Chapter 7
Epigenetic Regulation of Cardiac Regeneration

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7.1 Self-Regeneration of the Adult Human Heart

A small percentage of cardiomyocyte's proliferation occurs in adult human heart (Bergmann et al. 2009). This extraordinary phenomenon was demonstrated through the measurement of the amount of 14C incorporated into the DNA of cardiomyocytes of individuals born before and after the nuclear bomb tests and the comparison with the levels of atmospheric 14C. Interestingly, the detectable pool of new cardiomyocytes ranged from 1 % per year in young adults to 0.45 % in the elderly. While these renewal rates cannot support the endogenous myocardial self-regeneration in response to injury, they have nonetheless encouraged the study of any regulatory mechanism able to enhance it. Heart failure is an heterogeneous disease and recent scientific reports have demonstrated that the improvement of intercellular cross-talk is essential to foster a more effective adaptive response to injury (Tirziu et al. 2010). Less than a third of the total cell number of the adult myocardium consists of cardiomyocytes, which communicate with a broad pool of additional cell types, such as smooth muscle cells, endothelial cells, fibroblasts, mast cells, immune system-related cells and progenitor cells (Bu et al. 2009). The myocardial homeostasis implies different cell-to-cell and cell-to-matrix connections, which form a mature and self-regulated functional unit (Ausoni and Sartore 2009). These distinct cell pools also interact via a variety of soluble paracrine, autocrine and endocrine factors, which require a stable apparatus of gene expression (Lionetti et al. 2010a, b). Recent studies of developmental biology and integrative physiology have highlighted the role played by epigenetic modifications as common regulatory pathway of cell function and threshold of cell turnover in adult's heart (Romano and Lionetti 2013). The epigenetic signals in fact activate, maintain, and change the cardiac transcriptional status

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underlying the tissue ability to tolerate the microenvironmental stress without losing functional coherence. All epigenetic pathways remodel chromatin and ensure the maintenance of cardiac homeostasis during several cell divisions and across generations without altering the DNA sequence. Lack of gene function and the cell death follow the failure of balancing epigenetic signals. Specific signaling pathways may drive the phenotype of cardiac resident cells at transcriptional and post-transcriptional levels in healthy and damaged hearts (Lionetti and Ventura 2013a, b). In particular, the following pathways play a key role in the self-regulation of the epigenetic state of adult cardiac cells: DNA methylation, histone modifications, and RNA-based modulation (Issa and Baylin 1996).

7.2 The Epigenetic Memory of Cardiac Cells: A Prelude to Myocardial Regeneration

The epigenetic memory relies on the ability of cardiac cells to translate environmental cues in adaptive response and it remains unchanged after several cell divisions. High levels of DNA methylation lead to maintenance of cell epigenetic memory (Sanchez-Freire et al. 2014). In addition, histone modifications support epigenetic inheritance mechanisms of adult cardiac cells during transient or persistent exposure to different microenvironments (Schlingman et al. 2014). Histone modifications, which determine the structure and function of the chromatin, transmit stable epigenetic information between proliferating cells. Finally, pool of regulatory small noncoding RNAs (ncRNAs) regulate the expression of factors leading to histone modifications, such as the Polycomb-group proteins (Mathiyalagan et al. 2014).

7.2.1 DNA Methylation

In cardiac cells the magnitude of DNA methylation at the C5 position of CpG islands is a balance of two biological processes: (1) de novo methylation or (2) maintenance of methylation during cellular DNA replication. Based on recent findings, the maintenance of methylation assumes a relevant biological role as DNA replication occurs in cardiomyocytes during adulthood (Bergmann et al. 2009). Cardiac DNA demethylation is specifically regulated by Ten-eleven translocation methylcytosine dioxygenase enzymes (TET) that oxidizes the methyl group of specific methylcytosines (Kinney et al. 2011).

The balance between DNA methylation or demethylation affects the expression and function of genes which regulate cardiac function. For example, low levels of circulating methylated Long Interspersed Nucleotide Elements-1 (LINE-1) gene, a biomarker of systemic DNA methylation (Yang et al. 2004), predicts intolerance to myocardial ischemia in elderly individuals (Baccarelli et al. 2010). Moreover, DNA hypermethylation even limits contractile function of rodent cardiomyocytes chroni-

cally exposed to norepinephrine-induced hypertrophy (Xiao et al. 2014). Different levels of DNA methylation in normal and failing hearts suggest that changes of epigenomic patterns affect the ability to predict the development of heart failure in humans (Movassagh et al. 2011). It is conceivable that high levels of DNA methylation represent an adaptive mechanism underlying the response to injury by adult cardiomyocytes.

Despite this fascinating hypothesis, DNA methylation does not affect tyrosine kinase-type cell surface receptor HER3 and Homeobox B13 genes, which play a key role in the expression of adaptive cardiac features in response to sustained contractile dysfunction (Haas et al. 2013).

7.2.2 Histone Modifications

Histones are basic proteins of the chromatin (H1, H2A and B, H3 and H4) that form the nucleosome after binding DNA. Histone modifications include the following enzymatic processes: acetylation (Taylor and Liew 1976), phosphorylation (Akhtar and Itzhaki 1977; Liew and Sole 1978), methylation (Kaneda et al. 2009), ADP-ribosylation (Martinez-Zamudio and Ha 2012), biotinylation (Kuroishi et al. 2011), ubiquitination (Zhang 2003) and sumoylation (Wang and Dasso 2009).

The balance between histone acetyltransferases (HATs) or deacetylases (HDACs) modulates DNA transcription in adult cardiac cells of animal models and humans (Miyamoto et al. 2006; Lee et al. 2007; Hariharan et al. 2010). In particular, nuclear HATs play a key role in cardiac hypertrophy (Qiao et al. 2014) and dilation (Miyamoto et al. 2006). However, loss of HATs activity causes the death of embryos due to serious cardiac congenital defects in p300 knock-in mice, where embryonal stem cells do not respond to cardiomyogenic factors (i.e.: BMP-2) (Shikama et al. 2003).

In 2010, we were one of the first to show that the early pharmacological inhibition of cardiac class I HDACs by intramyocardial injection of low dose of hyaluronan mixed estersof butyric and retinoic acid (HBR) induces the expression of paracrine factors that enhance angiogenesis (i.e.: vascular endothelial growth factor, VEGF) and survival/proliferation (i.e.: hepatocyte growth factor, HGF) of adult rat cardiomyocytes bordering the infarct zone. All these changes have preserved the cardiac function in a rodent model of myocardial infarction (Lionetti et al. 2010a, b). Other investigators have confirmed our data in vivo (Zhang et al. 2012). Preliminary evidences provided by us have also shown that HBR hampers cell proliferation and migration of cardiac fibroblasts, attenuates the differentiation to myofibroblasts and inhibits collagen expression (Cavallini et al. 2011). These evidences support the hypothesis that the inhibition of class I HDAC) differently affects the myocardial epigenetic memory depending on the cell type.

Histone H3 phosphorylation at serine-10, a different histone modification, is detectable in proliferating cardiomyocytes of heart exposed to unloading conditions (Canseco et al. 2015) or stimulated with growth factors (Illi et al. 2005). In addition,

Histone H3 Ser-10 phosphorylation leads to transcription of Mef2, a transcription factor that induces cell growth (Awad et al. 2013).

Mono-, di- or tri-methylation of arginine or lysine residues of histone H3 and H4 leads to active or repressed states of chromatin in murine adult cardiac cells (Chaturvedi et al. 2014). Loss of methylation at lysine K4 of histone H3 (H3K4) increases intracellular calcium levels resulting in improved contractile function (Stein et al. 2011). The role of histone biotinylation, ADP-ribosylation and sumoylation in myocardial adaptive response to stressors is still unknown.

7.2.3 RNA-Based Epigenetic Modulators

MicroRNAs (miR) are small non-coding RNAs, long about 22 nucleotides, that degrade the complementary mRNA after binding the RNA-induced silencing complex (RISC) (Brennecke et al. 2005). To date, miRNAs play a role as pathophysiological hallmark of cardiac disease/repair at post-trascriptional level. For example, high circulating levels of miR-1, 21, 133a, 208 and 499 are detectable after myocardial injury (Zile et al. 2011).

In addition, plasma levels of cardiac muscle-enriched miR (i.e.: miR-133a and 208a) increase in patients with coronary artery disease (Fichtlscherer et al. 2010). The profile of circulating miRNAs may be helpful to identify cardiovascular patients even at earlier and later stages of disease. They are also helpful to track the short- and long-term effects of different regenerative approach. In fact, high levels of miR-323-3p and -652 are detected in untreated patients affected by acute coronary syndrome compared to healthy controls up to 4 months post-hospitalization (Pilbrow et al. 2014) and significantly improve the risk stratification in combination with established biomarkers of cardiac dysfunction, such as high levels of N-terminal of the prohormone brain natriuretic peptide (NT-proBNP) and low left ventricular ejection fraction (Pilbrow et al. 2014). Recent studies even highlighted the role of miRNAs as epigenetic paracrine mediators of cell-to-cell/cell-to-matrix interactions in adult heart. Cardioprotective and proangiogenic miRNAs are released into exosomes, nanosized extracellular vesicles, which are detectable in myocardial interstitium and have a strong therapeutic potential (Cervio et al. 2015).

Long non-coding RNAs (LncRNAs), long about 200 nucleotides, play a role in regulating cardiac physiological traits at both transcriptional and post-transcriptional levels, even if they lack significant protein-coding potential (Guttman et al. 2009). Despite LncRNAs expression being affected by cardiovascular disorders (Ounzain et al. 2015), their epigenetic role in mediating myocardial regeneration is still not well defined. Emerging evidences suggest that LncRNAs modulate the expression of miRNAs during hypertrophic response of adult cardiomyocytes to stressors, as angiotensin II (Wang et al. 2014).

Deregulated epigenetic patterns through LncRNAs expression underlies the lack of angiogenic ability of coronary endothelial cells in response to hypoxia. For example, lower levels of metastasis-associated lung adenocarcinoma transcript 1

(MALAT1), a lncRNA highly expressed in hypoxic adult endothelial cells, inhibit endothelial cell proliferation and VEGF-dependent vessel growth (Michalik et al. 2014). A recent study demonstrated that lncRNAs have a differential abundance in exosomes, indicating a selective loading by producing cells (Gezer et al. 2014).

7.3 Epigenetic Modifications in Regenerating Myocardium

Epigenetic modifications are emerging as endogenous mechanisms sustaining cardiac self-renewing properties and are therapeutic candidates for regeneration of heart failure.

7.3.1 DNA Methylation

Leferovich et al. (2001) have observed a high cardiac regenerative potential in MRL/MpJ mice, which show cells arrested in the G2/M phase and high activity of matrix metalloproteinases. In particular, the heart of adult MRL/MpJ mice shows high levels of DNA methylation and retains embryonic features (Górnikiewicz et al. 2013). However, the heart of these mice does not heal after myocardial infarction (Cimini et al. 2008) and I/R injury (Abdullah et al. 2005). This observation suggests that the microenvironment promotes the switch on DNA methylation peak from embryonic to adult profile therefore limiting the regenerative potential of the myocardium. This model may be helpful to detect the genes responsible of the low regenerative capacity of adult heart. Recent studies have demonstrated that Notch-responsive promoters, which support cardiomyocytes proliferation in zebrafish heart (Zhao et al. 2014), show higher levels of permanent CpG DNA methylation in adult murine cardiomyocytes of infarcted hearts compared to healthy ones (Felician et al. 2014). Notch-responsive promoters are permanently silenced by DNA methylation.

Conversely, another study has shown that the pharmacological inhibition of DNA methylation in rodent stem cells induces protein expression of homeobox protein Nkx2.5, transcription factor GATA binding protein 4 (GATA4) and cardiac troponin T which trigger the differentiation to cardiac lineage (Naeem et al. 2013). Therefore, the modulators of levels of DNA methylation are therapeutic candidate to enhance myocardial regeneration.

7.3.2 Histone Modifications

HATs activation increases acetylation of lysine K9 and K14 of histone H3 at physiological level, reduces levels of DNA CpG methylation, and recovers the ability of mesenchymal stem cells to proliferate and differentiate to cardiac lineage in the presence of oxidative stress (Vecellio et al. 2014).

Conversely, the inhibition of HDACs induces the proliferation of adult rodent cardiomyocytes both in vitro and in vivo (Majumdar et al. 2012). In small and large animal models of heart failure, myocardial histone acetylation increase the expression and release of paracrine factors, such as the hepatocyte growth factor (HGF) (Iwasaki et al. 2005) or the vascular endothelial growth factor (VEGF) (Tao et al. 2011).

Some growth factors may in turn activate HDACs. For example, VEGF play an epigenetic role by inducing the degradation of HDAC7 via phospholipase C gamma-inositol-1,4,5-trisphosphate kinase signal pathway, which prevents the HDAC7-mediated inhibition of cell proliferation, as observed in mature endothelial cells (Margariti et al. 2010).

Recent studies have highlighted the epigenetic role of other important paracrine factors involved in myocardial angiogenesis. Li et al. (2014) have demonstrated that carbon monoxide enhances the levels of histone acetylation, which in turn improves endothelial cell migration, and increases the angiogenic ability of human endothelial cells (Lionetti et al. 2010a, b; Agostini et al. 2015) following the treatment with inhibitors of class I HDACs. These data refute previous in vitro studies demonstrating that the maintenance of HDAC activity induces angiogenesis (Kim et al. 2001; Mottet et al. 2007).

Furthermore, Mezentseva et al. (2013) have demonstrated in vitro that low levels of methylation at lysine9 of histone H3 leads to reprogramming of bone marrow stem cells towards a cardiac lineage.

These studies encourage the development of novel and safer histone modulators also to optimize the cardiac differentiation of circulating bone marrow-derived stem cells engrafted in the failing heart.

7.3.3 RNA-Based Epigenetic Modulators

The cardiac delivery of miR-590 and -199 promoted cell cycle re-entry of adult cardiomyocytes and enhanced cardiomyocyte proliferation in infarcted murine heart (Eulalio et al. 2012). These data are in accord with previous studies suggesting that endogenous miRNAs act as endogenous regulators of cell reprogramming and as therapeutic targets in the setting of novel avenue in cell-free cardiac regeneration. However, miRNAs even may exert negative effect on cell function.

Bonauer et al. (2009) have shown that high levels of miR-92a inhibits the formation of new blood vessels, which play a relevant role in the functional recovery of murine infarcted heart. In light of this evidence, the synthesis of miRNA antagonists may be a helpful tool to promote myocardial regeneration. In fact, treatment with selective LNA-modified anti-miR-15 prevented loss of hypoxic cardiomyocytes, reduced infarct scar size and preserved cardiac function in murine infarcted heart (Hullinger et al. 2012). Interestingly, stable myocardial down-regulation of miR-24 enhances angiogenesis and blood perfusion in the myocardium surrounding the

infarct area and improves cardiac function despite promoting apoptosis of fibroblasts and cardiomyocytes (Meloni et al. 2013).

To date, it remains unclear whether the modulation of single miR-dependent pathway is sufficient to trigger cardiomyocyte's proliferation in regenerating the adult infarcted heart. The selective inhibition of miRNA-15 even increases the rate of proliferation of adult cardiomyocytes and significantly improves the cardiac function of infarcted murine heart (Porrello et al. 2013). Conversely, other investigators found that an effective proliferation of adult cardiomyocytes require the combined action of several miRNAs, such as miR17-92 cluster (Chen et al. 2013). Jayawardena et al. (Jayawardena et al. 2012) have demonstrated in mice that a cocktail of miR-1, -133, -208 and -499 restores the post-ischemic loss of cardiomyocyte's pool with a population of fibroblast-derived cardiomyocytes due to gene reprogramming. All these findings suggest that mir-crine mechanisms hold great promise as therapeutic candidates for the development of personalized myocardial regeneration.

Regulatory miRNAs are naturally released by cardiomyocytes, endothelial cells, fibroblasts or cardiac/endothelial progenitor cells, as demonstrated in adult mice (Brás-Rosário et al. 2013). Exosomes containing miRNAs are released into the extracellular microenvironment by adult cardiomyocytes (Wang et al. 2014), cardiac fibroblasts (Bang et al. 2014), endothelial cells (Ong et al. 2014) and cardiac progenitor cells (Vrijsen et al. 2010).

Exosomes secreted by human cardiac progenitor cells (CPCs) contain higher amounts of miRNA210, 132 and 146a-3p compared with human cardiac fibroblasts (Barile et al. 2014). Each miRNA acts on specific pathways; miRNA210 inhibits cardiomyocyte's apoptosis through the down regulation of ephrin A3 and protein-tyrosine phosphatase 1B (PTP1B) expression, while miRNA132 increases the angiogenic ability of mature endothelial cells through the down regulation of RasGAP-p120 expression. The single intramyocardial injection of CPCs-derived exosomes hampers cardiac remodeling and preserves left ventricular ejection fraction of infarcted rat heart in a dose-dependent manner. Conversely, fibroblasts-derived exosomes did not exert any cardioprotective effects. Other investigators have found similar findings in a murine model of heart failure (Ibrahim et al. 2014).

Some investigators are developing new methods to increase the endogenous release of cardioprotective exosomes containing miRNAs. Original study found that high levels of hypoxia-inducible factor-1 (HIF-1), a transcription factor that protects against ischemia and highly expressed in human failing heart (Lionetti et al. 2014), increase the release of exosomes containing miRNA126 and 210 (Ong et al. 2014). Therefore, the exosomes have the potential to circumvent many limitations of stem cells transplantation for therapeutic applications in cardiac regenerative medicine.

Recently, it has been shown that exosomes deliver LncRNAs (Gezer et al. 2014). To date, a few studies have characterized the regenerative potential of LncRNAs in the adult heart. For example, the LncRNA steroid receptor RNA activator 1 (SRA1) regulates the expression of myogenic differentiation 1 (MyoD1) (Caretti et al. 2006) and is essential for myocardial function. Further investigations should be encouraged to better address the therapeutic potential of exosomes containing LncRNAs.

7.4 Conclusions and Perspectives

Studies conducted so far have provided convincing experimental evidences that different epigenetic mechanisms underlie the changes of the cardiac physiological traits during myocardial regeneration. The pleiotropic non-invasive modulation of the epigenetic threshold of resident cardiac cells by drugs or diet is a frontier of investigation that should be encouraged in order to overcome the limitations that hinder an efficient structural and functional recovery of the adult heart in an epigenetic manner. In fact, emerging scientific evidences have shown that it is possible to modulate the cardiac epigenome and to induce cardiac benefits by the regular intake of lower doses of active plant compounds, such as barley beta-glucan (Agostini et al. 2015), or by the administration of selected exosomes (Barile et al. 2014). To best of our knowledge, the cardiac repair following the administration of the potential epigenetic modulators will be more effective at lower doses and mainly focused to histone or RNA-based modifications. Further translational investigations should be conducted in large animal models of heart failure and humans to better address dose, timing and route of administration.

References

Abdullah I, Lepore JJ, Epstein JA, Parmacek MS, Gruber PJ (2005) MRL mice fail to heal the heart in response to ischemia reperfusion injury. Wound Repair Regen 13:205–208

Agostini S, Chiavacci E, Matteucci M, Torelli M, Pitto L, Lionetti V (2015) Barley beta-glucan promotes MnSOD expression and enhances angiogenesis under oxidative microenvironment. J Cell Mol Med 19:227–238

Akhtar RA, Itzhaki S (1977) Studies in vitro of the effects of adenosine 3′:5′-cyclic monophosphate on the phosphorylation of nuclear proteins in isolated rat heart nuclei. Biochem J 161:487–494

Ausoni S, Sartore S (2009) The cardiovascular unit as a dynamic player in disease and regeneration. Trends Mol Med 15:543–552

Awad S, Kunhi M, Little GH, Bai Y, An W, Bers D, Kedes L, Poizat C (2013) Nuclear CaMKII enhances histone H3 phosphorylation and remodels chromatin during cardiac hypertrophy. Nucleic Acids Res 41:7656–7672

Baccarelli A, Wright R, Bollati V, Litonjua A, Zanobetti A, Tarantini L, Sparrow D, Vokonas P, Schwartz J (2010) Ischemic heart disease and stroke in relation to blood DNA methylation. Epidemiology 21:819–828

Bang C, Batkai S, Dangwal S, Gupta SK, Foinquinos A, Holzmann A, Just A, Remke J, Zimmer K, Zeug A, Ponimaskin E, Schmiedl A, Yin X, Mayr M, Halder R, Fischer A, Engelhardt S, Wei Y, Schober A, Fiedler J, Thum T (2014) Cardiac fibroblast-derived microRNA passenger strand enriched exosomes mediate cardiomyocyte hypertrophy. J Clin Invest 124:2136–2146

Barile L, Lionetti V, Cervio E, Matteucci M, Gherghiceanu M, Popescu LM, Torre T, Siclari F, Moccetti T, Vassalli G (2014) Extracellular vesicles from human cardiac progenitor cells inhibit cardiomyocyte apoptosis and improve cardiac function after myocardial infarction. Cardiovasc Res 103:530–541

Bergmann O, Bhardwaj RD, Bernard S, Zdunek S, Barnabé-Heider F, Walsh S, Zupicich J, Alkass K, Buchholz BA, Druid H, Jovinge S, Frisén J (2009) Evidence for cardiomyocyte renewal in humans. Science 324:98–102

Bonauer A, Carmona G, Iwasaki M, Mione M, Koyanagi M, Fischer A, Burchfield J, Fox H, Doebele C, Ohtani K, Chavakis E, Potente M, Tjwa M, Urbich C, Zeiher AM, Dimmeler S (2009) MicroRNA-92a controls angiogenesis and functional recovery of ischemic tissues in mice. Science 324:1710–1713

Brás-Rosário L, Matsuda A, Pinheiro AI, Gardner R, Lopes T, Amaral A, Gama-Carvalho M (2013) Expression profile of microRNAs regulating proliferation and differentiation in mouse adult cardiac stem cells. PLoS One 8:e63041

Brennecke J, Stark A, Russell RB, Cohen SM (2005) Principles of microRNA-target recognition. PLoS Biol 3, e85

Bu L, Jiang X, Martin-Puig S, Caron L, Zhu S, Shao Y, Roberts DJ, Huang PL, Domian IJ, Chien KR (2009) Human ISL1 heart progenitors generate diverse multipotent cardiovascular cell lineages. Nature 460:113–117

Canseco DC, Kimura W, Garg S, Mukherjee S, Bhattacharya S, Abdisalaam S, Das S, Asaithamby A, Mammen PP, Sadek HA (2015) Human ventricular unloading induces cardiomyocyte proliferation. J Am Coll Cardiol 65:892–900

Caretti G, Schiltz RL, Dilworth FJ, Di Padova M, Zhao P, Ogryzko V, Fuller-Pace FV, Hoffman EP, Tapscott SJ, Sartorelli V (2006) The RNA helicases p68/p72 and the noncoding RNA SRA are coregulators of MyoD and skeletal muscle differentiation. Dev Cell 11:547–560

Cavallini C, Tassinari R, Bonavita F, Lionetti V, Ventura C (2011) Hyaluronan mixed esters of butyric and retinoic acid act transcriptionally on cardiac fibroblasts decreasing myocardial scarring in infarcted hearts. FASEB J 25: 2:2

Cervio E, Barile L, Moccetti T, Vassalli G (2015) Exosomes for intramyocardial intercellular communication. Stem Cells Int 2015:482171

Chaturvedi P, Kalani A, Givvimani S, Kamat PK, Familtseva A, Tyagi SC (2014) Differential regulation of DNA methylation versus histone acetylation in cardiomyocytes during HHcy in vitro and in vivo: an epigenetic mechanism. Physiol Genomics 46:245–255

Chen J, Huang ZP, Seok HY, Ding J, Kataoka M, Zhang Z, Hu X, Wang G, Lin Z, Wang S, Pu WT, Liao R, Wang DZ (2013) mir-17-92 cluster is required for and sufficient to induce cardiomyocyte proliferation in postnatal and adult hearts. Circ Res 112:1557–1566

Cimini M, Fazel S, Fujii H, Zhou S, Tang G, Weisel RD, Li RK (2008) The MRL mouse heart does not recover cardiac function after a myocardial infarction. Cardiovasc Pathol 17:32–39

Eulalio A, Mano M, Dal Ferro M, Zentilin L, Sinagra G, Zacchigna S, Giacca M (2012) Functional screening identifies miRNAs inducing cardiac regeneration. Nature 492:376–381

Felician G, Collesi C, Lusic M, Martinelli V, Ferro MD, Zentilin L, Zacchigna S, Giacca M (2014) Epigenetic modification at notch responsive promoters blunts efficacy of inducing notch pathway reactivation after myocardial infarction. Circ Res 115:636–649

Fichtlscherer S, De Rosa S, Fox H, Schwietz T, Fischer A, Liebetrau C, Weber M, Hamm CW, Röxe T, Müller-Ardogan M, Bonauer A, Zeiher AM, Dimmeler S (2010) Circulating microRNAs in patients with coronary artery disease. Circ Res 107:677–684

Gezer U, Özgür E, Cetinkaya M, Isin M, Dalay N (2014) Long non-coding RNAs with low expression levels in cells are enriched in secreted exosomes. Cell Biol Int 38:1076–1079

Górnikiewicz B, Ronowicz A, Podolak J, Madanecki P, Stanisławska-Sachadyn A, Sachadyn P (2013) Epigenetic basis of regeneration: analysis of genomic DNA methylation profiles in the MRL/MpJ mouse. DNA Res 20:605–621

Guttman M, Amit I, Garber M, French C, Lin MF, Feldser D, Huarte M, Zuk O, Carey BW, Cassady JP, Cabili MN, Jaenisch R, Mikkelsen TS, Jacks T, Hacohen N, Bernstein BE, Kellis M, Regev A, Rinn JL, Lander ES (2009) Chromatin signature reveals over a thousand highly conserved large non-coding RNAs in mammals. Nature 458:223–227

Haas J, Frese KS, Park YJ, Keller A, Vogel B, Lindroth AM, Weichenhan D, Franke J, Fischer S, Bauer A, Marquart S, Sedaghat-Hamedani F, Kayvanpour E, Köhler D, Wolf NM, Hassel S, Nietsch R, Wieland T, Ehlermann P, Schultz JH, Dösch A, Mereles D, Hardt S, Backs J, Hoheisel JD, Plass C, Katus HA, Meder B (2013) Alterations in cardiac DNA methylation in human dilated cardiomyopathy. EMBO Mol Med 5:413–429

Hariharan N, Maejima Y, Nakae J, Paik J, Depinho RA, Sadoshima J (2010) Deacetylation of FoxO by Sirt1 plays an essential role in mediating starvation-induced autophagy in cardiac myocytes. Circ Res 107:1470–1482

Hullinger TG, Montgomery RL, Seto AG, Dickinson BA, Semus HM, Lynch JM, Dalby CM, Robinson K, Stack C, Latimer PA, Hare JM, Olson EN, van Rooij E (2012) Inhibition of miR-1 5 protects against cardiac ischemic injury. Circ Res 110:71–81

Ibrahim AG, Cheng K, Marbán E (2014) Exosomes as critical agents of cardiac regeneration triggered by cell therapy. Stem Cell Rep 2:606–619

Illi B, Scopece A, Nanni S, Farsetti A, Morgante L, Biglioli P, Capogrossi MC, Gaetano C (2005) Epigenetic histone modification and cardiovascular lineage programming in mouse embryonic stem cells exposed to laminar shear stress. Circ Res 96:501–508

Issa JP, Baylin SB (1996) Epigenetics and human disease. Nat Med 2:281–282

Iwasaki M, Adachi Y, Nishiue T, Minamino K, Suzuki Y, Zhang Y, Nakano K, Koike Y, Wang J, Mukaide H, Taketani S, Yuasa F, Tsubouchi H, Gohda E, Iwasaka T, Ikehara S (2005) Hepatocyte growth factor delivered by ultrasound-mediated destruction of microbubbles induces proliferation of cardiomyocytes and amelioration of left ventricular contractile function in Doxorubicin-induced cardiomyopathy. Stem Cells 23:1589–1597

Jayawardena TM, Egemnazarov B, Finch EA, Zhang L, Payne JA, Pandya K, Zhang Z, Rosenberg P, Mirotsou M, Dzau VJ (2012) MicroRNA-mediated in vitro and in vivo direct reprogramming of cardiac fibroblasts to cardiomyocytes. Circ Res 110:1465–1473

Kaneda R, Takada S, Yamashita Y, Choi YL, Nonaka-Sarukawa M, Soda M, Misawa Y, Isomura T, Shimada K, Mano H (2009) Genome-wide histone methylation profile for heart failure. Genes Cells 14:69–77

Kim MS, Kwon HJ, Lee YM, Baek JH, Jang JE, Lee SW, Moon EJ, Kim HS, Lee SK, Chung HY, Kim CW, Kim KW (2001) Histone deacetylases induce angiogenesis by negative regulation of tumor suppressor genes. Nat Med 7:437–443

Kinney SM, Chin HG, Vaisvila R, Bitinaite J, Zheng Y, Estève PO, Feng S, Stroud H, Jacobsen SE, Pradhan S (2011) Tissue-specific distribution and dynamic changes of 5-hydroxymethylcytosine in mammalian genomes. J Biol Chem 286:24685–24693

Kuroishi T, Rios-Avila L, Pestinger V, Wijeratne SS, Zempleni J (2011) Biotinylation is a natural, albeit rare, modification of human histones. Mol Genet Metab 104:537–545

Lee TM, Lin MS, Chang NC (2007) Inhibition of histone deacetylase on ventricular remodeling in infarcted rats. Am J Physiol Heart Circ Physiol 293:H968–H977

Leferovich JM, Bedelbaeva K, Samulewicz S, Zhang XM, Zwas D, Lankford EB, Heber-Katz E (2001) Heart regeneration in adult MRL mice. Proc Natl Acad Sci U S A 98:9830–9835

Li M, Gallo D, Csizmadia E, Otterbein LE, Wegiel B (2014) Carbon monoxide induces chromatin remodelling to facilitate endothelial cell migration. Thromb Haemost 111:951–959

Liew CC, Sole MJ (1978) Studies of nuclear proteins in the heart of the cardiomyopathic Syrian hamster—phosphorylation of histones. J Mol Cell Cardiol 10:847–855

Lionetti V, Ventura C (2013a) New chemicals drive mesenchymal stem cells toward cardiac cells. Recent Pat Regen Med 3:47–55

Lionetti V, Ventura C (2013b) Regenerative medicine approach to repair the failing heart. Vascul Pharmacol 58:159–163

Lionetti V, Bianchi G, Recchia FA, Ventura C (2010a) Control of autocrine and paracrine myocardial signals: an emerging therapeutic strategy in heart failure. Heart Fail Rev 15:531–542

Lionetti V, Cantoni S, Cavallini C, Bianchi F, Valente S, Frascari I, Olivi E, Aquaro GD, Bonavita F, Scarlata I, Maioli M, Vaccari V, Tassinari R, Bartoli A, Recchia FA, Pasquinelli G, Ventura C (2010b) Hyaluronan mixed esters of butyric and retinoic acid affording myocardial survival and repair without stem cell transplantation. J Biol Chem 285:9949–9961

Lionetti V, Matteucci M, Ribezzo M, Di Silvestre D, Brambilla F, Agostini S, Mauri P, Padeletti L, Pingitore A, Delsedime L, Rinaldi M, Recchia FA, Pucci A (2014) Regional mapping of myocardial hibernation phenotype in idiopathic end-stage dilated cardiomyopathy. J Cell Mol Med 18:396–414

Majumdar G, Adris P, Bhargava N, Chen H, Raghow R (2012) Pan-histone deacetylase inhibitors regulate signaling pathways involved in proliferative and pro-inflammatory mechanisms in H9c2 cells. BMC Genomics 13:709

Margariti A, Zampetaki A, Xiao Q, Zhou B, Karamariti E, Martin D, Yin X, Mayr M, Li H, Zhang Z, De Falco E, Hu Y, Cockerill G, Xu Q, Zeng L (2010) Histone deacetylase 7 controls endothelial cell growth through modulation of beta-catenin. Circ Res 106:1202–1211

Martinez-Zamudio R, Ha HC (2012) Histone ADP-ribosylation facilitates gene transcription by directly remodeling nucleosomes. Mol Cell Biol 32:2490–2502

Mathiyalagan P, Okabe J, Chang L, Su Y, Du XJ, El-Osta A (2014) The primary microRNA-208b interacts with Polycomb-group protein, Ezh2, to regulate gene expression in the heart. Nucleic Acids Res 42:790–803

Meloni M, Marchetti M, Garner K, Littlejohns B, Sala-Newby G, Xenophontos N, Floris I, Suleiman MS, Madeddu P, Caporali A, Emanueli C (2013) Local inhibition of microRNA-24 improves reparative angiogenesis and left ventricle remodeling and function in mice with myocardial infarction. Mol Ther 21:1390–1402

Mezentseva NV, Yang J, Kaur K, Iaffaldano G, Rémond MC, Eisenberg CA, Eisenberg LM (2013) The histone methyltransferase inhibitor BIX01294 enhances the cardiac potential of bone marrow cells. Stem Cells Dev 22:654–667

Michalik KM, You X, Manavski Y, Doddaballapur A, Zörnig M, Braun T, John D, Ponomareva Y, Chen W, Uchida S, Boon RA, Dimmeler S (2014) Long noncoding RNA MALAT1 regulates endothelial cell function and vessel growth. Circ Res 114:1389–1397

Miyamoto S, Kawamura T, Morimoto T, Ono K, Wada H, Kawase Y, Matsumori A, Nishio R, Kita T, Hasegawa K (2006) Histone acetyltransferase activity of p300 is required for the promotion of left ventricular remodeling after myocardial infarction in adult mice in vivo. Circulation 113:679–690

Mottet D, Bellahcène A, Pirotte S, Waltregny D, Deroanne C, Lamour V, Lidereau R, Castronovo V (2007) Histone deacetylase 7 silencing alters endothelial cell migration, a key step in angiogenesis. Circ Res 101:1237–1246

Movassagh M, Choy MK, Knowles DA, Cordeddu L, Haider S, Down T, Siggens L, Vujic A, Simeoni I, Penkett C, Goddard M, Lio P, Bennett MR, Foo RS (2011) Distinct epigenomic features in end-stage failing human hearts. Circulation 124:2411–2422

Naeem N, Haneef K, Kabir N, Iqbal H, Jamall S, Salim A (2013) DNA methylation inhibitors, 5 azacytidine and zebularine potentiate the transdifferentiation of rat bone marrow mesenchymal stem cells into cardiomyocytes. Cardiovasc Ther 31:201–209

Ong SG, Lee WH, Huang M, Dey D, Kodo K, Sanchez-Freire V, Gold JD, Wu JC (2014) Cross talk of combined gene and cell therapy in ischemic heart disease: role of exosomal microRNA transfer. Circulation 130:S60–S69

Ounzain S, Micheletti R, Beckmann T, Schroen B, Alexanian M, Pezzuto I, Crippa S, Nemir M, Sarre A, Johnson R, Dauvillier J, Burdet F, Ibberson M, Guigó R, Xenarios I, Heymans S, Pedrazzini T (2015) Genome-wide profiling of the cardiac transcriptome after myocardial infarction identifies novel heart-specific long non-coding RNAs. Eur Heart J 36:353–368a

Pilbrow AP, Cordeddu L, Cameron VA, Frampton CM, Troughton RW, Doughty RN, Whalley GA, Ellis CJ, Yandle TG, Richards AM, Foo RS (2014) Circulating miR-323-3p and miR-652: candidate markers for the presence and progression of acute coronary syndromes. Int J Cardiol 176:375–385

Porrello ER, Mahmoud AI, Simpson E, Johnson BA, Grinsfelder D, Canseco D, Mammen PP, Rothermel BA, Olson EN, Sadek HA (2013) Regulation of neonatal and adult mammalian heart regeneration by the miR-15 family. Proc Natl Acad Sci U S A 110:187–192

Qiao W, Zhang W, Gai Y, Zhao L, Fan J (2014) The histone acetyltransferase MOF overexpression blunts cardiac hypertrophy by targeting ROS in mice. Biochem Biophys Res Commun 448:379–384

Romano SL, Lionetti V (2013) From cell phenotype to epigenetic mechanisms: new insights into regenerating myocardium. Can J Physiol Pharmacol 91:579–585

Sanchez-Freire V, Lee AS, Hu S, Abilez OJ, Liang P, Lan F, Huber BC, Ong SG, Hong WX, Huang M, Wu JC (2014) Effect of human donor cell source on differentiation and function of cardiac induced pluripotent stem cells. J Am Coll Cardiol 64:436–448

Schlingman DJ, Mack AH, Kamenetska M, Mochrie SG, Regan L (2014) Routes to DNA accessibility: alternative pathways for nucleosome unwinding. Biophys J 107:384–392

Shikama N, Lutz W, Kretzschmar R, Sauter N, Roth JF, Marino S, Wittwer J, Scheidweiler A, Eckner R (2003) Essential function of p300 acetyltransferase activity in heart, lung and small intestine formation. EMBO J 22:5175–5185

Stein AB, Jones TA, Herron TJ, Patel SR, Day SM, Noujaim SF, Milstein ML, Klos M, Furspan PB, Jalife J, Dressler GR (2011) Loss of H3K4 methylation destabilizes gene expression patterns and physiological functions in adult murine cardiomyocytes. J Clin Invest 121:2641–2650

Tao Z, Chen B, Tan X, Zhao Y, Wang L, Zhu T, Cao K, Yang Z, Kan YW, Su H (2011) Coexpression of VEGF and angiopoietin-1 promotes angiogenesis and cardiomyocyte proliferation reduces apoptosis in porcine myocardial infarction (MI) heart. Proc Natl Acad Sci U S A 108:2064–2069

Taylor PB, Liew CC (1976) Acetylation of nuclear proteins in the isolated perfused rat heart. Basic Res Cardiol 71:27–35

Tirziu D, Giordano FJ, Simons M (2010) Cell communications in the heart. Circulation 122:928–937

Vecellio M, Spallotta F, Nanni S, Colussi C, Cencioni C, Derlet A, Bassetti B, Tilenni M, Carena MC, Farsetti A, Sbardella G, Castellano S, Mai A, Martelli F, Pompilio G, Capogrossi MC, Rossini A, Dimmeler S, Zeiher A, Gaetano C (2014) The histone acetylase activator pentadecylidenemalonate 1b rescues proliferation and differentiation in the human cardiac mesenchymal cells of type 2 diabetic patients. Diabetes 63:2132–2147

Vrijsen KR, Sluijter JP, Schuchardt MW, van Balkom BW, Noort WA, Chamuleau SA, Doevendans PA (2010) Cardiomyocyte progenitor cell-derived exosomes stimulate migration of endothelial cells. J Cell Mol Med 14:1064–1070

Wang K, Liu F, Zhou LY, Long B, Yuan SM, Wang Y, Liu CY, Sun T, Zhang XJ, Li PF (2014) The long noncoding RNA CHRF regulates cardiac hypertrophy by targeting miR-489. Circ Res 114:1377–1388

Wang X, Huang W, Liu G, Cai W, Millard RW, Wang Y, Chang J, Peng T, Fan GC (2014) Cardiomyocytes mediate anti-angiogenesis in type 2 diabetic rats through the exosomal transfer of miR-320 into endothelial cells. J Mol Cell Cardiol 74:139–150

Wang Y, Dasso M (2009) SUMOylation and deSUMOylation at a glance. J Cell Sci 122:424952

Xiao D, Dasgupta C, Chen M, Zhang K, Buchholz J, Xu Z, Zhang L (2014) Inhibition of DNA methylation reverses norepinephrine-induced cardiac hypertrophy in rats. Cardiovasc Res 101:373–382

Yang AS, Estécio MR, Doshi K, Kondo Y, Tajara EH, Issa JP (2004) A simple method for estimating global DNA methylation using bisulfite PCR of repetitive DNA elements. Nucleic Acids Res 32, e38

Zhang L, Qin X, Zhao Y, Fast L, Zhuang S, Liu P, Cheng G, Zhao TC (2012) Inhibition of histone deacetylases preserves myocardial performance and prevents cardiac remodeling through stimulation of endogenous angiomyogenesis. J Pharmacol Exp Ther 341:285–293

Zhang Y (2003) Transcriptional regulation by histone ubiquitination and deubiquitination. Genes Dev 17:2733–2740

Zhao L, Borikova AL, Ben-Yair R, Guner-Ataman B, MacRae CA, Lee RT, Burns CG, Burns CE (2014) Notch signaling regulates cardiomyocyte proliferation during zebrafish heart regeneration. Proc Natl Acad Sci U S A 111:1403–1408

Zile MR, Mehurg SM, Arroyo JE, Stroud RE, DeSantis SM, Spinale FG (2011) Relationship between the temporal profile of plasma microRNA and left ventricular remodeling in patients after myocardial infarction. Circ Cardiovasc Genet 4:614–619