Chapter 2

Epigenetics, Protein Kinases and Heart Failure

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1. Introduction

During the past century, the diagnosis and treatment of heart failure has changed substantially. Heart failure, once viewed primarily as resulting from rheumatic valvular heart disease, presents currently as the main complications of diabetes, viral cardiomyopathy, coronary artery disease, myocardial infarction and hypertension. Although the etiologies are diverse, virtually all instances of heart failure can be characterized by a sequence of specific molecular changes in the myocardium. These molecular changes result in decrements in cardiac myocyte contractility (negative inotropy), the hallmark of heart failure. In the normal heart, contractility is mainly regulated and, therefore, maintained by G protein-coupled receptor (GPCR) signaling (Huang et al., 2011). GPCR signaling is stimulated when neurotransmitters, hormones and drugs possessing positive inotropic activity bind to β-adrenergic receptors (Leone et al., 2002). One of the main signaling problems in heart failure is that the GPCR β-adrenoceptor kinases -2 and -5 (GRK2 and GRK5) phosphorylate the GPCRs and make them insensitive to positive inotropic β-adrenoceptor ligands (Hata et al., 2004). As such, phosphorylation of the GPCRs functionally uncouples them from their G-proteins, thereby downregulating the cardiomyocyte intracellular signaling cascades which support myocardial contractility. GRK2 is the most abundant cardiac GRK and GRK2 activity is increased in several cardiovascular diseases involving impaired cardiac function and heart failure (Petrofski and Koch, 2003). GRK2 regulates myocardial signaling and heart development and the targeting of GRK activity by small molecule inhibitors of GRK2 is thought to have the potential to become an effective strategy for treatment of heart failure (Iaccarino and Koch, 2003).

1.1. Protein kinases and molecular mechanisms of negative inotropy and heart failure

Increased synthesis and release of endogenous β-adrenoceptor ligands such as norepinephrine, epinephrine and dopamine (catecholamines), mediated by sympathetic neural activity, is an attempt to drive the heart pump to compensate for heart failure. Another compensation attempts to drive the cardiac pump by upregulating the protein kinases known as cAMP-dependent protein kinase (PKA), protein kinases activated by the same signal transduction pathways as phospholipase C (PKC), phosphoinositide-3 kinases (PI3K) and mitogen-activated protein kinases (MAPK). These protein kinases phosphorylate intracellular enzymes that are functionally coupled to the signaling pathways which produce positive inotropic effects. Another compensation attempts to drive the pump by amplifying effects of the thyroid hormones on ion-channel and pump activities; however, upregulation of the thyroid hormones tri-iodothyronine (T3) and tetra-iodothyronine (T4) is also known to produce negative inotropic effects which are thought to be mediated by a T4 metabolite, 3-iodothyronamine (Axelband et al., 2011). The amplification of the effects of T3and T4 results in protein kinase mediated phosphorylation of proteins which promote calcium ion influx via L-type calcium channels and calcium removal by sarcoplasmic reticular Ca^{2+} (SERCA) pumping. Calcium influx increases contractility and results in positive inotropic effects whereas removal prevents calcium overload and, thereby, limits negative inotropy. In addition, thyroid hormones increase protein kinase-mediated phosphorylation of the sarcoplasmic reticular protein phospholemman. Phosphorylation of phospholemman is reported to stimulate sarcolemmal sodium-potassium pumping, (Na^+/K^+ ATPase) activity. Na^+/K^+ ATPase activity limits excessive depolarization associated with increases in intracellular Na^+ and may thereby prevent the arrhythmias likely to be associated with depolarization (Bers et al., 2009). Adenylyl cyclase regulates cAMP mediated signaling and the cAMP in cardiomyocytes stimulates PKA mediated phosphorylation of proteins which produces positive inotropic effects (Movsesian, 2003). Desensitization of cardiac adenylyl cyclase signaling is known to occur in heart failure. Whereas such desensitization may protect against adrenergic overstimulation, it also limits positive inotropy by downregulating β-adrenoceptors and by upregulating both the β-adrenoceptor GRKs and the inhibitory alpha subunit of G-proteins (Gi). In turn, upregulation of β-adrenoceptor GRKs decreases cardiomyocyte cAMP and the downstream positive inotropic signaling effects of PKA (El-Armouche et al., 2003). Hence, β-adrenergic antagonists are very effective treatments for heart failures; however, it remains unclear whether β-adrenoceptor blockers resensitize the β-adrenergic receptor system (Lohse et al., 2003).

1.2. Hormonal and immunologic mediators of negative inotropy and heart failure

Angiotensin II (Ang II) is also an important modulator of myocardial inotropy and the inotropic effects of Ang II are mediated by a balance of positive and negative inotropic signaling mechanisms. Heart failure is characterized by chronic exposure to high circulating levels of Ang II wherein the inotropic balance favors negative inotropy linked to activation of p38- mitogen-activated protein kinase which is activated by environmental stressors such as pro-inflammatory cytokine (p38 MAPK) signaling. Ang II also plays a role

in cardiac remodeling involving hypertrophy and apoptosis mediated by Ca^{2+}/calmodulin dependent protein kinase II (CaMKII) (Palomeque et al., 2009). The sarcoplasmic reticular ryanodine receptor-2 Ca^{2+} release channel (RyR2) can be phosphorylated by several protein kinases such as PKA and CaMKII (Currie, 2009). Abnormal phosphorylation of RyR2 is also reported to contribute to the negative inotropy associated with heart failure. Although phosphorylation of RyR2 by CaMKII can produce positive inotropic effects, upregulated CaMKII activity may increase Ca^{2+} release from the sarcoplasmic reticulum, resulting in negative inotropy and cardiac arrhythmias (Currie, 2006). Negative inotropy and β-adrenergic receptor insensitivity may also result from production and release of the inducible isoform of nitric oxide synthase (iNOS) (Deswal et al., 2001). In addition, pro-inflammatory cytokines such as TNF-α appear to have the capacity to produce negative inotropy and cardiac remodeling whereby TNF-α (Levine et al., 1990) IL-6 (Kell et al., 2002) and IL-8 (Damas et al., 2000) are reported to induce apoptosis of cardiac myocytes. High circulating levels of IL-6 are associated with low cardiac muscle contractility and high levels of TNF-α have been found to be negatively correlated with a favorable clinical outcome of heart failure patients. The negative inotropy, remodeling (hypertrophy) and apoptosis associated with exposures to TNF-α seem to be mediated by activation of TNF receptor-1 (TNFR1) and inhibited by stimulation of TNF receptor-2 (TNFR2). Activity of both TNFR1 and TNFR2 are reported to be upregulated in heart failures (Chrysohoou e tal., 2009; Hamid et al., 2009). Stimulation of TNFR1 is shown to have the capacity to downregulate the rapid delayed-rectifier K^+ current (I_{Kr}) and, thereby, to delay cardiac repolarization, increasing the duration of the cardiac action potential in cardiomyocytes (Wang et al., 2004). Increased duration of the ventricular action potential is thought to be an attempt to maintain cardiac muscle contractility and limit the negative inotropy associated with heart failure. We have reported insensitivity to an agonist and to an antagonist of K_{ATP} channels, which would serve to limit the aforementioned increased duration of the ventricular action potential, in cardiomyocytes undergoing eccentric hypertrophic remodeling after volume overloading such as that which could occur during the development of heart failure associated with aortic valve regurgitation (Alvin et al., 2011). However, this increased duration of action potential also increases susceptibility to cardiac arrhythmia in hypertrophy-induced heart failure. Increased duration of the cardiac action potential is also observed in the TNF1.6 mouse model in which heart failure is produced by overexpression of TNF-α. Therefore, it seems that TNF-α induced increments in duration of the cardiac action potential and insensitivity to mechanisms that could limit the increased action potential duration may be important features of the hypertrophic remodeling associated with heart failures. Another mechanism for TNF-α induced negative inotropy is thought to result from overexpression of collagen and underexpression of the gap junction protein connexin-43 (Janczewski et al., 2004).

1.3. Intracellular transduction molecules, negative inotropy and heart failure

Myocardial infarction in mice together with estrogen receptor-β knockout is reported to increase the cardiomyocyte MAPK activities of the c-Jun, N-terminal kinase (JNK) activator protein-1 (AP-1) transcription factor component. These signaling molecules are activated by

environmental stressors such as pro-inflammatory cytokines in females and the MAPK extracellular signal-related kinases (ERK1 and ERK2 also known as MAPK3 and MAPK1, respectively), cell-proliferation, growth-promoting signaling pathway in males. Estrogen protection of the myocardium associated with upregulated PI3K and the original mouse-strain Ak, thymic lymphoma transforming (Akt) cell proliferation and anti-apoptosis signaling activities in female mice suggests deficiencies of ER-β and PI3K/Akt mediated anti-apoptosis signaling in such infarction induced heart failures (Wang et al., 2009). Cardiomyocyte protection by adrenomedullin, a vasoactive peptide discovered in pheochromacytomas, also appears to involve upregulated PI3K/Akt and MAPK/ERK signaling pathways (Yanagawa and Nagaya, 2007). The myocardial protection reported with ginseng and related ginsenoside treatments are likely to decrease negative inotropy and the duration of cardiac action potentials, arrhythmias, remodeling and apoptosis by interactions with these protein kinases (Maslov and Konkovskaia, 2009). We have reported that MAPK ERK and PI3K/Akt signaling molecules play important roles in eccentrically-hypertrophied rat hearts subjected to volume overload (Teos et al., 2008; Zhao et al., 2010; Alvin et al., 2011). Substantial evidence is, therefore, emerging that the protein kinases in cardiomyocytes are invaluable biomarkers for heart failure, as well as for the effectiveness of cardiac protection by novel heart failure treatments.

2. Epigenetics, chromatin condensation for transcription and protein kinases

Chromatin participating in the transcription of DNA to mRNA is represented in the nucleus of eukaryotic cells by euchromatin, a lightly staining, unfolded structure which facilitates binding to the DNA of various gene regulatory proteins and RNA polymerases which induce transcription. The euchromatin that is not transcribed is transformed into more tightly and intensely staining heterochromatin which appears to protect the genes while they are not being actively transcribed. According to the "accessibility hypothesis," the cell uses transformation from euchromatin to heterochromatin as a method of regulating the expression and replication of genes (Yancopoulos et al., 1986). Constitutive euchromatin is the portion that is always being actively transcribed, thereby representing "housekeeping genes" which encode proteins supporting the cell survival functions (*International Human Genome Sequencing Consortium*, 2004). The condensation of chromatin for transcription is regulated by epigenetic modifications made to DNA, histones and to other regulatory proteins (Martin and Cardoso, 2010).

Epigenetics at work in the normal heart is demonstrated by the regulation of cyclic AMP-dependent protein kinase (protein kinase A, PKA), a positive-inotropic agent in cardiac myocytes and related heart tissue cells. PKA induces relaxation of the histone-3 and histone-5 (H3 and H5) associated DNA segments which condense the chromatin for transcription of the structural and functional proteins supporting normal cardiomyocyte contractility (Marion et al., 1985).

2.1. Epigenetic marks for monitoring cardiovascular disease progression

A potential use of epigenetic modifications on histones and related DNA segments is to provide a code sensitive to the progression of diseases that are impacted by environment-gene interaction. An example of this usage of epigenetic marks has been demonstrated in patients with end-stage heart failure. Heart failure in these patients appears to be correlated with differential methylation of the DNA and histones at lysine residue 36 (H3K36me3) in their cardiomyocytes. (Movassagh et al., 2011). Another example of how epigenetic marks can be useful is reported in cardiac hypertrophy wherein mRNA expression of multiple cardiomyopathy-related genes was found to be associated with a unique, specific pattern of acetylation and methylation of histone H3 in the cardiomyocytes (Gaikwad et al., 2010).

3. Histone acetylation/methylation and cardiomyopathy

3.1. Repressor element-1 silencing transcription factor (REST) and cardiomyocyte hypertrophy

Cardiomyocyte hypertrophy is a compensatory response to maintain contractility and heart function. Inhibition of expression of the gene for repressor element-1 silencing transcription factor (REST), a fetal gene transcription repressor in adult ventricular myocytes (Schoenherr and Anderson, 1995), has been linked to cardiomyocyte hypertrophy (Bingham et al., 2007). Endothelin-1 is an endogenous vasoconstrictor molecule known to be a stimulator of cardiomyocyte hypertrophy (Harada et al., 1997; Drawnel et al., 2013). Treatment of cardiomyoblast (H9C2) cells with endothelin-1 is shown to inhibit REST and the amount of hypertrophy seems to be positively correlated with acetylation of histone H4 and methylation of histone H3 lysine 4 (H3K4) (Bingham et al., 2007). Endothelin-1 also appears to employ REST to increase expression of the genes for brain and atrial natriuretic peptides, hormones that help compensate for heart failure by decreasing the circulating blood volume (Bingham et al., 2007).

3.2. Histone deacetylase GATAD1 and cardiomyopathy

Autosomal-recessive dilated cardiomyopathy in a human family has been shown to be associated with abnormal expression and subcellular localization of GATAD1 (Theis et al., 2011). GATAD1 is a gene product that is highly expressed during embryonic development of the mouse eye (Kim et al., 2010) and is a regulator of gene expression in cardiomyocytes. GATAD1 appears to function as a histone deacetylase, evidenced by its binding to a histone modification site (Theis et al., 2011). Interestingly, dilated cardiomyopathy has been produced in mice following inhibition of histone deacetylases (Theis et al., 2011). Deletion of histone deacetylase-3 (HDAC3) in embryonic cardiomyocytes is associated with a nonlethal cardiac hypertrophy and cardiomyopathy induced while animals are fed a normal diet. However, more severe hypertrophy, cardiomyopathy and heart failure occurs when these animals are fed a high-fat diet, associated with downregulation of several genes which regulate lipid metabolism (Sun et al., 2011).

3.3. DNMT inhibition, gene methylation, cardiomyocyte differentiation and cardiomyopathy

Decreased availability of β-catenin is expected to decrease developmental Wnt pathway transcription, an effect shown to be associated with inhibition of cardiomyocyte hypertrophy and left ventricular remodeling in animal models of heart failure (Bergmann, 2010). DNA methyltransferases (DNMT) are epigenetic regulators of cell differentiation and metabolism and treatment of mesenchymal stem cells with the DNMT inhibitor and demethylating agent 5-aza-2′-deoxycytidine (5-Aza) is shown to induce expression of the cardiac tissue-specific genes Nkx2.5 and α-MHC. 5-Aza treatment also upregulates glycogen synthase kinase-β (GSK3β) and downregulates the cell adhesion protein β-catenin, whereas it increases expression of GSK3α. This upregulation of GSK3β also stimulates cardiomyocyte differentiation (Cho et al., 2009). That GSK3 inhibitors (e.g., lithium) were found to be effective at blocking this 5-Aza induced upregulation implies that GSK3 is required for cardiomyocyte differentiation. That downregulation of β-catenin is closely associated with GSK3β-stimulated cardiomyocyte differentiation (Cho et al., 2009) suggests an unclear relationship between β-catenin and GSK3β and a significant difference between the 5-Aza induced mechanisms for differentiation of mesenchymal stem cells and the DNMT-dependent mechanism for inhibiting cardiomyocyte hypertrophy, ventricular remodeling and heart failure.

3.4. Methylation of PKCε and heart failure

Epidemiological studies have suggested correlations between prenatal exposure to adverse intrauterine conditions and risk for ischemic heart disease, a primary cause of heart failure, in adulthood. Hypoxia is a fetal stress known to decrease expression of the PKCε gene and increased susceptibility to myocardial ischemia and reperfusion injury in adult rat offspring (Xue and Zhang, 2009). This predilection for myocardial ischemia is associated with increased methylation of the PKCε promoter at two fetal transcription factor specific protein-1 (Sp1) binding sites. Hypoxia seems to affect the binding of Sp1 only to methylated, and not to unmethylated, sites. However, methylation of both Sp1 sites seems to decrease PKCε binding to Sp1 in both fetal and adult cardiomyocytes (Xiong et al., 2012). The DNMT inhibitor 5-Aza has been shown to block the hypoxia-induced increase in methylation of both Sp1 binding sites and to restore PKCε mRNA and protein levels to normal. These effects appear to be greater in male than in female fetal hearts. Decreased expression of PKCε mRNA was found only in the male hearts, with interactions between both estrogen receptor proteins and the Sp1 binding site (Patterson et al., 2010). Moreover, activation of PKCε is reported to cause return of high susceptibility of adult rat hearts to ischemic injury following prenatal exposure to hypoxia (Patterson et al., 2010). PKCε is also an activator of ATP-activated potassium (K_{ATP}) channels, a metabolic energy conservation measure associated with reduced energy supply stress in cardiomyocytes (Kim et al., 2006). PKCε induced K_{ATP} activation is reported to shorten the cardiomyocyte action potential and limit calcium ion influx and overload in cardiac myocyte sarcolemmal and mitochondrial membranes (Inagaki et al., 2006). PKCε also seems to mediate ischemic preconditioning, a cardioprotective countermeasure against myocardial ischemia/infarction (Inagaki et al., 2006).

Epigenetically-induced decrements in expression of PKCε, associated with aberrant methylation patterns, are also reported in relation to heart failure following experimental exposures to cocaine and nicotine (Barik, 2007). Prenatal exposure to cocaine is reported to downregulate PKCε expression in hearts, effects which persist into adulthood and increase susceptibility to myocardial ischemia and infarction and reperfusion injury (Zhang et al., 2007). Cocaine treatment of the fetal rat heart for as little as 48 h produces significant decrements in PKCε protein and mRNA expression, associated with increases in CpG methylation at two binding sites of the Sp1 in the PKCε promoter region, confirmed by the use of the DNMT inhibitor 5-Aza or procainamide to inhibit this methylation (Meyer et al., 2009). Moreover, cocaine administered to pregnant rats during gestational days 15-20 produced offspring whose fetal hearts had significantly decreased expression of PKCε mRNA and protein. We have shown that IGF-1 antagonizes the positive-inotropic effect of Ang II in atrial myocytes of aging male rats and that prenatal exposure to cocaine abolishes this response (Haddad et al., 2005), an effect that could also be mediated by decreased expression of PKCε. In addition, methylation of CpGs at the binding sites for activator protein-1 (Ap1), a human protein transcription factor, was decreased in these fetal hearts and was increased significantly by prenatal exposure to cocaine (Zhang et al., 2007). The CpG dinucleotides at Bhlhb2, Pparg, E2f, and Egr1 binding sites of the PKCε gene promoter are reported to be densely methylated in both male and female adult rats and prenatal exposure to cocaine appears to decrease methylation of the CpG dinucleotides at the two Sp1 binding sites and this binding was increased significantly by cocaine exposure, but only in the male offspring (Zhang et al., 2009).

Interestingly, prenatal exposure to nicotine, which increases release of norepinephrine, is also associated with inhibition of PKCε gene expression in the fetal rat heart, an effect which, like cocaine, persists into adulthood (Lawrence et al., 2008). This finding appears to link maternal smoking to heart failure in the offspring. Nicotine treatment of pregnant rats starting at day 4 of gestation is shown to increase methylation of the Egr-1 binding site at the PKCε gene promoter and decrease PKCε protein and mRNA expression in fetal hearts (Lawrence et al., 2011). Moreover, methylation of the Egr-1 binding site decreases Egr-1 binding to the PKCε promoter and cardiomyocyte deletion of the Egr-1 binding site decreases PKCε promoter activity and treatment of isolated fetal hearts with norepinephrine produces similar effects including increased Egr-1 binding site methylation and inhibition of PKCε gene expression. The DNMT inhibitor 5-Aza inhibited the norepinephrine-induced increase in methylation of the Egr-1 binding site and restored both Egr-1 binding and PKCε gene expression, implicating sympathetic neural overactivity in this nicotine-induced heart failure (Lawrence et al., 2011).

3.5. TNF-α methylates SERCA genes and inhibits cardiac relaxation

The aforementioned epigenetic mechanisms for heart failure are relevant mainly to the negative inotropy associated with heart failure resulting from systolic dysfunction and the majority of current treatments at increasing contractility to counteract systolic dysfunction. However, heart failure associated with diastolic dysfunction may be more common and most heart failure is, therefore, resistant to current treatments and may contribute to ethnicity-related predilections for heart failure in specific populations such as African-Americans (Khan

et al., 2012). The focus of diastolic dysfunctional heart failure is an inability of cardiomyocytes to relax, a function critical to ventricular filling and, according to Starling's law of the heart, maintenance of contractility and stroke volume. In that regard, treatment with the pro-apoptotic cytokine tumor necrosis factor-α (TNF-α) is reported to methylate and decrease the cardiomyocyte sarcoplasmic reticulum calcium pump (SERCA-2A) gene activity, mRNA expression and protein transcription, thereby providing an epigenetic mechanism for diastolic heart failure (Kao et al., 2010). The promoter region of the SERCA-2A gene has a high content of CpG islands and treatment with the antihypertensive, smooth muscle relaxer apresoline, commonly used in cases of human heart failure, is reported to improve cardiac function in rat hearts by the mechanism of demethylating and increasing SERCA-2A gene expression of mRNA and protein and, thereby, improving cardiac relaxation, a key factor in heart failure associated with diastolic dysfunction (Kao et al., 2011).

3.6. Methylation patterns, cardiomyocyte apoptosis and cardiomyopathy

Aberrant methylation patterns also appear to play roles in myotonic dystrophy, a group of multisystemic diseases characterized by cardiac conduction abnormalities and CTG expansions. These CTG expansions are found to be substantially larger in cardiomyocytes than in cells of other affected tissues. The expanded repeat is reported to be a genetic biomarker for methylation wherein the CpG-free expanded CTG repeat appears to maintain a distinct pattern of CpG methylation at the myotonic dystrophy (DM1) locus, which varies markedly with age and tissue-specificity (López Castel et al., 2011). Promoter DNA methylation and Sp1 binding are also reported to be regulators of expression of human myotonic dystrophy kinase-related Cdc42 binding kinases (MRCKs) and family members of human myotonic dystrophy kinase (DMPK), RhoA-binding kinase(ROK), and citron kinase (Ng et al., 2004). These findings demonstrate an important interaction between epigenetic methylation marks (CpG-free expanded CTG repeats) and a genetic skeletal muscle disease that often produces cardiac anomalies.

Epigenetic mechanisms are also apparent in diabetic cardiomyopathy, another common cause of heart failure. Tumor suppressor protein p53-induced expression of the cyclin dependent kinasae inhibitor p21(WAF1/CIP1) is a mediator of cell cycle arrest at the G1 phase (Harper et al., 1993). When cardiomyocytes from diabetic patients and rats are subjected to oxidative stress, the change in methylation pattern and overexpression of p21(WAF1/CIP1) appears to contribute to cardiomyopathy by inducing cardiomyocyte apoptosis (Mönkemann et al., 2002).

Methylation of the insulin receptor IGF-1 and IGF-1 receptor genes at their promoter regions in cardiomyocytes of diabetic (db/db) mice is reported to be substantial and may, therefore, function as epigenetic markers for the development of diabetic cardiomyopathy. Unfortunately, their methylation patterns seem to be inadequate for differentiating db/db diabetic cardiomyocytes from those of normal control animals (Nikoshkov et al., 2011). On the other hand, the methylation patterns of three angiogenesis-related genes (angiomotin-like-2, Rho GTPase activating protein-24 and platelet/endothelial cell adhesion molecule-1) is reported to be able to differentiate left ventricular cardiomyocytes of normal control subjects from those of patients diagnosed with idiopathic end-stage cardiomyopathy (Movassagh et al., 2010).

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3.7. Acetylation, cardiomyocyte survival and stress-apoptosis signaling

Stress and cardiomyocyte hypertrophy are associated with lysine acetylation of many different proteins, including histones. Lysine 53 of p38 mitogen-activated protein kinase (MAPK) appears to be acetylated and activated by histone deacetylase-3 (HDAC3) which increases the affinity of p38 MAPK for ATP, in parallel with decreased intracellular ATP concentrations in cardiomyocytes undergoing stress and hypertrophy (Pillai et al., 2011). In hypertrophied cardiomyocytes, phosphorylation of HDACs, specifically those shuttled between nucleus and cytoplasm by HDAC kinases, is associated with disinhibition of various transcription factors such as nuclear factor of activated T-cells and myocyte enhancer factor-2 (MEF2). On the other hand, the antioxidant thioredoxin 1 (Trx1) is shown to inhibit cardiomyocyte hypertrophy by decreasing cysteine residues in HDACs (Oka et al., 2009), thereby demonstrating a putative HDAC-dependent epigenetic mechanism for oxidative stress in cardiac hypertrophy and associated heart failure.

3.8. Lysine acetylation, shear stress and endothelial cell apoptosis

Blood vessel wall stress appears to be a potent epigenetic activator of sarcomeric protein transcription in vascular smooth muscle cells, a likely environment-gene interaction contributor to hypertension, the hallmark of cardiovascular disease and an important precursor to heart failure. In that regard, blood vessel wall shear stress is shown to enhance the lysine acetylation of histone H3 at position 14 (K14), as well as serine phosphorylation at position 10 (S10) and lysine methylation at position 79 (K79), and cooperated with trichostatin (TSA), an antifungal agent that is also an HDAC inhibitor, inducing acetylation of histone H4 and phosphoacetylation of S10 and K14 of histone H3. In addition, endothelial stem cells exposed to shear stress appear to strongly activate transcription from the vascular endothelial growth factor (VEGF) receptor 2 promoter. This effect was paralleled by an early induction of smooth muscle actin, smooth muscle protein 22-alpha, platelet-endothelial cell adhesion molecule-1, VEGF receptor-2, myocyte enhancer factor-2C (MEF2C), and α-actin. Moreover, the transcription factors MEF2C and Sma/MAD homolog protein 4 were isolated from shear stress-treated embryonic stem cells complexed with the cAMP response element-binding protein acetyltransferase (Illi et al., 2005). HDAC3 is known to play a crucial role in the differentiation of endothelial cells (Spallotta et al., 2010). In mature endothelial cells, HDAC3 appears to be a pro-survival molecule by activating the Akt intracellular signal transduction pathway (Zampetaki et al., 2010) and when HDAC3 is downregulated in apolipoprotein E-knockout mice, atherosclerosis and rupture is found in the isografted vessels (Zeng et al., 2006). However, at aortic bifurcations, where the endothelial cells are highly susceptible to atherosclerosis, HDAC3 expression is increased with phosphorylation of HDAC3 at serine/threonine. HDAC3 appears to bind to Akt and upregulates HDAC3 to increase phosphorylation of Akt in these endothelial cells. Increased Akt kinase activity combined with knockdown of HDAC3 could, therefore, promote both atherosclerosis and endothelial cell apoptosis by epigenetic mechanisms (Zampetaki et al., 2010). Such epigenetic mechanisms for endothelial dysfunction appear to be important, yet poorly understood, contributors to cardiovascular disease

which is found to be correlated with ethnicity and may, therefore, help explain health and disease disparities such as the predilections for hypertension and heart failure in African-Americans (Khan et al., 2012).

A number of studies, during the past decade or so, have shown that HDAC inhibitors may be effective as inhibitors of cardiomyocyte hypertrophy. Class I HDACs are shown to inhibit cardiomyocyte hypertrophy associated with inhibition of the gene encoding dual-specificity phosphatase 5 (DUSP5) DUSP5, a nuclear phosphatase that downregulates hypertrophic signaling by ERK1/2 (Ferguson et al., 2013). Expression of DUSP5 in cardiomyocytes produces inhibition of hypertrophy resulting from administering prohypertrophic factors (Ferguson et al., 2013). Inhibition of DUSP5 by class I HDACs requires activity of both ERK and MAPK, that has been characterized as a self-reinforcing mechanism for promotion of cardiac ERK signaling (Ferguson et al., 2013). Moreover, in cardiomyocytes treated with highly selective class I HDAC inhibitors, nuclear ERK1/2 signaling is suppressed in a manner that is shown to be dependent on DUSP5. Research on HDAC inhibitors as therapeutic agents for heart failure has focused on the left ventricle. However, there is also research demonstrating that HDAC inhibitors may also be able to treat right heart failure by mechanisms that decrease pulmonary arterial smooth muscle contractility. A specific benzamide class I HDAC inhibitor, MGCD0103 is shown to decrease pulmonary arterial pressure more dramatically than the type 5 cGMP phosphodiesterase inhibitor and vasodilator tadalafil, a standard-of-care therapy for human pulmonary hypertension (Cavasin et al., 2012). Although this class I HDAC inhibitor only modestly reduced right ventricular hypertrophy, it had multiple beneficial effects on the right ventricle, which included suppression of pathological gene expression, inhibition of proapoptotic caspase activity, and repression of proinflammatory protein expression (Cavasin et al., 2012).

4. Therapeutic potential of HDAC inhibitors

Although the findings that cardiomyocytes expressing an HDAC mutation lack phosphorylation sites and are refractory to the development of hypertrophy (Zhang et al., 2002), mutant mice lacking the class II HDAC HDAC9 do exhibit stress-induced cardiomyocyte hypertrophy (Antos et al., 2003). These findings, although somewhat confounding, at least, suggest that modulating specific kinase activities could be key to elucidating the mechanisms of cardiac hypertrophy, as well as to developing novel heart failure treatments. Class II HDACs are known to inhibit the activity of myocyte enhancer factor-2, a mediator of responsiveness to environmental stressors and cardiac hypertrophy by binding to a stress-responsive kinase that is specific for conserved serines. (Zhang et al., 2002). The antiepileptic drug valproic acid is a class II HDAC inhibitor shown to inhibit the catalytic activity of HDAC6 (Lemon et al., 2011). Both HDAC6 and HDAC8 are apparently upregulated in experimental animal models of salt (DOCA)-sensitive hypertension and valproic acid is also shown to be an inhibitor of cardiac hypertrophy in hypertensive rats (Kee et al., 2013). PKC signaling is shown to support nuclear export of class II HDAC5 in cardiomyocytes and inhibition of PKC prevents shuttling of HDAC5 from nucleus to cytosol by a chromosome region maintenance protein (CRM1, also known as transportin-1) in response to a number of agents that promote cardiomyocyte hypertrophy (Vega et al., 2004). An HDAC5 mutant that cannot be phosphorylated is also

refractory to PKC signaling and inhibits cardiomyocyte hypertrophy mediated by PKC agonists. These findings are consistent with those that demonstrate protein kinase D (PKD) phosphorylation and enhancement of HDAC5 nuclear export because PKD is a downstream effector of PKC (Vega et al., 2004). HDAC5 is only one of several transcriptional regulators of pathological cardiac hypertrophy the activities of which are regulated by subcellular distribution. For example, transcription factors belonging to the nuclear factor of activated T cells (NFAT) and GATA families of transcription factors that specifically bind to the GATA sequences of DNA are subject to CRM1-dependent nuclear export (Harrison et al., 2004). These pro-hypertrophic proteins are rapidly relocalized to the nucleus in response to pro-hypertrophic growth signaling (Harrison et al., 2004). CRM1 activity is, apparently, not required for the normal cardiomyocyte gene activation mediated by thyroid hormones (T3, T4) and IGF-1, agonists that fail to trigger the nuclear export of HDAC5. These results suggest a selective role for CRM1 in disinhibition of pathological cardiac hypertrophy and that targeting CRM1-dependent nuclear export in cardiomyocytes may ameliorate stress induced cardiomyocyte hypertrophy and provide novel therapeutic strategies for heart failure (Harrison et al., 2004).

5. Summary and conclusions

Heart failure is the result of complex environment-gene interactions between regulators of intracellular transduction molecules, intracellular/extracellular receptors and endocrine/ paracrine/ autocrine hormone-like signaling molecules. Figure 1 summarizes the main epigenetic mechanisms of cardiomyocyte hypertrophy, negative inotropy and heart failure. β-adrenoceptor activation of PKA is shown to interact directly with histones and related DNA segments at promoter regions of genes for mRNA transcription to support normal cardiac contractility. Prenatal exposures to hypoxia, cocaine or nicotine produces predilections for heart failure shown to persist into adulthood, associated with increased methylation and decreased expression of PKCε. Norepinephrine is also shown to decrease expression of PKCε, thereby implicating sympathetic overactivity in an epigenetic mechanism for cardiomyocyte hypertrophy and heart failure. Inhibition of a cardiomyocyte differentiation signaling molecule REST by endothelin-1 is shown to contribute to cardiomyocyte hypertrophy. An autosomal-recessive dilated cardiomyopathy is associated with HDAC inhibition produced by upregulation of another differentiation signaler, GATAD1. Hypermethylation of SERCA-2A gene by the pro-inflammatory cytokine TNF-α is shown to produce diastolic dysfunction, a common cause of heart failure in African-Americans and particularly resistant to current treatments. Lithium-induced inhibition of DNMTs appears to induce hypomethylation which upregulates GS3Kβ, associated with downregulation of β-catenin and inhibition of Wnt pathway signaling for differentiation, providing a mechanism for inhibiting cardiac hypertrophy and heart failure. HDAC-dependent mechanisms for stimulating stress-apoptosis pathway signaling and inducing cardiac hypertrophy have been demonstrated in cardiomyoctes, endothelial cells and vascular smooth muscle cells. These findings demonstrate multiple roles of histone and related DNA acetylations and methylations for regulating cardiomyocyte contractility. These epigenetic mechanisms provide insights that could be translated to novel clinical interventions for the prevention and treatment of heart failure.

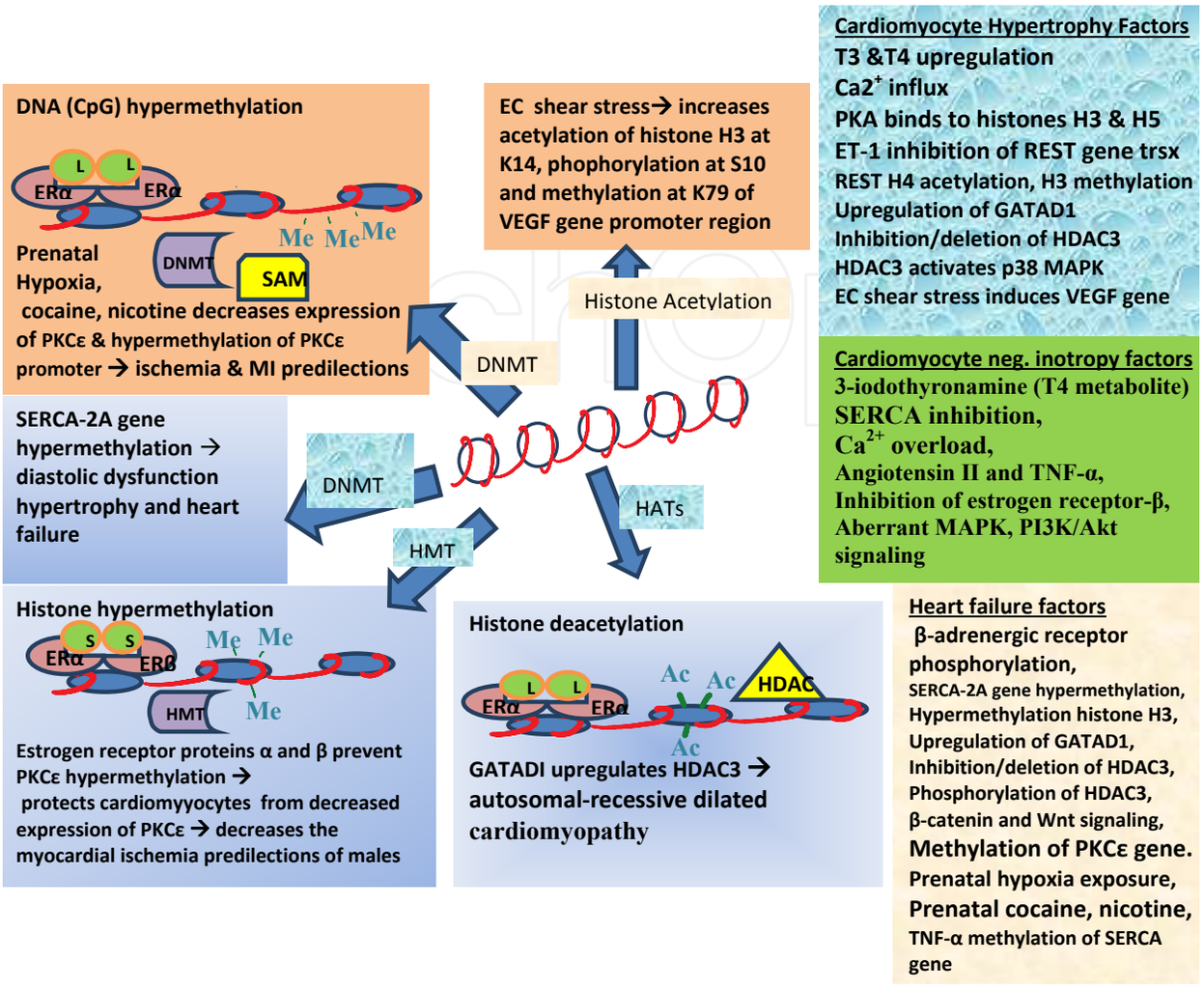

Figure 1. Main mechanisms for epigenetic alterations in heart failure. Each alteration involves many enzymes but the main players to cause methylation or acetylation are shown by arrows. These are not separate mechanisms and the enzymes do not act alone. Several enzymes act at a promoter simultaneously. 1) DNA methylatrasferase (DNMT) upregulation with S-adenosyl methionine (SAM) methylates the CpG islands at the PKCε promoter region which increases susceptibility of neonatal and adult cardiomyocytes to ischemia after prenatal exposure to hypoxia, cocaine or nicotine. 2) Upregulation of DNMTs is also associated with SERCA-2A gene hypermethylation which increases diastolic dysfunction and susceptilibity to heart failure. 3) Estrogen receptor binding to Sp1 fetal transcription factor binding sites (S) upregulates histone methyltransferases (HMTs) which protects cardiomyocytes from downregulation of PKCε expression. 4) Histone deacetylase-3 (HDAC3) upregulation is associated with expression of GATAD1, a fetal transcription factor, associated with autosomal-recessive dilated cardiomyopathy in humans and in animal models of cardiomyopathy and heart failure. 5) Histone acetylases (HACs) and histone methyltransferases (HMTs) are upregulated at a vascular endothelial growth factor (VEGF) promoter region by exposure of adult endothelial cell (EC) and embryonic stem cells to shear stress. Cardiomyocyte pro-hypertrophy factors include thyroid hormones (T3, T4), Ca2+ overload, protein kinase A (PKA) binding to histones, endothlein-1 (ET-1) inhibition of the gene for repressor element-1 silencing transcription factor (REST-1), REST gene acetylation at histone H4 and methylation at histone H3, upregulation of fetal transcription factor GATAD1, inhibition of HDAC3 and activation of p38 MAPK.

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