment of the infracted heart. In another study by Yu et al [129], the regeneration potential of BMSC isolated from different age groups of rhesus monkeys (<5; 8–10; >12yrs) was tested and it was found that BMSC showed significant changes in the microRNA expressions and heat shock proteins, but non-significant changes in histone modifications. Although aging is associated with poor response of BMSC, the angiogenesis in ischemic tissues can be improved by exercise [130, 131]. Cheng et al [132] have shown that in mice of advanced age exercise training can activate ischemia induced neovascularization by reactivating Akt-dependent hypoxia induced factor (HIF)-1α. In an attempt to explain, why vascular regeneration declines with aging, Di Q et al [133] used deferoxamine which is a stabilizer of HIF-1α and demonstrated that age related decline is due to impairment of cross activation between β_3 and VEGFR-2 in EPCs which is partially associated with decreased HIF-1α stability.

We have shown that ablation of MMP9 leads to the profound conversion of cardiac stem cells into cardiomyocytes [134, 135]. On the basis of our observation and available literature we postulate that microRNAs can lead to the conversion of stem cells to cardiomyocytes through regulating the expression of MMP and DNA methyltransferases (Fig 3). The upregulation of MMP9 gene expression leads to the stiffness of extracellular matrix and consequently fibrosis and affect the differentiation of stem cells into cardiomyocytes.

Epigenetic biomarkers

Micro RNAs have been proposed as biomarkers for the diagnosis of various cardiac diseases, as there is an altered expression of genes and micro RNAs in diseased states and they not only reside in the cells but are abundant in circulating fluids. Disease specific signatures of microRNAs have been identified for coronary artery disease (CAD)[29], heart failure [136] and atherosclerosis [137, 138]. microRNAs can be detected by using conventional techniques such as Nothern Blotting, qRTPCR and advanced techniques such as deep sequencing and genome wide microRNA profiling techniques [139, 140]. Chen et al [141] have shown that circulating microRNAs are quite stable at low and high pH, freeze thaw cycles and boiling which makes them suitable candidates as biomarkers [141]. The stability of microRNAs has been attributed to 1) the formation of protein-microRNA complexes [142, 143]; 2) binding with high density lipoproteins (HDL) and; 3) encapsulation in microvesicles [144, 145] and exosomes [146–148]

Mir423-5p has been shown to be a promising potential candidate in blood samples and cardiomyocytes of patients with heart failure [136] along with mir145, -155, -92a, -17 and -126, in CAD patients [26]. Kuwabara et al [149] have showed that in patients with cardiovascular disease, there is an increased level of mir1 and mir133a in the serum [149]. The altered expression of microRNAs is due to 1) changes in the microRNA production 2) post-transcriptional processing and 3) release of microRNAs from cells. To potentiate the use of microRNA as biomarkers, 20 clinical studies have been performed for circulating microRNAs for MI [36, 96, 150]. The studies show that mir1, -133a/b, -208, -499 [151–153] can be used as potential candidates for biomarker studies in myocardial damage. For vascular wall damage, mir126 [154] mir92a [155] and mir145 [156] have been found to be

suitable biomarkers. Mir146, mir155 and mir223 have been found to be associated with leukocytes and platelets and can also be monitored for early stress and myocardial damage [29, 36, 157]. Although microRNAs appear to be very promising as biomarkers still their clinical applications have been delayed due to 1) their laborious isolation and detection procedures as compared to other biomarkers and 2) the time of detection i.e. the time for which they appear in the circulation. However, the combined selection of traditional detection kits and circulating mi-RNAs together, can be used for increasing the specificity and sensitivity of the cardiac diagnostic procedures.

Effect of environment on epigenetics of cardiac disorders

Heart function is affected by environmental factors such as life style, dietary habits, exercise, smoking, alcohol intake and depression. The adverse phenotype faced in later stages of life are due to epigenetic changes, induced by the exposure to harsh conditions in early stages of life [158, 159]. The maternal diet during pregnancy, can dramatically affect the DNA methylation pattern of specific genes which lead to permanent phenotypic changes such as body weight, blood pressure and coat color [160, 161]. The humans who were exposed to Dutch Hunger [23, 162] famine *in utero* had different DNA methylation patterns as compared to those who were not affected. The risk of cardiac disease is much more in individuals who have prenatal exposure to tobacco smoke. Breton et al [163] have showed altered DNA methylation in LINE-1 (Long Interspersed Elements) and Alu elements in children exposed to smoke in prenatal conditions [163]. The study shows that *in utero* exposure to tobacco smoke for a long time results in altered DNA methylation. Monozygotic twins gradually become different in their behavior due to epigenetic drift with advancing age caused by different life styles [164]. The pollution created by traffic is associated with increased CVD risk and DNA methylation of LINE-1 and Alu elements [165, 166]. It has been suggested that microRNAs are important regulators of circadian rhythm which can help in the understanding of biological clock [167] and the fact that risk of CVD is more in the early morning than in the late afternoon or late evening further speculates the role of epigenetics.

Future prospects and challenges

In the past few years, microRNAs have emerged as one of the important epigenetic mechanisms behind cardiac dysfunction (myocardial infarction, heart failure, cardiovascular disease and hypertrophy) and regeneration (cardiomyocyte progenitor cells and stem cell proliferation to caridiomyocytes). MicroRNAs have the potential to become pharmacological targets, as dysregulation of a single microRNA can lead to cardiac disorders and their expression can be modulated for stem cell proliferation. This perpetuates the development of therapeutic and diagnostic strategies by regulating the expression of target genes through microRNAs and their detection in body fluids much before the onset of the disease. Understanding the role of histone modifications in differential regulation of genes under diseased conditions can help in mechanistic and disease therapeutics. DNA methylation underlying developmental processes and progression of disease are important phenomena in disease pathologies. However, the translational means of this research are still in their infancy and less efficient. Though, keeping in mind their immense potential, ongoing exploration and refinement of experimental approaches is the need of the hour and good reason to be optimistic.

Abbreviations

ABCA1	ATP Binding Cassette Transporter A1

CAD	Coronary Artery Disease
CREBP	cAMP (Adenosine 3′5′ Cyclic Monophosphate) Response Element-Binding Protein
CVD	Cardiovascular Disease
DNMT	DNA methyltransferase
DOTIL	Disruption of Telomeric silencing protein
FHL1	Four and a Half LIM domains 1
GNASAS	Guanine Nucleotide Binding Protein (G Protein), Alpha Stimulating Activity Polypeptide
HAT	Histone Acetyl transferase
HDAC	Histone Deacetylase
HF	Heart Failure
HSD11B2	Hydroxysteroid (11-beta) dehydrogenase 2
IL-10	Interleukin 10
INSIGF	Insulin Induced Gene
JMJD	Jumonji Domain
LIM1	Lin11/Isl1/Mec3 transcription factors
LINE-1	Long Interspersed Neucleotide Elements
MEG3	Maternally expressed Gene 3
MI	Myocardial Infarction
MTHFR	Methylene tetrahydrofolate reductase
SRF	Serum Response Factor
TBX5	T Box 5
UTX	Ubiquitously transcribed tetratricopeptide repeat, X chromosome
VCAM-1	Vascular Cell Adhesion Molecule 1

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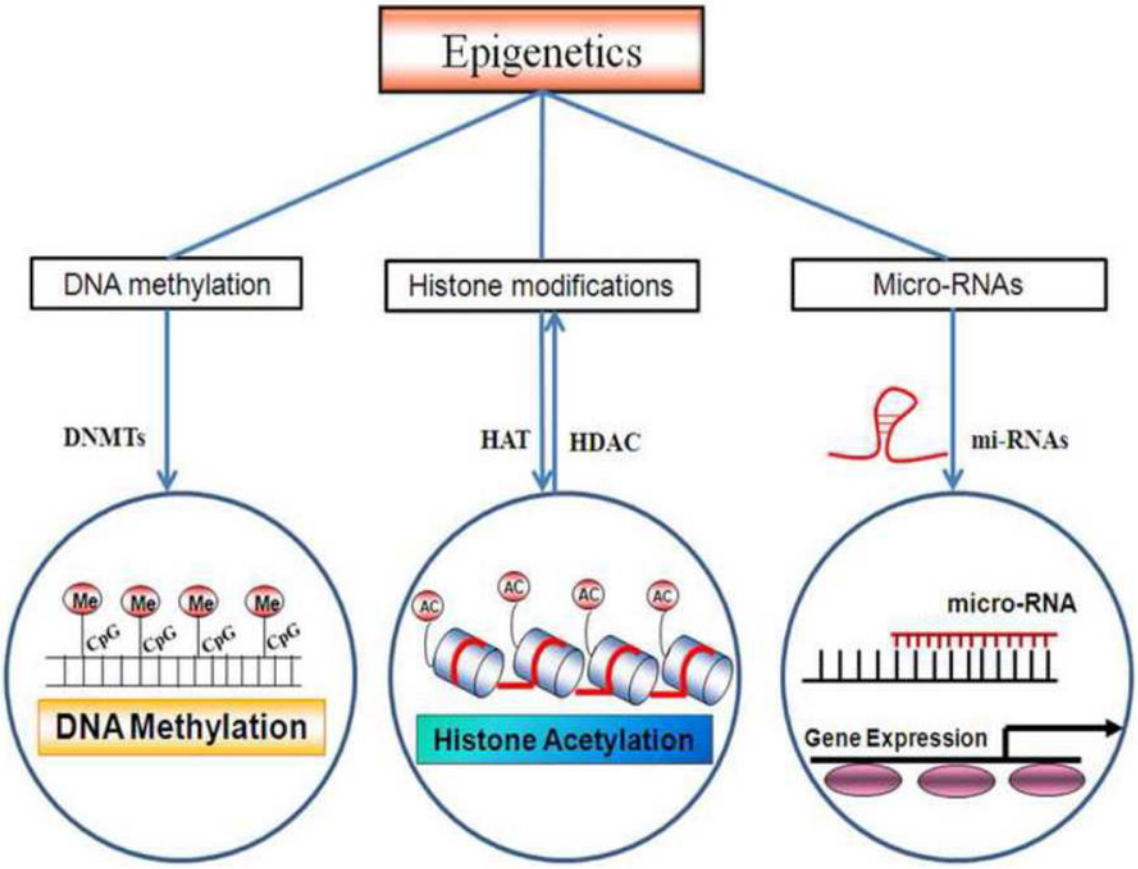

Figure 1.
Epigenetic mechanisms and role of DNA methylation, histone modification by acetylation and methylation and post-transcriptional control by microRNA. Mthyl trasferase is involved in gene methylation. HAT and HDAC are involved in histone acetylation and chromatin remodeling. microRNAs are involved in post-transcriptional control of the genes.

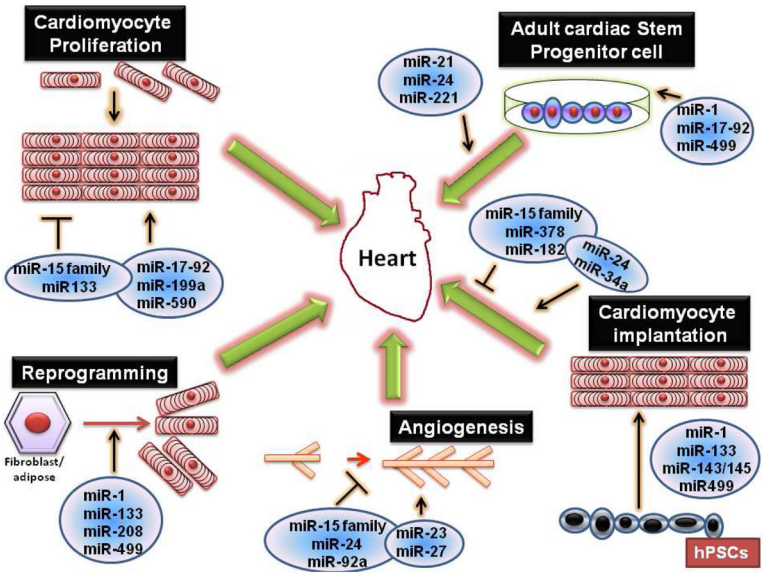

Figure 2.

The role of microRNAs in cardiac regeneration. mir15 family suppresses the proliferation of cardiomyocytes and angiogenesis while mir1, -133 and -499 induces fibroblast/adipocyte reprogramming into cardiomyocytes. Mir1 and -499 also promote the conversion of cardiac stem progenitor cells into cardiomyocytes and they can be injected for cardiac regeneration. hPSC or human pleuripotent stem cells can be converted to cardiomyocytes by the induction of mir1, mir133 and mir499.

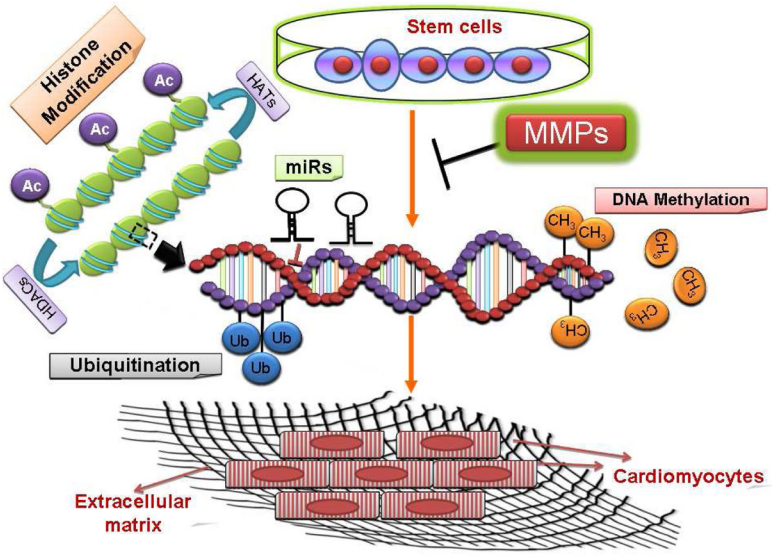

Figure 3.

Proposed hypothesis for the conversion of stem cells into cardiomyocytes. Based on our previous reports we postulate that microRNAs alter the expression of MMP/TIMP genes which are responsible for matrix degradation and the expression of DNA methyltransferases which consequently lead to altered DNA methylation status. This helps in the conversion of embryonic stem cells to cardiomyocytes.

Table 1

Overview of epigenetic modifications: DNA methylation and Histone modifications in cardiomyopathy.

Epigenetic Modification	Target	Binding domains	Altered Gene expression	Phenotype	References
DNA methylation	CPG islands	None	Upregulation or Downregulation	Heart failure and altered gene expression of angiogenic factors	[70, 134]
Histone modifications					
Methylation	H3K4me3, H3K4me2, H3K9me3, H3K27me3, H3K79me	PTIP, DOTIL	Upregulation or Downregulation	Fetal cardiac gene activation; angiogenesis and heart failure; dialated cardiomyopathy	[59–61, 143]
Demethylation	H3K4me3, H3K4me3, H3K36me3, H3K27me3	JMJD2A, UTX	Upregulation or Downregulation	Cardiac hypertrophy stimulation; heart malformation and embryo lethality	[59, 62–64]
Acetylation	H3K4, K19, H4K5, K8, K12, K16	CREBP- binding protein (CBP)/p300	Upregulation	Cardiac hypertrophy regulation	[49, 135, 136]
Deacetylation	Histone tails	Class II HDACs (HDAC-4,5,9)		Inhibit the activity of myocyte enhancer factor 2 (MEF2); negative regulation of cardiac hypertrophy	[55, 137]
Phosphorylation	H3 S10/S28/S10, H4Y41 and H2B, HDACs	Aurora, Rsk2, Msk1, IKKα, PIMI, Akt, CaMK11, JAK2, AMPK, PKD	Upregulation or Downregulation	Mototic activity, cellular proliferation, cardiac hypertrophy regulation; transcriptional activation	[8, 138–142]
Ribosylation	Histones and PARP-1, HDACs and brg1	PARP-1	Upregulation	Cardiac hypertrophy and heart failure; form a complex with Brg1 and HDACs and increase expression of fetal β-MHC	[52]

Table 2

miRNAs involved in cardiac degeneration:

micro-RNA	Fold expression	Site of expression	Methodology	Reference
Acute myocardial infarction				
mir-1 mir-133a mir-208b mir-4993p	Upregulated 300 times Upregulated 70 times Upregulated 3000 times Upregulated 250 times	Human Plasma	qRT-PCR	[37]
mir-223	Downregulated	Human Plasma	qRT-PCR	[33]
mir-21	Downregulated in infarcted cells Upregulated in border cells	Rat Myocytes	qRT-PCR	[32]
mir-122 mir-375	Downregulated	Human Plasma	qRT-PCR	[26]
mir-423-5p	Upregulated 3–10 fold	Human Plasma	qRT-PCR	[11]
mir-663-b, -1291, mir-306, -145	Downregulated Upregulated	Human PBMCs	Array	[12]
mir-328	Upregulated in plasma	Human plasma	qRT-PCR	[144]
Coronary Artery Disease				
mir-17-92 cluster, -126, -145, -155, mir-133, -208a	Downregulated Upregulated	Human Plasma	Array	[27]
mir-92a, -126, - 133a, -208a, -499	Upregulated 2–20 fold, ACS vs CAD	Human Plasma	qRT-PCR	[116]
mir-134, -135a, - 147, -198, -370 mir-147	Upregulated 3.1–12 fold Downregulated 4 fold	Human PBMCs	qRT-PCR	[30]
mir-340, mir-624	Upregulated >1.5 fold	Human Platelets	Array	[31]
Heart failure				
mir-24, -125b, - 195, -199a, -214	Upregulated 1.3–3 fold	Human left ventricle	Northern blots	[38]
mir-214 mir-19a/b	Upregulated 2–2.8 fold Downregulated 2–2.7 fold	Human left ventricle	Bead based hybridization	[39]
mir-29b, -142-3p mir-107, -125b, -139, -142-5p, -497	Upregulated >2 fold Down regulated >2 fold	Human PBMCs	qRT-PCR	[145]
mir-125, -181b, - 214, -342	Upregulated	Human left ventricle	Array	[146]
mir-100, -195,	Upregulated	Human heart tissue	Array	[40]
mir-92, -133b mir-21, -129, -212	Downregulated Upregulated >1.5 fold	Human heart tissue	Array	[147]

Table 3

mi-RNAs involved in cardiac regeneration

micro-RNA	Functions	Reference
miR-1	Increases differentiation of hPSCs into cardiomyocytes and enhances direct reprogramming of human fibroblasts into cardiomyocytes	[89, 91, 92, 148]
miR-499	Overexpression enhances hPSC cardiac differentiation and CPC cardiac differentiation	[89, 92]
miR-199a	Expression in cardiomyocytes induces proliferation and facilitates cardiac regeration following myocardial infarction in adult mice	[82]
miR-15 family	Inhibition of this microRNA enhances cardiomyocyte survival following ischemia-reperfusion injury in vitro and in vivo, as well as in response to palmitate in vitro; inhibition also facilitates cardiac regeration following myocardial infarction in adult mice	[83, 149–152]
miR-199b	In vivo inhibition of miR-199b normalized significantly attenuated cardiac functional impairment, fibrosis and NFAT activity; the target of miR-199b is Dyrk1	[153]
mir-29	miR-29 downregulation prenvents aortic dialation	[154]
miR-21	Antagomir-21 decreased the development of cardiac fibrosis and improved cardiac function	[155–157]
miR-98	Overexpression of miR-98 reduced AngII mediated fibrosis and amount of apoptotic myocytes	[158]
miR-17-92 cluster	Overexpression enhances cardiac progenitor cell proliferation and facilitates cardiac regeneration following myocardial MI in adult mice	[90]
miR-23, miR-24	Overexpression Enhances cardiomyocyte survival following MI n in adult mice and enhances angiogenesis	[159, 160]
mir-27	Enhances angiogenesis	[160]
miR-34a	Expression levels gradually increase with age in mice and humans and inihibition enhances cardiomyocyte survival following MI in adult mice	[154]
miR-92a, miR-133	Enhances mesodermal differentiation of hPSCs and enhances direct reprogramming of human fibroblasts into cardiomyocytes;	[91, 121, 148]
miR-143/145	Knockdown prevents cardiogenesis in murine embryonic stem cell differentiation	[161]
miR-378	Inhibition of this microRNA improves cardiomyocyte survival in an in vitro model of hypoxia-reperfusion	[162]
miR-590	Expression of this microRNA in cardiomyocytes induces proliferation and facilitates cardiac regeration following MI in adult mice	[82]