Biochimica et Biophysica Acta 1832 (2013) 2414–2424

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbadis

Review

Regulation of fetal gene expression in heart failure ☆

Ellen Dirkx [a,b], Paula A. da Costa Martins [a], Leon J. De Windt [a,*]

[a] Dept of Cardiology, CARIM School for Cardiovascular Diseases, Faculty of Health, Medicine and Life Sciences, Maastricht University, The Netherlands
[b] ICIN-Netherlands Heart Institute, Royal Netherlands Academy of Sciences, Utrecht, The Netherlands

ARTICLE INFO

Article history:
Received 3 January 2013
Received in revised form 15 July 2013
Accepted 23 July 2013
Available online 10 September 2013

Keywords:
Gene regulation
Transcription factors
Epigenetics

ABSTRACT

During the processes leading to adverse cardiac remodeling and heart failure, cardiomyocytes react to neurohumoral stimuli and biomechanical stress by activating pathways that induce pathological hypertrophy. The gene expression patterns and molecular changes observed during cardiac hypertrophic remodeling bare resemblance to those observed during fetal cardiac development. The re-activation of fetal genes in the adult failing heart is a complex biological process that involves transcriptional, posttranscriptional and epigenetic regulation of the cardiac genome. In this review, the mechanistic actions of transcription factors, microRNAs and chromatin remodeling processes in regulating fetal gene expression in heart failure are discussed.

1. Introduction

Heart failure is a serious clinical disorder that represents the primary cause of hospitalization and death in Europe and in the United States. In numerous types of heart diseases including ischemic diseases, hypertension, aortic stenosis, valvular dysfunctions and genetic forms of cardiomyopathies, hypertrophic remodeling is a common observable process. Sustained pathological hypertrophy still represents the major clinical predictor of heart failure and sudden cardiac death in humans [1,2].

At the cellular level, pathological hypertrophy can be largely regarded as the response of cardiomyocytes to biomechanical stress, including pressure or volume overload, reactive oxygen species, cytokines, and circulating neurohormones, which all can activate an intrinsic web of interconnected signaling modules within cardiomyocytes. Many of the stress signaling pathways culminate in the nucleus with the activation of a set of transcription factors, co-regulators, and microRNAs (miRNAs, miRs) [3,4], leading to alterations in cardiac gene expression. The molecular changes observed during the process of pathological hypertrophy resemble those observed during fetal cardiac development, and therefore cardiac hypertrophy is often described as being accompanied by the reactivation of a "fetal gene program" [5–7]. Although elements of this program might be salutary adaptations to stress, it has become increasingly clear that the aberrant expression of fetal genes involved in contractility, calcium handling, and myocardial energetics leads to maladaptive changes in cardiac function. Activation of the "fetal gene program" is believed to play a causative role in adverse cardiac remodeling and the pathogenesis of heart failure, both in humans and in mouse models [8]. Interestingly, upon treatment of heart failure patients, improvement of ventricular function is often accompanied with decreased expression of cardiac fetal genes in patients before and after treatment with beta-blockers [9] and before and after mechanical unloading with ventricular assist devices [10–12]. Although mechanical unloading in combination with current pharmacotherapies has shown effectiveness in prolonging survival of heart failure patients, the prognosis of affected individuals remains poor. More insight in the molecular mechanisms underlying the pathogenesis of this disease is still a prerequisite to design pharmacotherapeutic strategies that may directly counter the less salutary aspects of the "fetal gene programs". Additionally, molecular markers that track the fetal gene program could serve as useful clinical biomarkers of heart failure progression. Indeed, circulating NT-proBNP, derived from activation of the human *NPPB* gene, has already become a standard biomarker in this area [13]. Here, we will provide an overview of the mechanistic link between gene regulation in the developing heart and gene regulatory paradigms in the adult failing heart at the transcriptional, epigenetic and posttranscriptional level as illustrated in Fig. 1.

2. Transcriptional regulation of the fetal gene program

Transcription factors have been demonstrated to play an important role in embryonic development of the heart. In humans, mutations of several transcription factors have been associated with a variety of congenital heart diseases including atrial septal defect, ventricular septal defect, tricuspid valve abnormalities and atrioventricular block (Table 1). Although NKX2-5, and GATA4 are among the most studied cardiac transcription factors implicated in patients with congenital heart disease (CHD), the transcriptional regulators described in the

☆ This article is part of a Special Issue entitled: Heart failure pathogenesis and emerging diagnostic and therapeutic interventions.

* Corresponding author.
E-mail address: l.dewindt@maastrichtuniversity.nl (L.J. De Windt).

0925-4439/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.bbadis.2013.07.023

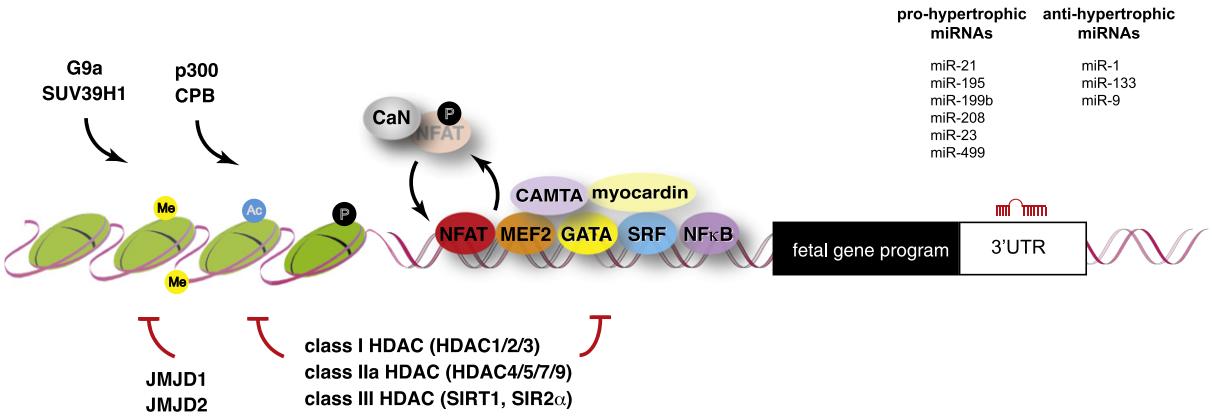

Fig. 1. Simplified overview of the gene regulatory mechanisms underlying re-expression of fetal gene programs in the adult myocardium. Epigenetic regulators influence chromatin condensation surrounding fetal genes, including the methylation status of chromosomal DNA and methylation, acetylation and phosphorylation status of histones. In addition, several transcriptional regulators drive direct activation of fetal gene programs. In turn, some of the transcription factors are regulated by histone acetyl transferases (HATs) and histone deacetylases (HDACs). Finally, post-transcriptional mechanisms by specific miRNA species have been demonstrated to directly influence fetal gene programs and cardiac hypertrophy.

sections below play roles in both cardiac development and hypertrophic remodeling of the adult failing heart.

2.1. Nuclear factor of activated T-cells (NFAT)

The calcineurin-regulated NFAT transcription factor family consists of four members, NFATc1–c4. Upon dephosphorylation by calcineurin, the NFAT transcription factor members translocate to the nucleus and bind a consensus site consisting of (A/T)GGAAA [14]. During cardiogenesis, calcineurin-NFATc1 signaling is expressed in the murine endocardium and second heart field and plays a major role in valve elongation and semilunar valve development [15]. Using PCR amplification and DNA sequencing is has been shown that differential duplication of an intronic region in the NFATc1 gene is associated with ventricular septal defects (Table 1) [16].

In the postnatal myocardium, NFAT is known to regulate the expression of "fetal genes", including brain natriuretic peptide (BNP, *Nppb*), the cardiac metabolic gene adenylosuccinate synthetase 1 (*Adss1*) and CnAβ (*Ppp3cb*), which are direct transcriptional targets of NFAT, activated in synergy with GATA4 [17,18]. Also in cultured cardiomyocytes, upon calcineurin or agonist stimulation, dominant negative NFAT reduced ANF (ANF, *Nppa*) expression [19,20]. Furthermore, inhibition of the transient receptor potential cation channel TRPC6 reduces AngII-induced hypertrophy and NFAT activity in cardiomyocytes [21]. In mice overexpressing TRPC6 in the heart, hypertrophic remodeling was induced, accompanied by increased activation of calcineurin/NFAT signaling [22]. In human heart failure, TRPC6 was enhanced, indicating the importance of these channels during cardiac pathology. Interestingly, in the TRPC6 promoter region, two functional NFAT binding sites have been reported [22]. Thus, TRPC6 induces calcineurin/NFAT signaling, which in turn again regulates expression of TRPC6, thereby creating a positive feedback mechanism. Another NFAT target gene is *Rcan1.4*, harboring 15 consensus NFAT binding sites in the nucleotide sequence upstream of exon 4 [23]. Depending on its phosphorylation status and expression levels, *Rcan1* can both enhance and decrease calcineurin/NFAT activity [24–27].

In addition, NFAT also has been shown to regulate expression of miRNAs. For example, it has been reported that *miR-199b* is a direct downstream target of the NFATc2 isoform in the heart [28]. MiR-199b targets Dyrk1a, a nuclear kinase involved in NFAT phosphorylation. Likewise, miR-23 is a direct NFATc3 target and is induced upon cardiac hypertrophy [29]. This miRNA was shown to target MUscle specific Ring Finger protein (MuRF1) and Foxo3a, both of which are independently involved in hypertrophic remodeling. Furthermore, deletion of miR-23 attenuated cardiac hypertrophy [30,31].

Table 1
Mutations in cardiac transcription factors associated with congenital heart disease.

Transcription factor	Associated CHD phenotype	References
NFATC1	VSD	[16]
MEF2A	CAD	[35]
GATA4	ASD, AVSD, VSD, PS, AR, VPS, PDA, TOF, AF	[49–65]
GATA6	PTA, TOF, ASD	[70–72]
NKX2-5	ASD,VSD, AVSD, TOF, SVAS, LVNC, PA, PS, PDA, MV anomalies, conduction defects, DORV, PAPVR, TAPVR, heterotaxy, TGA	[64,65,78–91]
NKX2-6	PTA	[92]

Abbreviations used: AF, atrial fibrillation; AR, aortic regurgitation; ASD, atrial septal defect; AVSD, atrioventricular septal defect; CAD: coronary artery disease; DORV, double outlet right ventricle; LVNC, left ventricular noncompaction; MV, mitral valve; PA, pulmonary atresia; PAPVR, partial anomalous pulmonary venous return; PDA, patent ductus arteriosus; PS, pulmonary stenosis; PTA, persistent truncus arteriosus; SVAS, supravalvar aortic stenosis; TAPVR, total anomalous venous return; TGA, transposition of the great arteries; TOF, tetralogy of Fallot; VSD, ventricular septal defect.

2.2. Myocyte enhancer factor 2 (MEF2)

The MEF2 family of transcription factors is encoded by the four genes MEF2A–D. MEF2 proteins bind the DNA sequence CAT(A/T)$_4$TA(G/A) as homo- or heterodimers. Although MEF2A through MEF2D are expressed in many types of cells, their specific functions are assigned to transcriptional regulation in the immune system, neurons, and striated muscle. At E7.5, MEF2B and MEF2C are initially expressed in the cardiac mesoderm, followed a day later by expression of MEF2A and MEF2D. MEF2C transcripts are detected in the somites at E8.5, concomitant with the onset of myocyte differentiation in the myotome. Targeted disruption of MEF2C has been shown to evoke death at E9.5 due to arrested cardiac looping and right ventricular formation during murine embryogenesis [32]. Also, systematic mutational screening of the entire MEF2A gene identified a 21-base pair deletion in exon 11 in patients with an autosomal dominant form of coronary artery disease, *adCAD1* [33].

In the postnatal heart, MEF2 genes play a role in hypertrophic remodeling. The MEF2-binding A/T-rich DNA sequences have been identified within the promoter regions of a number of fetal and cardiac genes involved in contractility including muscle creatine kinase gene (*Ckm*), α-myosin heavy chain (*Myh6*), myosin light chain 1/3 (*Myl1*), myosin light chain 2v (*Myl2*), skeletal α-actin (*Acta1*), sarcoplasmic reticulum Ca^{2+}-ATPase (*Atp2a2*), cardiac troponin T (*Tnnt2*), cardiac troponin C (*Tnnc1*), cardiac troponin I (*Tnni3*), desmin (*Des*), and dystrophin (*Dmd*). Accordingly, forced expression of MEF2A, MEF2C and MEF2D in transgenic mice proved sufficient to drive intolerance to pressure overload, ventricular chamber dilation and contractile dysfunction

[34–36]. Transgenic mice expressing a dominant-negative MEF2C showed attenuated postnatal myocardial growth in calcineurin-induced hypertrophy. Furthermore, it has been shown that the depletion of MEF2C by siRNA attenuates both the hypertrophic cardiac growth and the upregulation of *Nppa* in response to pressure overload [37]. Also, in dominant-negative MEF2D mice, no cardiac hypertrophy, fibrosis or fetal gene activation was observed in response to pressure overload. Together, these data demonstrate a role for MEF2 transcription factors in both cardiac development and pathological hypertrophy.

2.3. GATA-binding proteins

GATA transcription factors are characterized by the conserved double zinc fingers that are required for binding to the specific consensus DNA sequence (A/T)GATA(A/G). There are 6 GATA transcription factors known, among which GATA4, GATA5 and GATA6 are expressed in the heart with an overlapping pattern [38–40], and interact with each other in outflow tract development and cardiac morphogenesis in the developing embryo. GATA4 is highly expressed in cardiac myocytes, endocardial and epicardial cells of the heart, and is crucial for normal cardiac development, as mouse embryos lacking GATA4 die by E8.5 with defective heart tube morphogenesis from a lack of ventral folding [41,42]. Inactivation of GATA4 in endothelial cells causes failure of endocardial cushion formation and remodeling, which leads to embryonic lethality by E12.5 [43]. Select deletion of GATA4 in cardiomyocytes results in myocardial thinning and hypoplastic endocardial cushions during embryonal development [44]. Functional analysis of the cis-regulatory elements has revealed that by binding to promotor sequences, GATA4 directly regulates expression of several cardiac-specific genes, such as *Myh6*, *Myl1*, *Tnnc1*, *Tnni3*, *Nppa*, *Nppb*, cardiac-restricted ankyrin repeat protein (*Ankrd1*), cardiac sodium–calcium exchanger (*Slc8a1*), cardiac M2 muscarinic acetylcholine receptor (*Chrm2*), A1 adenosine receptor (*Adora1*), and carnitine palmitoyl transferase I β (*Cpt1b*) [44]. Patients with deletions in the GATA4 locus display several severe forms of congenital heart disease, including atrioventricular septal defects, aortic regurgitation, outflow tract alignment defects, dextrocardia, tetralogy of Fallot, patent ductus arteriosus and pulmonary stenosis [45–58]. Linkage analyses demonstrated an association between GATA4 mutations and multiple cardiac defects including, atrial septal defects and ventricular septal defects [59–61].

In the adult heart, cardiac-specific overexpression of GATA4 induces hypertrophic remodeling [62]. Furthermore, expression of a dominant negative GATA-engrailed fusion protein or antisense GATA4 mRNA each blocked GATA4-directed features of cardiac hypertrophy induced by phenylephrine and endothelin-1 in culture [62]. GATA4 is also directly phosphorylated by extracellular signal-regulated kinase 1/2 (ERK1/2) and p38 mitogen-activated protein kinase (p38 MAPK) as a means of enhancing hypertrophic gene expression. Knock-in mice in which a known MAPK phosphorylation site at serine 105 (S105) in GATA4 that augments activity was mutated to alanine (homozygous GATA4-S105A mutant mice), showed a compromised stress response of the myocardium [63]. Cardiac hypertrophy in response to phenylephrine agonist infusion for 2 weeks was largely blunted in GATA4-S105A mice, as was the hypertrophic response to pressure overload at 1 and 2 weeks of applied stimulation. Also in these GATA4-S105A mice, down-regulation of fetal genes such as *Nppb*, *Nppa* and *Myh7* was observed [63]. Furthermore, deletion of GATA4 specifically in cardiac myocytes by using a Cre-loxP approach resulted in spontaneous heart failure with aging, and younger adult targeted mice subjected to stress stimulation failed to mount an effective hypertrophic response. Gene expression profiling revealed up-regulation of the fetal genes *Nppb*, *Nppa* and *Myh7* as well as genes associated with apoptosis and fibrosis [64,65]. These studies suggest that GATA4 is a crucial regulator of adaptive cardiac growth in response to pathologic and even physiologic stress stimulation by activating fetal genes.

GATA6 also participates in postnatal heart disease and GATA6 mutations have been shown to be associated with atrial septal defects, tetralogy of Fallot and persistent truncus arteriosus [66–68]. GATA6-deleted mice develop less cardiac hypertrophy following angiotensin II/phenylephrine infusion, while GATA6 overexpression induces hypertrophy with aging and are predisposed to greater hypertrophy with pressure overload stimulation [69]. Also, $GATA6^{-/-}$ hearts showed a greater induction of mRNA for fetal genes associated with heart failure and stress stimulation. Combinatorial deletion of GATA4 and GATA6 from the adult heart resulted in dilated cardiomyopathy and lethality by 16 weeks of age [69]. GATA5 is able to interact with GATA4 and GATA6, but whether GATA6 alone or in combination with GATA4 or GATA5 regulates the hypertrophic response in the adult heart is not known yet and requires additional research [70].

2.4. Cardiac homeobox transcription factor Csx/NKX2-5

The official name of NKX2-5 is NK-2 transcription factor related, locus 5 (also known as Csx). This name refers to the grouping of homeodomain-containing proteins of 20 different classes, one of which is the NK-2 class. Within the NK-2 genes expressed in vertebrates, a subset, referred to as the “cardiac group”, includes NKX2-3, NKX2-5, NKX2-6, NKX2-7, NKX-8, and NKX2-10. The homeodomain of NKX2-5 has a helix-turn-helix motif that binds to the DNA consensus sites 5′-TNAAGTG-3′ and 5′-TTAATT-3′. Amongst the NK-2 genes, NKX2-5 plays a crucial role during embryonic heart development. NKX2-5 is expressed in the heart throughout life and it is the earliest known marker for myocardial progenitor cells. During embryogenesis in the mouse heart, NKX2-5 transcripts commit to the cardiac lineage and are first detected at the end of gastrulation, where they are highly expressed in myocardial progenitor cells [71–73]. Although NKX2-5 knock-out mice start to form a heart and express a variety of cardiac specific genes, they fail to progress much beyond the linear heart tube stage. The heart of NKX2-5 mutant mouse embryos does not loop, nor develop endocardial cushions nor trabeculae [73]. Mice carrying NKX2-5 mutations have phenotypes that include compromised physiology, morphology, and function of the heart. Also in humans, mutations in NKX2-5 have been associated with a variety of congenital heart diseases including atrial septal defect, ventricular septal defect, tricuspid valve abnormalities and atrioventricular block (Table 1) [60,61,74–87]. Analysis of NKX2-5 mutations in patients with congenital heart disease frequently show reduced DNA binding and transcriptional activation by NKX2-5 [79,87].

So far, a great deal is known about the role of NKX2-5 in heart development, but less is known about the other cardiac Nk-2 proteins. For example, although persistent truncus arteriosus is associated with a homeodomain mutation of NKX2.6 (Table 1) [88], mice with a null mutation for NKX2-6 do not have a cardiac phenotype and it has been postulated that the loss of NKX2-6 was compensated by an increase in NKX2-5 expression [89]. However, it is still unclear which, if any, combination of cardiac NK-2 genes can contribute to heart development.

In adult NKX2-5 null mice, the expression of the fetal genes *Nppa* and *Nppb*, but also *Myl2*, *Mef2c*, *Hand1*, *Msx2*, and *Irx4* were significantly perturbed. A ventricular-restricted knock-out of NKX2-5 using a MLC2v-Cre deleter strain resulted in aberrant expression of atrial and conduction system genes in adult ventricular myocardium [90,91]. Although NKX2-5 transgenic mice only increase fetal gene expression in the heart without cardiac hypertrophic remodeling, NKX2-5 interacts with other factors that are known to regulate hypertrophy such as the transcription factors GATA4 and serum response factor (SRF), or the co-factor calmodulin-binding transactivator (*Camta1*) [92,93]. Taken together, these reports support the contention that NKX2-5 plays a critical role in fetal gene reactivation and cardiac hypertrophy in the adult heart.

2.5. The SMAD (small mother against decapentaplegic) family

The SMAD family can be divided into three different functional classes: the receptor-activated R-SMADs (SMAD1, -2, -3, -5, and -8), the co-mediator Co-SMADs (SMAD4), and the inhibitory I-SMADs (SMAD6 and -7). R-SMADs are predominantly localized at the cytoplasm, Co-SMADs are distributed in the cytoplasm and the nucleus, and I-SMADs are found mostly in the nucleus [94]. SMADs bind to the consensus sequence 5′-GTCT-3′ with low affinity. Therefore, the binding of only SMADs to this sequence is not thought to be sufficient to significantly affect promoter activity of a target gene. Consequently, recruitment of SMADs to a DNA binding site is regulated by cooperation with other transcription factors, which creates a large spectrum of sequence-specific binding patterns.

During cardiac development, SMADs regulate endocardial cushion formation, valve morphogenesis, and outflow tract formation downstream of TGF-β and bone morphogenic protein (BMP) [95,96]. The primary function of BMP is in heart development. Treatment of embryonic cells with BMP induces differentiation into cardiomyocytes via SMAD1/4 signaling. Overexpression of SMAD6, an inhibitory SMAD protein that specifically inhibits BMP-mediated SMAD activation, prevents myocyte differentiation [97], and SMAD6 knock-out mice have several cardiovascular abnormalities [98]. Finally, it was observed that SMAD proteins together with GATA4 regulate expression of the NKX2-5 cardiac enhancer, which implicates a role for SMADs regulating the cardiac cell lineage [99].

In the adult heart, SMADs are critically involved in multiple aspects of pathophysiology, including the regulation of cardiac fibrosis, apoptosis and hypertrophic remodeling. In response to cardiomyopathy and myocardial infarction, increased expression of (transforming growth factor-β) TGF-β and Smad2, 3, and 4 in the heart has been observed [100]. In cardiac fibroblasts, phosphorylation of SMAD2 was associated with fibrosis and scar formation [101]. By overexpressing SMAD7 (I-SMAD) in fibroblasts, collagen synthesis could be inhibited [102]. Furthermore, decreased levels of TGF-β and SMAD2 activity is associated with decreased fibrosis after angiotensin II receptor stimulation [103]. Also, by using TGF-β1 heterozygous mice, both myocardial infarction-mediated fibrosis and aging associated fibrosis were diminished [104]. In addition to the fibrotic effects associated with TGF-β/SMAD signaling in the heart, SMAD1 mediates the anti-apoptotic action of BMP-2 in neonatal cardiomyocytes [105]. This anti-apoptotic effect of BMP-2/SMAD1 signaling could be repressed by SMAD6 [106]. Targeted disruption of SMAD4 leads to an increased heart weight/body weight ratios compared with their littermates and increased expression of *Nppa*, *Nppb* and *Myh7*. Moreover, cardiac-specific SMAD4-deficient mice display increased left ventricular and intraventricular septal wall thicknesses compared to wild type mice [107]. In line, the TGF-β family member growth-differentiation factor 15 (*Gdf15*) was demonstrated to function as an anti-hypertrophic regulator [108]. TGF-β1 itself has been shown to have pro-hypertrophic effects in the adult heart. Deletion of TGF-β1 in the mouse reduced angiotensin II-induced hypertrophy and TGF-β1 overexpression mice develop cardiac hypertrophy, supporting a prohypertrophic role for TGF-β1, likely by signaling through MAPK signaling, including TGF-β-activated kinase 1 (TAK1). In line, TAK1 overexpression mice have been shown to develop cardiac hypertrophy and induction of fetal gene programs [109].

2.6. Serum response factor (SRF)

SRF belongs to an ancient DNA-binding protein family, which shares a highly conserved DNA-binding and dimerization domain termed the MADS box (similar to the MEF2 transcription factor family described earlier). SRF binds to the SRF-binding consensus element known as the CArG box and is essential for cardiac differentiation and maturation [110–114]. A CArG box consists of the sequence $CC(AT)_6GG$ and found primarily in promoters of genes involved in cell growth, migration, differentiation, cytoskeleton organization and energy metabolism. In the developing heart, cardiac-specific deletion of SRF disrupts proper heart development and results in severe defects in the contractile apparatus of cardiomyocytes, which leads to death in the embryonic gastrulation stage [112,114]. Also, SRF deletion in mouse embryos results in impaired gastrulation and lethality before mesoderm formation, which suggests an essential role for SRF in mesoderm formation during mouse embryogenesis [110].

In the postnatal heart, cardiac-specific overexpression of the SRF gene with a double mutation (dmSRF) attenuated the total SRF binding activity and resulted in dilated cardiomyopathy and death within the first 12 days after birth [111]. In line with this, cardiac-specific and tamoxifen-inducible disruption of SRF in mice results in decreased levels of proteins involved in sarcomeric function (force generation and transmission), fibrotic development, and changes in the cytoarchitecture of cardiomyocytes without hypertrophic compensation [115]. Furthermore, loss of SRF in the postnatal heart leads to increased expression of the fetal genes *Nppa*, *Nppb* and *Myh7* and a decreased expression of sarcoplasmic reticulum Ca^{2+}-ATPase 2a (SERCA2) and the cardiac sodium–calcium exchanger (NCX1) [116]. Additionally, mRNA array analysis of SRF deletion in cardiomyocytes resulted in downregulation of genes encoding sarcomeric proteins and other cardiac transcription factors [117]. Due to the cardiac changes upon deletion of SRF, these mice developed severe dilated cardiomyopathy, which lead to heart failure and death around 10 weeks after triggering SRF loss [118]. Interestingly, increased expression of insulin-like growth factor, which has been shown to improve myocardial function in pathological situations [119,120], was also able to protect the heart against dilated cardiomyopathy evoked by SRF deletion [116]. Also in humans it has been suggested that late alterations of SRF function is associated with cardiomyopathies, which eventually could lead to heart failure [121,122].

SRF associates with tissue-specific regulatory cofactors, including GATA4 and NKX2-5, which independently regulate hypertrophic gene expression in the adult heart [93]. Furthermore, SRF regulates microRNA expression levels. Cardiac-specific overexpression of SRF led to altered expression of a number of microRNAs, such as a downregulation of miR-1, miR-133a and upregulation of miR-21, microRNAs known to be involved in cardiac hypertrophic remodeling. Reduced cardiac SRF expression levels resulted in an increased expression of miR-1, miR-133a and a decreased expression of miR-21 [123].

3. Epigenetic control of the fetal gene program

In eukaryotes, packaging of genomic DNA into chromatin is a central mechanism for gene regulation. The fundamental unit of the chromatin is the nucleosome, which envelops a histone octamer. To repress gene expression, nucleosomes interact to create a highly compact structure that limits access of transcriptional machinery to genomic DNA. During organ development, but also in the process of hypertrophic remodeling, epigenetic changes regulate gene activity. These epigenetic modifications of chromatin include methylation of genomic DNA as well as acetylation, methylation, and phosphorylation of histone proteins.

3.1. Histone acetyltransferases (HATs)

Acetylation of the amino-terminal histone tails by histone acetyltransferases (HATs) relaxes nucleosomal structure by weakening the interaction of the positively charged histone tails with the negatively charged phosphate backbone of the DNA, allowing access of transcriptional activators and induction of gene expression. In cardiac muscle, the most extensively studied HATs are p300 and the closely related coactivator, CREB-binding protein (CBP). P300 relaxes chromatin structure and promotes gene activation by modifying chromatin and associated transcription factors. The first evidence for a role of HATs in cardiac muscle was shown by deletion of p300, which perturbed heart development and cell proliferation. P300 knockout mice died between

E9 and E11.5 and show reduced cardiac structure accompanied by decreased expression of β-MHC and α-actinin, and reduced trabeculation [124]. Furthermore, a gene knock-in approach demonstrated that the HAT domain of p300 seems to be essential for heart formation [125].

In the adult heart, p300 and CBP play a critical role in cardiac hypertrophic remodeling that is dependent on their histone acetyltransferase activity [126]. In cardiomyocytes, agonist-induced hypertrophy leads to increased levels of p300 [127]. Furthermore, overexpression of both p300 and CBP stimulates agonist-induced cardiac hypertrophic growth, while dominant-negative mutants of p300 block agonist-mediated cardiac hypertrophy [126,128].

Besides acetylation of histone tails, p300 also affects the activity of hypertrophy-responsive transcription factors, such as GATA4, SRF, and MEF2 [129–132]. Together, these studies show a role for p300 and CBP in stimulating activity of fetal genes during the process of cardiac hypertrophy.

3.2. Histone deacetylases (HDACs)

Histone deacetylases (HDACs) catalyze the removal of acetyl groups from conserved lysine residues in histone tails, thereby inducing chromatin condensation and transcriptional repression. HDACs are classified into four subfamilies (classes I, IIa, IIb, and IV). Class I HDACs consist of HDAC1, 2, 3, and 8 proteins, which are localized predominantly in the cell nucleus, and display high enzymatic activity toward histone substrates. Most class I HDACs are part of multiprotein nuclear complexes crucial for transcriptional repression, and have important functional roles in regulating cellular proliferation and survival (reviewed in [133]). Class II HDACs are further subdivided into class IIa (HDAC4, 5, 7, and 9) and class IIb (HDAC6 and 10) [134]. Class IIa HDACs function more as signal transducers that shuttle between the cytoplasm and the nucleus and have only weak enzymatic activity. Shuttling of class IIa HDACs is dependent on phosphorylation of two serine-containing motifs found exclusively in the amino-terminal extensions of HDAC4, HDAC5, HDAC7, and HDAC9. In higher eukaryotes 14-3-3 proteins have also been shown to be involved in nucleo-cytoplasmic shuttling of HDAC5, where phosphorylated HDAC5 binds to 14-3-3 and is transported out of the nucleus [135,136]. Class IIa HDACs can also regulate gene repression by interacting with class I HDACs and other transcriptional repressors such as nuclear receptor corepressor (N-CoR), silencing mediator of RA and thyroid hormone receptors (SMRT), heterochromatin protein 1, and C-terminal-binding protein. Class III HDACs, or sirtuins, require NAD + for deacetylation and regulate transcriptional repression. Sirtuins have no sequence similarity to class I and II HDACs.

3.2.1. Class I HDACs

HDACI plays a critical role during embryonic development. HDAC1 knock-out mice die before E10.5 secondary to severe proliferation defects, indicated by elevated levels of the cyclin-dependent kinase inhibitors p21(WAF1/CIP1) and p27(KIP1) [137,138]. A recent study also implicates HDAC2 in cardiac hypertrophy. Cardiac overexpression of a heart-enriched nuclear protein, homeodomain-only protein (HOP), induces hypertrophic remodeling and subsequent heart failure. By recruiting HDAC2, HOP is able to repress SRF. In the same study, it was reported that HDAC inhibitors could block HOP-induced cardiac growth, suggesting HOP as a potential target of HDACs during cardiac hypertrophy [139]. Also cardiac-specific HDAC3 KO mice showed massive cardiac hypertrophy accompanied by up-regulation of genes involved in fatty acid uptake and oxidation [140]. However, deletion of HDAC3 by a Cre deleter strain under control of the Muscle creatine kinase (Mck) promoter, did not lead to such significant myocardial dysfunction on normal chow, but develop severe cardiomyopathy leading to death when animals were fed a high fat diet [141]. The differences between the two mouse models likely relates to the time point of HDAC3 deletion. Using the MHC-Cre deleter strain, HDAC is removed during mid-gestation, while the MCK-Cre deleter strain is active in heart and skeletal muscle at postnatal time points. The more severe phenotype produced by earlier deletion of HDAC3 suggests that HDAC3 is also important during cardiac development. The cardiac changes observed in both mouse models seem to be due to increased activity of PPARα. Also, HDAC3 can bind and deacetylate MEF2 and thereby lead to transcriptional repression of MEF2 [142].

3.2.2. Class IIa HDACs

A decade ago, it has become clear that class IIa HDACs play a prominent role as signal-responsive suppressors during the development of cardiac hypertrophy. Adenoviral overexpression of constitutive active mutants of HDAC4, HDAC5, and HDAC9 has been shown to prevent agonist-induced cardiomyocyte hypertrophy [143]. At early age, mice lacking either HDAC5 or HDAC9 are viable and show no evidence of cardiac abnormalities. Only when HDAC5/9 null mice reach the age of 6 months, they develop spontaneous cardiac hypertrophy. However, the majority of HDAC5/9 double-mutant mice die during late embryogenesis with thin-walled myocardium and ventricular-septal defects, suggesting that these HDACs are required for proper heart development. In the adult heart, either single HDAC5- or HDAC9-deficiency leads to the development of hypertrophy [144,145]. In contrast, in response to chronic β-adrenergic stimulation with isoproterenol, which is known to induce hypertrophy by acting through the cAMP-signaling pathway, these mice seem to have a normal cardiac morphology. Such findings indicate that both HDAC5 and HDAC9 function as antagonists of hypertrophic remodeling, specifically dependent on intracellular calcium signaling.

Furthermore, class IIa HDACs have the potential to repress hypertrophy-responsive genes recruited via MEF2. Class IIa HDACs are able to bind to MEF2 via an18-amino-acid motif present only in these HDACs. In HDAC knockout mice, cardiac hypertrophy is associated with enhanced activation of MEF2. A complex between class IIa HDACs and MEF2 will be formed, resulting in repression of genes harboring MEF2-binding sites. As discussed earlier, p300 has also been shown to associate with MEF2. Thus, both class IaI HDACs and HATs can bind MEF2, which then acts as platform to respond to positive or negative transcriptional signals by exchanging HATs and class IIa HDACs. Not only do HATs and class IIa HDACs compete for association with MEF2, also MEF2 and calmodulin (CaM) compete with each other to associate with class IIa HDACs. Upon binding of Ca^{2+} to CaM, CaM is able to interact with class IIa HDACs, which lead to HDAC phosphorylation and displacement of MEF2 accompanied by derepression of MEF2 target genes [146]. In addition, CaM is also able to bind to Cabin1, a transcriptional repressor of MEF2. Upon increased intracellular calcium levels, CaM competes with MEF2 for binding to Cabin1, resulting in the dissociation of MEF2 from its repressor Cabin1 and, consequently, in MEF2 activation [147]. Additionally, class IIa HDACs are able to repress other transcription factors either directly or indirectly, including SRF, GATA, NFAT, and myocardin, all of which have a known role in hypertrophic remodeling [148–152].

3.2.3. Class III HDACs

Class III HDACs (sirtuins) function in a wide array of cellular processes, including gene silencing, longevity, and DNA damage repair. There are several sirtuin isoforms distributed in different subcellular compartments, including the nucleus (SIRT1, -2, -6, and -7), cytoplasm (SIRT1 and -2), and mitochondria (SIRT3, -4, and -5) [153]. SIRT1 has been shown to deacetylate many transcription factors, including p53, and Forkhead box O (FoxO) transcription factors. In the adult heart, SIRT1 expression levels are increased by stress, such as pressure overload and nutrient starvation [154,155]. Another member of the sirtuin family, SIR2α, was reported in nuclei of cardiac myocytes. Inhibition of SIR2α by the inhibitors nicotinamide or sirtinol induced myocyte apoptosis. In line with this, also expression of dominant-negative SIR2α leads to increased levels of apoptosis [156]. Furthermore,

overexpression of SIR2α protected cardiomyocytes from apoptosis, which was associated with an increased size of cardiomyocytes. Furthermore, SIR2α expression levels were elevated in hearts from dogs with heart failure induced by rapid pacing superimposed on stable, severe hypertrophy. Thus, increased SIR2α expression during heart failure may play a cardioprotective role in vivo.

3.3. Histone methylation

Histone methylation can lead to both facilitation and repression of gene expression. For instance, methylation of H3K4 is associated with active genes, while dimethylation or trimethylation of H3K9 primarily occurs during gene repression. In the healthy heart, the methylation status is balanced by activities of histone methyltransferases (e.g. G9a and SUV39H1) and histone demethylases (e.g. JMJD1 and JMJD2) [157]. In human failing hearts, differential methylation patterns of H3K4 and H3K9 have been observed in various gene clusters.

3.3.1. Jumonji

Jumonji (Jmj) contains the conserved Jumonji C (JmjC) domain and belongs to a histone demethylase family. Jmj has a methyltransferase rather than a demethylase activity and it recruits G9a and GLP methyltransferases to methylate H3K9 and repress the cyclin D1 promoter [158]. Jmj KO mice have been shown to suffer from various cardiac defects. One study showed that Jmj KO mice are born ($p = 0$) with ventricular septal defects, ventricular noncompaction, and double outlet of the right ventricle [159], while another study demonstrated that Jmj KO embryos (with a C3H/He background), die between E11.5 and E12.5 and display abnormalities in the right heart and hyperplasia of the trabecular myocardium, resulting in obstruction of the ventricle [160].

In vitro experiments using neonatal cardiomyocytes demonstrate that Jmj associates with GATA4 and NKX2-5 to repress *Nppa* expression [161], and Jmj has also been demonstrated to repress *Myh6* expression by inhibiting MEF2A activity [162].

In human failing hearts, an upregulation of JMJC domain-containing demethylases has been observed [157]. Expression of JMJD1A, JMJD2A, and JMJD2B was 2-fold increased in failing versus nonfailing myocardium. In these human failing hearts, upregulation of JMJD1A correlated positively with increased *Nppa* and *Nppb* expression and inversely with H3K9me2 in the promoter region of *Nppa*. By performing ChIP assays, this study demonstrated that both JMJD1A and JMJD2A are bound to the promoter regions of *Nppa* in non-failing and failing myocardium of patients with ischemic or dilated cardiomyopathy [157].

3.3.2. HDAC4 and H3K9 demethylation

In response to G-protein coupled receptor (GPCR) activation, Ca^{2+}/calmodulin-dependent kinase II (CaMKII), protein kinase D, and reactive oxygen species, export of HDAC4, HDAC5, and HDAC9 from the nucleus to the cytosol is mediated. HDAC4 is able to repress gene transcription by interacting with MEF2, and at least part of its repressive function is related to keeping the respective promoter region in a deacetylated state. In human failing hearts, histone acetylation was not required for *Nppa* and *Nppb* gene activation, although pronounced nuclear export of HDAC4 was observed [157]. In contrast, H3K9 demethylation was closely associated with reactivation of fetal genes and nuclear export of HDAC4. Experiments in genetically modified mice revealed that nuclear HDAC4 export was required for H3K9 demethylation, HP1 dissociation, and *Nppa* gene activation in response to increased hemodynamic load [157]. In that study, protein–protein interaction of HDAC4 with the histone methyltransferase SUV39H1 was disrupted upon nuclear export of HDAC4 in response to targeted phosphorylation by CaMKIIδB, which might represent a potential underlying mechanism.

4. MicroRNA regulation of the fetal gene program

The discovery of the regulatory role of microRNAs (miRNAs, miRs) has added a new layer of complexity to our understanding of the embryonic development of the heart. Furthermore, recent studies established essential roles for miRNAs in cardiac hypertrophy and heart failure. miRNAs are 18–24 nucleotide single-stranded RNAs, encoded by the genome and suppress gene expression by translational repression. The importance of posttranscriptional regulation by miRNAs for embryonic development was underscored by generating loss of function mutations of enzymes essential for miRNA biogenesis such as Dicer, Drosha, Ago2, and DGCR8 [163–166]. Deletion of Dicer using Cre recombinase under control of the endogenous NKX2-5 regulatory region leads to defects in the ventricular myocardium, cardiac outflow tract morphogenesis and chamber septation, and results in pericardial edema and embryonic death at E12.5 in the mouse [167]. Also, in the adult heart, loss of Dicer induced pathological cardiac hypertrophy, accompanied by myofiber disarray, ventricular fibrosis, and strong induction of fetal gene transcripts [168]. DGCR8 inactivation in neural crest cells (using a Wnt1-Cre) revealed major cardiovascular defects at E18.5, including persistent truncus arteriosus, interrupted aortic arch, cervical aortic arch and aberrant origin of the right subclavian artery [169]. Together, these studies show that miRNA biogenesis is essential for normal cardiac development and survival. Although limited information is available about specific miRNAs that are involved in fetal gene regulation both during embryonic development and in heart failure, Thum and colleagues showed that fetal gene reactivation in human heart failure is, at least in part, driven by differential miRNA expression patterns [4]. Other examples are discussed below.

4.1. The miR-1~133 cluster

A subset of miRNAs, which are either specifically or highly expressed in cardiac and skeletal muscle, termed myomiRs, include miR-1, -133, -206 and -208 [170]. The miR-1 and miR-133 families are co-expressed. The miR-1 and miR-133 families are co-expressed. The miR-1 subfamily consists of two closely related transcripts, miR-1-1 and miR-1-2, and the miR-133 family comprises miR-133a-1, miR-133a-2 and miR-133b. Both in humans and in mice, miR-1-1 and miR-133a-2 form a cluster on chromosome 2 and miR-1-2 and miR-133a-1 form another cluster located on chromosome 18 [171].

During early stages of embryonic development, miR-1 and miR-133 are functionally expressed to promote mesoderm induction and suppress differentiation into the ectodermal or endodermal lineages in animals such as mice and zebrafish [171,172]. miR-1 promotes differentiation of cardiac progenitors and decreases cell proliferation in mammals and flies [173,174]. In contrast, miR-133 inhibits differentiation of skeletal myoblasts and maintains them in a proliferative state [171]. Furthermore, while miR-1-1 is initially strongly expressed in the less proliferative inner curvature of the heart loop and in atria, miR-1-2 is expressed mainly in the ventricles [175]. This suggests that the miR-1 and miR-133 family are regulated and expressed differentially during cardiac development.

Overexpression of miR-1 in a transgenic mouse model results in a phenotype characterized by thin-walled ventricles from premature differentiation and early withdrawal of cardiomyocytes from the cell cycle [173,176]. Conversely, miR-1-2 deficiency in mice results in 50% embryonic lethality accompanied by thickened chamber walls from prolonged hyperplasia and ventricle septal defects. The ventral septal defects might partially be mediated by the transcription factor heart and neural crest-derivatives-2 (*Hand2*), which regulates expansion of ventricular cardiomyocytes by inhibiting cardiomyocyte progenitor proliferation [174,177]. Overexpression of miR-133 after injury of a zebrafish heart resulted in reduced myocardial regeneration, while impaired expression of miR-133 was responsible for increased cardiomyocyte proliferation [178]. Along with miR-1, these two

opposing effects demonstrate the critical importance of correct timing and dosage of miRs for heart development [175]. Interestingly, several transcription factors, including SRF, MEF2, GATA4 and NKX2-5, have been shown to regulate the expression of the bicistronic miRNA pairs miR-1-1/miR-133a-2 and miR-1-2/miR-133a-1 [171,174]. SRF enhances the expression of these miRNAs in ventricular and atrial myocytes [179], whereas MEF2 binds to the intronic enhancer of these miRs to activate their expression in ventricular myocytes. In addition, several direct targets of miR-1 have been described in vivo, including the transcription factor *Hand2* and the Notch Delta ligand in *Drosophila*. Similarly, Delta-like 1 (Dll-1), a human homolog of the Notch Delta, is translationally repressed in miR-1 expressing mouse embryonic stem cells [180].

The upregulation of miR-1 to substantial levels in the postnatal heart suggested an important function in the regulation of postnatal heart function. Accordingly, it was shown that after aortic constriction-induced hypertrophy in a mouse model, miR-1 was singularly downregulated from day 1 until day 7 [181]. Consistently, also in other genetic models of cardiac hypertrophy, including the calcineurin and v-akt murine thymoma viral oncogene homolog 1 (AKT) transgenic hearts, miR-1 was downregulated [182,183]. Although in mice, a decreased expression of miR-1 has been reported, in humans the measurements of miR-1 have been less consistent. Although Ikeda et al. and Naga Prasad et al. report a reduction in miR-1 in ischemic and non-ischemic dilated cardiomyopathies [183,184], Matkovich et al. and Kumarswamy et al. report upregulation of miR-1 in human heart failure [185,186]. Thus in the failing human heart, measurements of miR-1 levels have been inconsistent and, accordingly, miR-1 function and targets in that context are less well explained.

Mechanistically, miR-1 has been shown to negatively regulate CaM, MEF2A, and GATA4, key components of calcium signaling pathways and fetal gene activation, which are two events that are necessary for agonist-induced cardiomyocyte hypertrophy in the mouse [183]. Indeed, another study reported that miR-1 overexpression also suppressed expression of the fetal gene ANF [181]. Furthermore, overexpression of miR-1 in cultured neonatal myocytes partially inhibited phosphorylation of ribosomal S6 protein and several genes that were related to cardiac growth, including Ras GTPase-activating protein, cyclin-dependent kinase 9, fibronectin, and Ras homolog expressed in brain [181].

Overexpression of miR-133 resulted in suppression of protein synthesis and inhibition of hypertrophic growth in PE- or endothelin-1-treated neonatal mouse cardiac myocytes. miR-133a is also known to regulate the expression of the transcription factor NFATc4 [187] and miR-133 overexpression leads to the de-repression of fetal genes, including *Nppa*, Acta1, and Myh7. In line with this, miR-133 deletion, using a targeted 3′ UTR decoy sequence, resulted in marked cell hypertrophy and increased protein synthesis, accompanied by increased fetal gene expression. Furthermore, treatment of mice with an antagomir against miR-133 resulted in cardiac hypertrophy and re-activation of the fetal gene program [182].

4.2. miR-208

The miR-208 family consists of two transcripts; miR-208a and miR-208b, which are both required for cardiomyocyte-specific gene expression, cardiomyocyte hypertrophy, and thyroid hormone sensitivity [3,188]. Both miRs share a high level of sequence homology but miR-208a is encoded by an intron of its host gene, *Myh6* [189], while miR-208b, is located within an intron of the gene encoding *Myh7* [190]. In mice, *Myh7* is expressed in the embryonic heart and *Myh6* is predominantly found at postnatal stages. A similar switch from miR-208b to miR-208a expression takes place right after birth, demonstrating a parallel expression of these microRNAs with their host genes. Therefore, miR-208a could be detected in the heart as early as E13.5 but only at very low levels, while miR-208b is much higher expressed in the developing heart [188]. Genomic deletion of miR-208a without impairing *Myh6* expression leads to ectopic expression of fast skeletal muscle markers in the adult heart, demonstrating that miR-208a affects cardiac differentiation. Interestingly, mice lacking miR-208a fail to increase *Myh7* levels under stress and do not develop hypertrophy [189], while deletion of miR-208b did not alter *Myh7* expression [191]. In response to pressure overload by thoracic aortic constriction or signaling by calcineurin, miR-208a deficient mice were resistant to hypertrophic remodeling, fibrosis and fetal gene activation. Upon stress, miR-208a inhibits the thyroid receptor-associated protein THRAP1, which in turn reduces thyroid receptor signaling, leading to increased *Myh7* expression and impaired cardiac function. Apart from Thrap1, myostatin and GATA4 levels are also regulated by miR-208a, which demonstrates the functional role of miR-208a in cardiac hypertrophic remodeling [188]. In line with this, cardiac-specific miR-208a overexpression induces cardiac hypertrophy. In miR-208a transgenic mice and in mice subjected to pressure overload, cardiac expression of miR-208b and its host gene, *Myh7*, are induced during hypertrophic remodeling [188]. Similar to *Myh6* and *Myh7*, a third myosin gene, *Myh7b*, also contains an intronic miR, miR-499. These three miRNA-gene units are involved in a complicated network regulating muscle function.

4.3. Other microRNAs regulating fetal gene reactivation

MiR-9 has been identified as an anti-hypertrophic miRNA by virtue of its ability to decrease myocardin expression, which has been identified as a NFATC3 target. Furthermore, infecting cardiomyocytes with a miR-9 adenovirus prevented isoproterenol-mediated upregulation of *Myh7* expression [137]. Also, miR-195 has been shown to be involved in the re-activation of the fetal gene program during pathological remodeling. miR-195 expression is increased in cardiac hypertrophy, and cardiac overexpression of miR-195 resulted in pathological cardiac growth and heart failure in transgenic mice, which was accompanied by an upregulation of *Nppa*, *Nppb* and *Myh7* [137].

5. Concluding remarks

In the adult failing heart, many fetal genes are re-employed, which in turn activate processes involved in pathological cardiac remodeling. The re-activation of "fetal" genes in the postnatal myocardium involves the combined activation of transcriptional processes, chromatin remodeling and post-transcriptional regulation. All these factors independently act on a different level of gene regulation, and together form a highly interconnected network. Both microRNAs and chromatin remodeling factors influence activation of transcription factors, which in their turn regulate the expression of several microRNA genes. These gene networks offer multiple therapeutic entry points to dampen the aberrant expression of fetal genes and adverse remodeling processes of the failing heart.

Acknowledgements

E.D. was supported by a Heart Failure Association fellowship of the European Society of Cardiology. P.D.C.M. was supported by a Leducq Career Development Award, a Dutch Heart Foundation grant NHS2010B261 and a MEERVOUD award 2011/09039/ALW from the Netherlands Research Organization (NWO). L.D.W. acknowledges support from *the Netherlands CardioVascular Research Initiative*: the Dutch Heart Foundation, Dutch Federation of University Medical Centers, the Netherlands Organization for Health Research and Development (ZonMW) and the Royal Netherlands Academy of Sciences. L.D.W. was further supported by a VIDI award 917-863-72 from the ZonMW; the Dutch Heart Foundation program grant NHS2007B167; the Fondation Leducq Transatlantic Network of Excellence program 08-CVD-03 and grant 311549 from the European Research Council (ERC).

References

[1] J.A. Towbin, N.E. Bowles, The failing heart, Nature 415 (2002) 227–233.
[2] M.F. Meijs, L.J. de Windt, N. de Jonge, M.J. Cramer, M.L. Bots, W.P. Mali, P.A. Doevendans, Left ventricular hypertrophy: a shift in paradigm, Curr. Med. Chem. 14 (2007) 157–171.
[3] E. van Rooij, L.B. Sutherland, X. Qi, J.A. Richardson, J. Hill, E.N. Olson, Control of stress-dependent cardiac growth and gene expression by a microRNA, Science 316 (2007) 575–579.
[4] T. Thum, P. Galuppo, C. Wolf, J. Fiedler, S. Kneitz, L.W. van Laake, P.A. Doevendans, C.L. Mummery, J. Borlak, A. Haverich, C. Gross, S. Engelhardt, G. Ertl, J. Bauersachs, MicroRNAs in the human heart: a clue to fetal gene reprogramming in heart failure, Circulation 116 (2007) 258–267.
[5] N. Frey, E.N. Olson, Cardiac hypertrophy: the good, the bad, and the ugly, Annu. Rev. Physiol. 65 (2003) 45–79.
[6] J.D. Molkentin, G.W. Dorn II, Cytoplasmic signaling pathways that regulate cardiac hypertrophy, Annu. Rev. Physiol. 63 (2001) 391–426.
[7] B.G. Petrich, Y. Wang, Stress-activated MAP kinases in cardiac remodeling and heart failure; new insights from transgenic studies, Trends Cardiovasc. Med. 14 (2004) 50–55.
[8] S. Hannenhalli, M.E. Putt, J.M. Gilmore, J. Wang, M.S. Parmacek, J.A. Epstein, E.E. Morrisey, K.B. Margulies, T.P. Cappola, Transcriptional genomics associates FOX transcription factors with human heart failure, Circulation 114 (2006) 1269–1276.
[9] B.D. Lowes, E.M. Gilbert, W.T. Abraham, W.A. Minobe, P. Larrabee, D. Ferguson, E.E. Wolfel, J. Lindenfeld, T. Tsvetkova, A.D. Robertson, R.A. Quaife, M.R. Bristow, Myocardial gene expression in dilated cardiomyopathy treated with beta-blocking agents, N. Engl. J. Med. 346 (2002) 1357–1365.
[10] B.C. Blaxall, B.M. Tschannen-Moran, C.A. Milano, W.J. Koch, Differential gene expression and genomic patient stratification following left ventricular assist device support, J. Am. Coll. Cardiol. 41 (2003) 1096–1106.
[11] Y. Chen, S. Park, Y. Li, E. Missov, M. Hou, X. Han, J.L. Hall, L.W. Miller, R.J. Bache, Alterations of gene expression in failing myocardium following left ventricular assist device support, Physiol. Genomics 14 (2003) 251–260.
[12] K.B. Margulies, S. Matiwala, C. Cornejo, H. Olsen, W.A. Craven, D. Bednarik, Mixed messages: transcription patterns in failing and recovering human myocardium, Circ. Res. 96 (2005) 592–599.
[13] A. Hammerer-Lercher, B. Halfinger, B. Sarg, J. Mair, B. Puschendorf, A. Griesmacher, N.A. Guzman, H.H. Lindner, Analysis of circulating forms of proBNP and NT-proBNP in patients with severe heart failure, Clin. Chem. 54 (2008) 858–865.
[14] B.J. Wilkins, L.J. De Windt, O.F. Bueno, J.C. Braz, B.J. Glascock, T.F. Kimball, J.D. Molkentin, Targeted disruption of NFATc3, but not NFATc4, reveals an intrinsic defect in calcineurin-mediated cardiac hypertrophic growth, Mol. Cell. Biol. 22 (2002) 7603–7613.
[15] C.Y. Lin, C.J. Lin, C.H. Chen, R.M. Chen, B. Zhou, C.P. Chang, The secondary heart field is a new site of calcineurin/Nfatc1 signaling for semilunar valve development, J. Mol. Cell. Cardiol. 52 (2012) 1096–1102.
[16] A. Yehya, R. Souki, F. Bitar, G. Nemer, Differential duplication of an intronic region in the NFATC1 gene in patients with congenital heart disease, Genome 49 (2006) 1092–1098.
[17] J.D. Molkentin, Calcineurin and beyond: cardiac hypertrophic signaling, Circ. Res. 87 (2000) 731–738.
[18] Y. Xia, J.B. McMillin, A. Lewis, M. Moore, W.G. Zhu, R.S. Williams, R.E. Kellems, Electrical stimulation of neonatal cardiac myocytes activates the NFAT3 and GATA4 pathways and up-regulates the adenylosuccinate synthetase 1 gene, J. Biol. Chem. 275 (2000) 1855–1863.
[19] A. Bubikat, L.J. De Windt, B. Zetsche, L. Fabritz, H. Sickler, D. Eckardt, A. Godecke, H.A. Baba, M. Kuhn, Local atrial natriuretic peptide signaling prevents hypertensive cardiac hypertrophy in endothelial nitric-oxide synthase-deficient mice, J. Biol. Chem. 280 (2005) 21594–21599.
[20] E. van Rooij, P.A. Doevendans, C.C. de Theije, F.A. Babiker, J.D. Molkentin, L.J. de Windt, Requirement of nuclear factor of activated T-cells in calcineurin-mediated cardiomyocyte hypertrophy, J. Biol. Chem. 277 (2002) 48617–48626.
[21] N. Onohara, M. Nishida, R. Inoue, H. Kobayashi, H. Sumimoto, Y. Sato, Y. Mori, T. Nagao, H. Kurose, TRPC3 and TRPC6 are essential for angiotensin II-induced cardiac hypertrophy, EMBO J. 25 (2006) 5305–5316.
[22] K. Kuwahara, Y. Wang, J. McAnally, J.A. Richardson, R. Bassel-Duby, J.A. Hill, E.N. Olson, TRPC6 fulfills a calcineurin signaling circuit during pathologic cardiac remodeling, J. Clin. Invest. 116 (2006) 3114–3126.
[23] J. Yang, B. Rothermel, R.B. Vega, N. Frey, T.A. McKinsey, E.N. Olson, R. Bassel-Duby, R.S. Williams, Independent signals control expression of the calcineurin inhibitory proteins MCIP1 and MCIP2 in striated muscles, Circ. Res. 87 (2000) E61–E68.
[24] S. Abbasi, J.D. Lee, B. Su, X. Chen, J.L. Alcon, J. Yang, R.E. Kellems, Y. Xia, Protein kinase-mediated regulation of calcineurin through the phosphorylation of modulatory calcineurin-interacting protein 1, J. Biol. Chem. 281 (2006) 7717–7726.
[25] R.B. Vega, B.A. Rothermel, C.J. Weinheimer, A. Kovacs, R.H. Naseem, R. Bassel-Duby, R.S. Williams, E.N. Olson, Dual roles of modulatory calcineurin-interacting protein 1 in cardiac hypertrophy, Proc. Natl. Acad. Sci. U. S. A. 100 (2003) 669–674.
[26] Q. Liu, J.C. Busby, J.D. Molkentin, Interaction between TAK1–TAB1–TAB2 and RCAN1-calcineurin defines a signalling nodal control point, Nat. Cell Biol. 11 (2009) 154–161.
[27] L. Genesca, A. Aubareda, J.J. Fuentes, X. Estivill, S. De La Luna, M. Perez-Riba, Phosphorylation of calcipressin 1 increases its ability to inhibit calcineurin and decreases calcipressin half-life, Biochem. J. 374 (2003) 567–575.
[28] P.A. da Costa Martins, K. Salic, M.M. Gladka, A.S. Armand, S. Leptidis, H. el Azzouzi, A. Hansen, C.J. Coenen-de Roo, M.F. Bierhuizen, R. van der Nagel, J. van Kuik, R. de Weger, A. de Bruin, G. Condorelli, M.L. Arbones, T. Eschenhagen, L.J. De Windt, MicroRNA-199b targets the nuclear kinase Dyrk1a in an auto-amplification loop promoting calcineurin/NFAT signalling, Nat. Cell Biol. 12 (2010) 1220–1227.
[29] E. van Rooij, L.B. Sutherland, N. Liu, A.H. Williams, J. McAnally, R.D. Gerard, J.A. Richardson, E.N. Olson, A signature pattern of stress-responsive microRNAs that can evoke cardiac hypertrophy and heart failure, Proc. Natl. Acad. Sci. U. S. A. 103 (2006) 18255–18260.
[30] Z. Lin, I. Murtaza, K. Wang, J. Jiao, J. Gao, P.F. Li, miR-23a functions downstream of NFATc3 to regulate cardiac hypertrophy, Proc. Natl. Acad. Sci. U. S. A. 106 (2009) 12103–12108.
[31] K. Wang, Z.Q. Lin, B. Long, J.H. Li, J. Zhou, P.F. Li, Cardiac hypertrophy is positively regulated by MicroRNA miR-23a, J. Biol. Chem. 287 (2012) 589–599.
[32] Q. Lin, J. Schwarz, C. Bucana, E.N. Olson, Control of mouse cardiac morphogenesis and myogenesis by transcription factor MEF2C, Science 276 (1997) 1404–1407.
[33] L. Wang, C. Fan, S.E. Topol, E.J. Topol, Q. Wang, Mutation of MEF2A in an inherited disorder with features of coronary artery disease, Science 302 (2003) 1578–1581.
[34] Y. Kim, D. Phan, E. van Rooij, D.Z. Wang, J. McAnally, X. Qi, J.A. Richardson, J.A. Hill, R. Bassel-Duby, E.N. Olson, The MEF2D transcription factor mediates stress-dependent cardiac remodeling in mice, J. Clin. Invest. 118 (2008) 124–132.
[35] J. Xu, N.L. Gong, I. Bodi, B.J. Aronow, P.H. Backx, J.D. Molkentin, Myocyte enhancer factors 2A and 2C induce dilated cardiomyopathy in transgenic mice, J. Biol. Chem. 281 (2006) 9152–9162.
[36] R.J. van Oort, E. van Rooij, M. Bourajjaj, J. Schimmel, M.A. Jansen, R. van der Nagel, P.A. Doevendans, M.D. Schneider, C.J. van Echteld, L.J. De Windt, MEF2 activates a genetic program promoting chamber dilation and contractile dysfunction in calcineurin-induced heart failure, Circulation 114 (2006) 298–308.
[37] A.H. Pereira, C.F. Clemente, A.C. Cardoso, T.H. Theizen, S.A. Rocco, C.C. Judice, M.C. Guido, V.D. Pascoal, I. Lopes-Cendes, J.R. Souza, K.G. Franchini, MEF2C silencing attenuates load-induced left ventricular hypertrophy by modulating mTOR/S6K pathway in mice, PLoS One 4 (2009) e8472.
[38] J.D. Molkentin, The zinc finger-containing transcription factors GATA-4, -5, and -6. Ubiquitously expressed regulators of tissue-specific gene expression, J. Biol. Chem. 275 (2000) 38949–38952.
[39] G. Nemer, M. Nemer, Transcriptional activation of BMP-4 and regulation of mammalian organogenesis by GATA-4 and -6, Dev. Biol. 254 (2003) 131–148.
[40] R.K. Patient, J.D. McGhee, The GATA family (vertebrates and invertebrates), Curr. Opin. Genet. Dev. 12 (2002) 416–422.
[41] C.T. Kuo, E.E. Morrisey, R. Anandappa, K. Sigrist, M.M. Lu, M.S. Parmacek, C. Soudais, J.M. Leiden, GATA4 transcription factor is required for ventral morphogenesis and heart tube formation, Genes Dev. 11 (1997) 1048–1060.
[42] J.D. Molkentin, E.N. Olson, GATA4: a novel transcriptional regulator of cardiac hypertrophy? Circulation 96 (1997) 3833–3835.
[43] J. Rivera-Feliciano, K.H. Lee, S.W. Kong, S. Rajagopal, Q. Ma, Z. Springer, S. Izumo, C.J. Tabin, W.T. Pu, Development of heart valves requires Gata4 expression in endothelial-derived cells, Development 133 (2006) 3607–3618.
[44] E.M. Zeisberg, Q. Ma, A.L. Juraszek, K. Moses, R.J. Schwartz, S. Izumo, W.T. Pu, Morphogenesis of the right ventricle requires myocardial expression of Gata4, J. Clin. Invest. 115 (2005) 1522–1531.
[45] T.L. Butler, G. Esposito, G.M. Blue, A.D. Cole, M.W. Costa, L.B. Waddell, G. Walizada, G.F. Sholler, E.P. Kirk, M. Feneley, R.P. Harvey, D.S. Winlaw, GATA4 mutations in 357 unrelated patients with congenital heart malformation, Genet. Test. Mol. Biomarkers 14 (2010) 797–802.
[46] Y. Chen, J. Mao, Y. Sun, Q. Zhang, H.B. Cheng, W.H. Yan, K.W. Choy, H. Li, A novel mutation of GATA4 in a familial atrial septal defect, Clin. Chim. Acta 411 (2010) 1741–1745.
[47] A. De Luca, A. Sarkozy, F. Consoli, R. Ferese, V. Guida, M.L. Dentici, R. Mingarelli, E. Bellacchio, G. Tuo, G. Limongelli, M.C. Digilio, B. Marino, B. Dallapiccola, Familial transposition of the great arteries caused by multiple mutations in laterality genes, Heart 96 (2010) 673–677.
[48] S.M. Dinesh, K. Lingaiah, M.R. Savitha, B. Krishnamurthy, D. Narayanappa, N.B. Ramachandra, GATA4 specific nonsynonymous single-nucleotide polymorphisms in congenital heart disease patients of Mysore, India, Genet. Test. Mol. Biomarkers 15 (2011) 715–720.
[49] S. Giglio, S.L. Graw, G. Gimelli, B. Pirola, P. Varone, L. Voullaire, F. Lerzo, E. Rossi, C. Dellavecchia, M.C. Bonaglia, M.C. Digilio, A. Giannotti, B. Marino, R. Carrozzo, J.R. Korenberg, C. Danesino, E. Sujansky, B. Dallapiccola, O. Zuffardi, Deletion of a 5-cM region at chromosome 8p23 is associated with a spectrum of congenital heart defects, Circulation 102 (2000) 432–437.
[50] V. Guida, F. Lepri, R. Vijzelaar, A. De Zorzi, P. Versacci, M.C. Digilio, B. Marino, A. De Luca, B. Dallapiccola, Multiplex ligation-dependent probe amplification analysis of GATA4 gene copy number variations in patients with isolated congenital heart disease, Dis. Markers 28 (2010) 287–292.
[51] X.Y. Liu, J. Wang, J.H. Zheng, K. Bai, Z.M. Liu, X.Z. Wang, X. Liu, W.Y. Fang, Y.Q. Yang, Involvement of a novel GATA4 mutation in atrial septal defects, Int. J. Mol. Med. 28 (2011) 17–23.
[52] G. Nemer, F. Fadlalah, J. Usta, M. Nemer, G. Dbaibo, M. Obeid, F. Bitar, A novel mutation in the GATA4 gene in patients with Tetralogy of Fallot, Hum. Mutat. 27 (2006) 293–294.
[53] T. Pehlivan, B.R. Pober, M. Brueckner, S. Garrett, R. Slaugh, R. Van Rheeden, D.B. Wilson, M.S. Watson, A.V. Hing, GATA4 haploinsufficiency in patients with interstitial deletion of chromosome region 8p23.1 and congenital heart disease, Am. J. Med. Genet. 83 (1999) 201–206.
[54] M.G. Posch, L.H. Boldt, M. Polotzki, S. Richter, S. Rolf, A. Perrot, R. Dietz, C. Ozcelik, W. Haverkamp, Mutations in the cardiac transcription factor GATA4 in patients with lone atrial fibrillation, Eur. J. Med. Genet. 53 (2010) 201–203.
[55] S.K. Rajagopal, Q. Ma, D. Obler, J. Shen, A. Manichaikul, A. Tomita-Mitchell, K. Boardman, C. Briggs, V. Garg, D. Srivastava, E. Goldmuntz, K.W. Broman, D.W.

Benson, L.B. Smoot, W.T. Pu, Spectrum of heart disease associated with murine and human GATA4 mutation, J. Mol. Cell. Cardiol. 43 (2007) 677–685.
[56] S.M. Reamon-Buettner, S.H. Cho, J. Borlak, Mutations in the 3′-untranslated region of GATA4 as molecular hotspots for congenital heart disease (CHD), BMC Med. Genet. 8 (2007) 38.
[57] M.K. Schluterman, A.E. Krysiak, I.S. Kathiriya, N. Abate, M. Chandalia, D. Srivastava, V. Garg, Screening and biochemical analysis of GATA4 sequence variations identified in patients with congenital heart disease, Am. J. Med. Genet. A 143A (2007) 817–823.
[58] J. Wang, Y. Lu, H. Chen, M. Yin, T. Yu, Q. Fu, Investigation of somatic NKX2-5, GATA4 and HAND1 mutations in patients with tetralogy of Fallot, Pathology 43 (2011) 322–326.
[59] V. Garg, I.S. Kathiriya, R. Barnes, M.K. Schluterman, I.N. King, C.A. Butler, C.R. Rothrock, R.S. Eapen, K. Hirayama-Yamada, K. Joo, R. Matsuoka, J.C. Cohen, D. Srivastava, GATA4 mutations cause human congenital heart defects and reveal an interaction with TBX5, Nature 424 (2003) 443–447.
[60] K. Hirayama-Yamada, M. Kamisago, K. Akimoto, H. Aotsuka, Y. Nakamura, H. Tomita, M. Furutani, S. Imamura, A. Takao, M. Nakazawa, R. Matsuoka, Phenotypes with GATA4 or NKX2.5 mutations in familial atrial septal defect, Am. J. Med. Genet. A 135 (2005) 47–52.
[61] A. Sarkozy, E. Conti, C. Neri, R. D'Agostino, M.C. Digilio, G. Esposito, A. Toscano, B. Marino, A. Pizzuti, B. Dallapiccola, Spectrum of atrial septal defects associated with mutations of NKX2.5 and GATA4 transcription factors, J. Med. Genet. 42 (2005) e16.
[62] Q. Liang, L.J. De Windt, S.A. Witt, T.R. Kimball, B.E. Markham, J.D. Molkentin, The transcription factors GATA4 and GATA6 regulate cardiomyocyte hypertrophy in vitro and in vivo, J. Biol. Chem. 276 (2001) 30245–30253.
[63] J.H. van Berlo, J.W. Elrod, B.J. Aronow, W.T. Pu, J.D. Molkentin, Serine 105 phosphorylation of transcription factor GATA4 is necessary for stress-induced cardiac hypertrophy in vivo, Proc. Natl. Acad. Sci. U. S. A. 108 (2011) 12331–12336.
[64] E. Bisping, S. Ikeda, S.W. Kong, O. Tarnavski, N. Bodyak, J.R. McMullen, S. Rajagopal, J.K. Son, Q. Ma, Z. Springer, P.M. Kang, S. Izumo, W.T. Pu, Gata4 is required for maintenance of postnatal cardiac function and protection from pressure overload-induced heart failure, Proc. Natl. Acad. Sci. U. S. A. 103 (2006) 14471–14476.
[65] T. Oka, M. Maillet, A.J. Watt, R.J. Schwartz, B.J. Aronow, S.A. Duncan, J.D. Molkentin, Cardiac-specific deletion of Gata4 reveals its requirement for hypertrophy, compensation, and myocyte viability, Circ. Res. 98 (2006) 837–845.
[66] K. Kodo, T. Nishizawa, M. Furutani, S. Arai, E. Yamamura, K. Joo, T. Takahashi, R. Matsuoka, H. Yamagishi, GATA6 mutations cause human cardiac outflow tract defects by disrupting semaphorin-plexin signaling, Proc. Natl. Acad. Sci. U. S. A. 106 (2009) 13933–13938.
[67] X. Lin, Z. Huo, X. Liu, Y. Zhang, L. Li, H. Zhao, B. Yan, Y. Liu, Y. Yang, Y.H. Chen, A novel GATA6 mutation in patients with tetralogy of Fallot or atrial septal defect, J. Hum. Genet. 55 (2010) 662–667.
[68] M. Maitra, S.N. Koenig, D. Srivastava, V. Garg, Identification of GATA6 sequence variants in patients with congenital heart defects, Pediatr. Res. 68 (2010) 281–285.
[69] J.H. van Berlo, J.W. Elrod, M.M. van den Hoogenhof, A.J. York, B.J. Aronow, S.A. Duncan, J.D. Molkentin, The transcription factor GATA-6 regulates pathological cardiac hypertrophy, Circ. Res. 107 (2010) 1032–1040.
[70] B. Laforest, M. Nemer, GATA5 interacts with GATA4 and GATA6 in outflow tract development, Dev. Biol. 358 (2011) 368–378.
[71] T.J. Lints, L.M. Parsons, L. Hartley, I. Lyons, R.P. Harvey, Nkx-2.5: a novel murine homeobox gene expressed in early heart progenitor cells and their myogenic descendants, Development 119 (1993) 969.
[72] E.G. Stanley, C. Biben, A. Elefanty, L. Barnett, F. Koentgen, L. Robb, R.P. Harvey, Efficient Cre-mediated deletion in cardiac progenitor cells conferred by a 3′ UTR-ires-Cre allele of the homeobox gene Nkx2-5, Int. J. Dev. Biol. 46 (2002) 431–439.
[73] I. Lyons, L.M. Parsons, L. Hartley, R. Li, J.E. Andrews, L. Robb, R.P. Harvey, Myogenic and morphogenetic defects in the heart tubes of murine embryos lacking the homeo box gene Nkx2-5, Genes Dev. 9 (1995) 1654–1666.
[74] D.W. Benson, G.M. Silberbach, A. Kavanaugh-McHugh, C. Cottrill, Y. Zhang, S. Riggs, O. Smalls, M.C. Johnson, M.S. Watson, J.G. Seidman, C.E. Seidman, J. Plowden, J.D. Kugler, Mutations in the cardiac transcription factor NKX2.5 affect diverse cardiac developmental pathways, J. Clin. Invest. 104 (1999) 1567–1573.
[75] S.M. Dinesh, L. Kusuma, R. Smitha, M.R. Savitha, B. Krishnamurthy, D. Narayanappa, N.B. Ramachandra, Single-nucleotide polymorphisms of NKX2.5 found in congenital heart disease patients of Mysore, South India, Genet. Test. Mol. Biomarkers 14 (2010) 873–879.
[76] D.A. Elliott, E.P. Kirk, T. Yeoh, S. Chandar, F. McKenzie, P. Taylor, P. Grossfeld, D. Fatkin, O. Jones, P. Hayes, M. Feneley, R.P. Harvey, Cardiac homeobox gene NKX2-5 mutations and congenital heart disease: associations with atrial septal defect and hypoplastic left heart syndrome, J. Am. Coll. Cardiol. 41 (2003) 2072–2076.
[77] L. Gioli-Pereira, A.C. Pereira, S.M. Mesquita, J. Xavier-Neto, A.A. Lopes, J.E. Krieger, NKX2.5 mutations in patients with non-syndromic congenital heart disease, Int. J. Cardiol. 138 (2010) 261–265.
[78] Y. Ikeda, Y. Hiroi, T. Hosoda, T. Utsunomiya, S. Matsuo, T. Ito, J. Inoue, T. Sumiyoshi, H. Takano, R. Nagai, I. Komuro, Novel point mutation in the cardiac transcription factor CSX/NKX2.5 associated with congenital heart disease, Circ. J. 66 (2002) 561–563.
[79] H. Kasahara, B. Lee, J.J. Schott, D.W. Benson, J.G. Seidman, C.E. Seidman, S. Izumo, Loss of function and inhibitory effects of human CSX/NKX2.5 homeoprotein mutations associated with congenital heart disease, J. Clin. Invest. 106 (2000) 299–308.
[80] D.B. McElhinney, E. Geiger, J. Blinder, D.W. Benson, E. Goldmuntz, NKX2.5 mutations in patients with congenital heart disease, J. Am. Coll. Cardiol. 42 (2003) 1650–1655.
[81] P. Ouyang, E. Saarel, Y. Bai, C. Luo, Q. Lv, Y. Xu, F. Wang, C. Fan, A. Younoszai, Q. Chen, X. Tu, Q.K. Wang, A de novo mutation in NKX2.5 associated with atrial septal defects, ventricular noncompaction, syncope and sudden death, Clin. Chim. Acta 412 (2011) 170–175.
[82] S.M. Reamon-Buettner, J. Borlak, Somatic NKX2-5 mutations as a novel mechanism of disease in complex congenital heart disease, J. Med. Genet. 41 (2004) 684–690.
[83] S.M. Reamon-Buettner, J. Borlak, NKX2-5: an update on this hypermutable homeodomain protein and its role in human congenital heart disease (CHD), Hum. Mutat. 31 (2010) 1185–1194.
[84] S.M. Reamon-Buettner, H. Hecker, K. Spanel-Borowski, S. Craatz, E. Kuenzel, J. Borlak, Novel NKX2-5 mutations in diseased heart tissues of patients with cardiac malformations, Am. J. Pathol. 164 (2004) 2117–2125.
[85] B. Stallmeyer, H. Fenge, U. Nowak-Gottl, E. Schulze-Bahr, Mutational spectrum in the cardiac transcription factor gene NKX2.5 (CSX) associated with congenital heart disease, Clin. Genet. 78 (2010) 533–540.
[86] J. Wang, X.Y. Liu, Y.Q. Yang, Novel NKX2-5 mutations responsible for congenital heart disease, Genet. Mol. Res. 10 (2011) 2905–2915.
[87] W. Zhu, I. Shiojima, Y. Hiroi, Y. Zou, H. Akazawa, M. Mizukami, H. Toko, Y. Yazaki, R. Nagai, I. Komuro, Functional analyses of three Csx/Nkx-2.5 mutations that cause human congenital heart disease, J. Biol. Chem. 275 (2000) 35291–35296.
[88] K. Heathcote, C. Braybrook, L. Abushaban, M. Guy, M.E. Khetyar, M.A. Patton, N.D. Carter, P.J. Scambler, P. Syrris, Common arterial trunk associated with a homeodomain mutation of NKX2.6, Hum. Mol. Genet. 14 (2005) 585–593.
[89] M. Tanaka, M. Schinke, H.S. Liao, N. Yamasaki, S. Izumo, Nkx2.5 and Nkx2.6, homologs of *Drosophila* tinman, are required for development of the pharynx, Mol. Cell. Biol. 21 (2001) 4391–4398.
[90] B.G. Bruneau, Z.Z. Bao, M. Tanaka, J.J. Schott, S. Izumo, C.L. Cepko, J.G. Seidman, C.E. Seidman, Cardiac expression of the ventricle-specific homeobox gene Irx4 is modulated by Nkx2-5 and dHand, Dev. Biol. 217 (2000) 266–277.
[91] M. Pashmforoush, J.T. Lu, H. Chen, T.S. Amand, R. Kondo, S. Pradervand, S.M. Evans, B. Clark, J.R. Feramisco, W. Giles, S.Y. Ho, D.W. Benson, M. Silberbach, W. Shou, K.R. Chien, Nkx2-5 pathways and congenital heart disease; loss of ventricular myocyte lineage specification leads to progressive cardiomyopathy and complete heart block, Cell 117 (2004) 373–386.
[92] K. Song, J. Backs, J. McAnally, X. Qi, R.D. Gerard, J.A. Richardson, J.A. Hill, R. Bassel-Duby, E.N. Olson, The transcriptional coactivator CAMTA2 stimulates cardiac growth by opposing class II histone deacetylases, Cell 125 (2006) 453–466.
[93] J.L. Sepulveda, S. Vlahopoulos, D. Iyer, N. Belaguli, R.J. Schwartz, Combinatorial expression of GATA4, Nkx2-5, and serum response factor directs early cardiac gene activity, J. Biol. Chem. 277 (2002) 25775–25782.
[94] P. ten Dijke, C.S. Hill, New insights into TGF-beta-Smad signalling, Trends Biochem. Sci. 29 (2004) 265–273.
[95] E.C. Delot, Control of endocardial cushion and cardiac valve maturation by BMP signaling pathways, Mol. Genet. Metab. 80 (2003) 27–35.
[96] Y. Nakajima, T. Yamagishi, S. Hokari, H. Nakamura, Mechanisms involved in valvuloseptal endocardial cushion formation in early cardiogenesis: roles of transforming growth factor (TGF)-beta and bone morphogenetic protein (BMP), Anat. Rec. 258 (2000) 119–127.
[97] K. Monzen, Y. Hiroi, S. Kudoh, H. Akazawa, T. Oka, E. Takimoto, D. Hayashi, T. Hosoda, M. Kawabata, K. Miyazono, S. Ishii, Y. Yazaki, R. Nagai, I. Komuro, Smads, TAK1, and their common target ATF-2 play a critical role in cardiomyocyte differentiation, J. Cell Biol. 153 (2001) 687–698.
[98] K.M. Galvin, M.J. Donovan, C.A. Lynch, R.I. Meyer, R.J. Paul, J.N. Lorenz, V. Fairchild-Huntress, K.L. Dixon, J.H. Dunmore, M.A. Gimbrone Jr., D. Falb, D. Huszar, A role for smad6 in development and homeostasis of the cardiovascular system, Nat. Genet. 24 (2000) 171–174.
[99] C.O. Brown III, X. Chi, E. Garcia-Gras, M. Shirai, X.H. Feng, R.J. Schwartz, The cardiac determination factor, Nkx2-5, is activated by mutual cofactors GATA-4 and Smad1/4 via a novel upstream enhancer, J. Biol. Chem. 279 (2004) 10659–10669.
[100] J. Hao, H. Ju, S. Zhao, A. Junaid, T. Scammell-La Fleur, I.M. Dixon, Elevation of expression of Smads 2, 3, and 4, decorin and TGF-beta in the chronic phase of myocardial infarct scar healing, J. Mol. Cell. Cardiol. 31 (1999) 667–678.
[101] B. Lei, H. Hitomi, T. Mori, Y. Nagai, K. Deguchi, H. Mori, T. Masaki, D. Nakano, H. Kobori, Y. Kitaura, A. Nishiyama, Effect of efonidipine on TGF-beta1-induced cardiac fibrosis through Smad2-dependent pathway in rat cardiac fibroblasts, J. Pharmacol. Sci. 117 (2011) 98–105.
[102] L.M. Sobral, F. Aseredo, M. Agostini, A. Bufalino, M.C. Pereira, E. Graner, R.D. Coletta, Molecular events associated with ciclosporin A-induced gingival overgrowth are attenuated by Smad7 overexpression in fibroblasts, J. Periodontal Res. 47 (2012) 149–158.
[103] J. Hao, B. Wang, S.C. Jones, D.S. Jassal, I.M. Dixon, Interaction between angiotensin II and Smad proteins in fibroblasts in failing heart and in vitro, Am. J. Physiol. Heart Circ. Physiol. 279 (2000) H3020–H3030.
[104] W.W. Brooks, C.H. Conrad, Myocardial fibrosis in transforming growth factor beta(1)heterozygous mice, J. Mol. Cell. Cardiol. 32 (2000) 187–195.
[105] M. Izumi, Y. Fujio, K. Kunisada, S. Negoro, E. Tone, M. Funamoto, T. Osugi, Y. Oshima, Y. Nakaoka, T. Kishimoto, K. Yamauchi-Takihara, H. Hirota, Bone morphogenetic protein-2 inhibits serum deprivation-induced apoptosis of neonatal cardiac myocytes through activation of the Smad1 pathway, J. Biol. Chem. 276 (2001) 31133–31141.
[106] A. Ishisaki, K. Yamato, S. Hashimoto, A. Nakao, K. Tamaki, K. Nonaka, P. ten Dijke, H. Sugino, T. Nishihara, Differential inhibition of Smad6 and Smad7 on bone morphogenetic protein- and activin-mediated growth arrest and apoptosis in B cells, J. Biol. Chem. 274 (1999) 13637–13642.

[107] J. Wang, N. Xu, X. Feng, N. Hou, J. Zhang, X. Cheng, Y. Chen, Y. Zhang, X. Yang, Targeted disruption of Smad4 in cardiomyocytes results in cardiac hypertrophy and heart failure, Circ. Res. 97 (2005) 821–828.
[108] J. Xu, T.R. Kimball, J.N. Lorenz, D.A. Brown, A.R. Bauskin, R. Klevitsky, T.E. Hewett, S.N. Breit, J.D. Molkentin, GDF15/MIC-1 functions as a protective and antihypertrophic factor released from the myocardium in association with SMAD protein activation, Circ. Res. 98 (2006) 342–350.
[109] D. Zhang, V. Gaussin, G.E. Taffet, N.S. Belaguli, M. Yamada, R.J. Schwartz, L.H. Michael, P.A. Overbeek, M.D. Schneider, TAK1 is activated in the myocardium after pressure overload and is sufficient to provoke heart failure in transgenic mice, Nat. Med. 6 (2000) 556–563.
[110] S. Arsenian, B. Weinhold, M. Oelgeschlager, U. Ruther, A. Nordheim, Serum response factor is essential for mesoderm formation during mouse embryogenesis, EMBO J. 17 (1998) 6289–6299.
[111] X. Zhang, J. Chai, G. Azhar, P. Sheridan, A.M. Borras, M.C. Furr, K. Khrapko, J. Lawitts, R.P. Misra, J.Y. Wei, Early postnatal cardiac changes and premature death in transgenic mice overexpressing a mutant form of serum response factor, J. Biol. Chem. 276 (2001) 40033–40040.
[112] J.M. Miano, N. Ramanan, M.A. Georger, K.L. de Mesy Bentley, R.L. Emerson, R.O. Balza Jr., Q. Xiao, H. Weiler, D.D. Ginty, R.P. Misra, Restricted inactivation of serum response factor to the cardiovascular system, Proc. Natl. Acad. Sci. U. S. A. 101 (2004) 17132–17137.
[113] A. Parlakian, D. Tuil, G. Hamard, G. Tavernier, D. Hentzen, J.P. Concordet, D. Paulin, Z. Li, D. Daegelen, Targeted inactivation of serum response factor in the developing heart results in myocardial defects and embryonic lethality, Mol. Cell. Biol. 24 (2004) 5281–5289.
[114] Z. Niu, W. Yu, S.X. Zhang, M. Barron, N.S. Belaguli, M.D. Schneider, M. Parmacek, A. Nordheim, R.J. Schwartz, Conditional mutagenesis of the murine serum response factor gene blocks cardiogenesis and the transcription of downstream gene targets, J. Biol. Chem. 280 (2005) 32531–32538.
[115] J. Fielitz, E. van Rooij, J.A. Spencer, J.M. Shelton, S. Latif, R. van der Nagel, S. Bezprozvannaya, L. de Windt, J.A. Richardson, R. Bassel-Duby, E.N. Olson, Loss of muscle-specific RING-finger 3 predisposes the heart to cardiac rupture after myocardial infarction, Proc. Natl. Acad. Sci. U. S. A. 104 (2007) 4377–4382.
[116] M. Touvron, B. Escoubet, M. Mericskay, A. Angelini, L. Lamotte, M.P. Santini, N. Rosenthal, D. Daegelen, D. Tuil, J.F. Decaux, Locally expressed IGF1 propeptide improves mouse heart function in induced dilated cardiomyopathy by blocking myocardial fibrosis and SRF-dependent CTGF induction, Dis. Models Mech. 5 (2012) 481–491.
[117] R.O. Balza Jr., R.P. Misra, Role of the serum response factor in regulating contractile apparatus gene expression and sarcomeric integrity in cardiomyocytes, J. Biol. Chem. 281 (2006) 6498–6510.
[118] A. Parlakian, C. Charvet, B. Escoubet, M. Mericskay, J.D. Molkentin, G. Gary-Bobo, L.J. De Windt, M.A. Ludosky, D. Paulin, D. Daegelen, D. Tuil, Z. Li, Temporally controlled onset of dilated cardiomyopathy through disruption of the SRF gene in adult heart, Circulation 112 (2005) 2930–2939.
[119] A. Serose, B. Prudhon, A. Salmon, M.A. Doyennette, M.Y. Fiszman, Y. Fromes, Administration of insulin-like growth factor-1 (IGF-1) improves both structure and function of delta-sarcoglycan deficient cardiac muscle in the hamster, Basic Res. Cardiol. 100 (2005) 161–170.
[120] M.Y. Donath, E.R. Froesch, J. Zapf, Insulin-like growth factor I and cardiac performance in heart failure, Growth Horm. IGF Res. 8 (Suppl. B) (1998) 167–170.
[121] F.J. Davis, M. Gupta, S.M. Pogwizd, E. Bacha, V. Jeevanandam, M.P. Gupta, Increased expression of alternatively spliced dominant-negative isoform of SRF in human failing hearts, Am. J. Physiol. Heart Circ. Physiol. 282 (2002) H1521–H1533.
[122] J. Chang, L. Wei, T. Otani, K.A. Youker, M.L. Entman, R.J. Schwartz, Inhibitory cardiac transcription factor, SRF-N, is generated by caspase 3 cleavage in human heart failure and attenuated by ventricular unloading, Circulation 108 (2003) 407–413.
[123] X. Zhang, G. Azhar, S.A. Helms, J.Y. Wei, Regulation of cardiac microRNAs by serum response factor, J. Biomed. Sci. 18 (2011) 15.
[124] T.P. Yao, S.P. Oh, M. Fuchs, N.D. Zhou, L.E. Ch'ng, D. Newsome, R.T. Bronson, E. Li, D.M. Livingston, R. Eckner, Gene dosage-dependent embryonic development and proliferation defects in mice lacking the transcriptional integrator p300, Cell 93 (1998) 361–372.
[125] N. Shikama, W. Lutz, R. Kretzschmar, N. Sauter, J.F. Roth, S. Marino, J. Wittwer, A. Scheidweiler, R. Eckner, Essential function of p300 acetyltransferase activity in heart, lung and small intestine formation, EMBO J. 22 (2003) 5175–5185.
[126] R.J. Gusterson, E. Jazrawi, I.M. Adcock, D.S. Latchman, The transcriptional co-activators CREB-binding protein (CBP) and p300 play a critical role in cardiac hypertrophy that is dependent on their histone acetyltransferase activity, J. Biol. Chem. 278 (2003) 6838–6847.
[127] R. Gusterson, B. Brar, D. Faulkes, A. Giordano, J. Chrivia, D. Latchman, The transcriptional co-activators CBP and p300 are activated via phenylephrine through the p42/p44 MAPK cascade, J. Biol. Chem. 277 (2002) 2517–2524.
[128] T. Yanazume, K. Hasegawa, T. Morimoto, T. Kawamura, H. Wada, A. Matsumori, Y. Kawase, M. Hirai, T. Kita, Cardiac p300 is involved in myocyte growth with decompensated heart failure, Mol. Cell. Biol. 23 (2003) 3593–3606.
[129] Y.S. Dai, B.E. Markham, p300 Functions as a coactivator of transcription factor GATA-4, J. Biol. Chem. 276 (2001) 37178–37185.
[130] T. Kakita, K. Hasegawa, T. Morimoto, S. Kaburagi, H. Wada, S. Sasayama, p300 protein as a coactivator of GATA-5 in the transcription of cardiac-restricted atrial natriuretic factor gene, J. Biol. Chem. 274 (1999) 34096–34102.
[131] T.I. Slepak, K.A. Webster, J. Zang, H. Prentice, A. O'Dowd, M.N. Hicks, N.H. Bishopric, Control of cardiac-specific transcription by p300 through myocyte enhancer factor-2D, J. Biol. Chem. 276 (2001) 7575–7585.
[132] V. Sartorelli, J. Huang, Y. Hamamori, L. Kedes, Molecular mechanisms of myogenic coactivation by p300: direct interaction with the activation domain of MyoD and with the MADS box of MEF2C, Mol. Cell. Biol. 17 (1997) 1010–1026.
[133] S. Minucci, P.G. Pelicci, Histone deacetylase inhibitors and the promise of epigenetic (and more) treatments for cancer, Nat. Rev. Cancer 6 (2006) 38–51.
[134] P.A. Marks, Histone deacetylase inhibitors: a chemical genetics approach to understanding cellular functions, Biochim. Biophys. Acta 1799 (2010) 717–725.
[135] T.G. Nishino, M. Miyazaki, H. Hoshino, Y. Miwa, S. Horinouchi, M. Yoshida, 14-3-3 regulates the nuclear import of class IIa histone deacetylases, Biochem. Biophys. Res. Commun. 377 (2008) 852–856.
[136] C.M. Grozinger, S.L. Schreiber, Regulation of histone deacetylase 4 and 5 and transcriptional activity by 14-3-3-dependent cellular localization, Proc. Natl. Acad. Sci. U. S. A. 97 (2000) 7835–7840.
[137] P. Ma, H. Pan, R.L. Montgomery, E.N. Olson, R.M. Schultz, Compensatory functions of histone deacetylase 1 (HDAC1) and HDAC2 regulate transcription and apoptosis during mouse oocyte development, Proc. Natl. Acad. Sci. U. S. A. 109 (2012) E481–E489.
[138] F. Ye, Y. Chen, T. Hoang, R.L. Montgomery, X.H. Zhao, H. Bu, T. Hu, M.M. Taketo, J.H. van Es, H. Clevers, J. Hsieh, R. Bassel-Duby, E.N. Olson, Q.R. Lu, HDAC1 and HDAC2 regulate oligodendrocyte differentiation by disrupting the beta-catenin–TCF interaction, Nat. Neurosci. 12 (2009) 829–838.
[139] H. Kook, J.J. Lepore, A.D. Gitler, M.M. Lu, W. Wing-Man Yung, J. Mackay, R. Zhou, V. Ferrari, P. Gruber, J.A. Epstein, Cardiac hypertrophy and histone deacetylase-dependent transcriptional repression mediated by the atypical homeodomain protein Hop, J. Clin. Invest. 112 (2003) 863–871.
[140] R.L. Montgomery, M.J. Potthoff, M. Haberland, X. Qi, S. Matsuzaki, K.M. Humphries, J.A. Richardson, R. Bassel-Duby, E.N. Olson, Maintenance of cardiac energy metabolism by histone deacetylase 3 in mice, J. Clin. Invest. 118 (2008) 3588–3597.
[141] Z. Sun, N. Singh, S.E. Mullican, L.J. Everett, L. Li, L. Yuan, X. Liu, J.A. Epstein, M.A. Lazar, Diet-induced lethality due to deletion of the Hdac3 gene in heart and skeletal muscle, J. Biol. Chem. 286 (2011) 33301–33309.
[142] S. Gregoire, L. Xiao, J. Nie, X. Zhang, M. Xu, J. Li, J. Wong, E. Seto, X.J. Yang, Histone deacetylase 3 interacts with and deacetylates myocyte enhancer factor 2, Mol. Cell. Biol. 27 (2007) 1280–1295.
[143] C.L. Zhang, T.A. McKinsey, S. Chang, C.L. Antos, J.A. Hill, E.N. Olson, Class II histone deacetylases act as signal-responsive repressors of cardiac hypertrophy, Cell 110 (2002) 479–488.
[144] S. Chang, T.A. McKinsey, C.L. Zhang, J.A. Richardson, J.A. Hill, E.N. Olson, Histone deacetylases 5 and 9 govern responsiveness of the heart to a subset of stress signals and play redundant roles in heart development, Mol. Cell. Biol. 24 (2004) 8467–8476.
[145] J.D. Molkentin, J.R. Lu, C.L. Antos, B. Markham, J. Richardson, J. Robbins, S.R. Grant, E.N. Olson, A calcineurin-dependent transcriptional pathway for cardiac hypertrophy, Cell 93 (1998) 215–228.
[146] H.D. Youn, C.M. Grozinger, J.O. Liu, Calcium regulates transcriptional repression of myocyte enhancer factor 2 by histone deacetylase 4, J. Biol. Chem. 275 (2000) 22563–22567.
[147] H.D. Youn, L. Sun, R. Prywes, J.O. Liu, Apoptosis of T cells mediated by Ca^{2+}-induced release of the transcription factor MEF2, Science 286 (1999) 790–793.
[148] M. Torrado, E. Lopez, A. Centeno, C. Medrano, A. Castro-Beiras, A.T. Mikhailov, Myocardin mRNA is augmented in the failing myocardium: expression profiling in the porcine model and human dilated cardiomyopathy, J. Mol. Med. (Berl.) 81 (2003) 566–577.
[149] D. Cao, Z. Wang, C.L. Zhang, J. Oh, W. Xing, S. Li, J.A. Richardson, D.Z. Wang, E.N. Olson, Modulation of smooth muscle gene expression by association of histone acetyltransferases and deacetylases with myocardin, Mol. Cell. Biol. 25 (2005) 364–376.
[150] F.J. Davis, M. Gupta, B. Camoretti-Mercado, R.J. Schwartz, M.P. Gupta, Calcium/calmodulin-dependent protein kinase activates serum response factor transcription activity by its dissociation from histone deacetylase, HDAC4. Implications in cardiac muscle gene regulation during hypertrophy, J. Biol. Chem. 278 (2003) 20047–20058.
[151] K. Watamoto, M. Towatari, Y. Ozawa, Y. Miyata, M. Okamoto, A. Abe, T. Naoe, H. Saito, Altered interaction of HDAC5 with GATA-1 during MEL cell differentiation, Oncogene 22 (2003) 9176–9184.
[152] Y.S. Dai, J. Xu, J.D. Molkentin, The DnaJ-related factor Mrj interacts with nuclear factor of activated T cells c3 and mediates transcriptional repression through class II histone deacetylase recruitment, Mol. Cell. Biol. 25 (2005) 9936–9948.
[153] M.C. Haigis, L.P. Guarente, Mammalian sirtuins—emerging roles in physiology, aging, and calorie restriction, Genes Dev. 20 (2006) 2913–2921.
[154] R.R. Alcendor, S. Gao, P. Zhai, D. Zablocki, E. Holle, X. Yu, B. Tian, T. Wagner, S.F. Vatner, J. Sadoshima, Sirt1 regulates aging and resistance to oxidative stress in the heart, Circ. Res. 100 (2007) 1512–1521.
[155] K. Shinmura, K. Tamaki, R. Bolli, Impact of 6-mo caloric restriction on myocardial ischemic tolerance: possible involvement of nitric oxide-dependent increase in nuclear Sirt1, Am. J. Physiol. Heart Circ. Physiol. 295 (2008) H2348–H2355.
[156] R.R. Alcendor, L.A. Kirshenbaum, S. Imai, S.F. Vatner, J. Sadoshima, Silent information regulator 2alpha, a longevity factor and class III histone deacetylase, is an essential endogenous apoptosis inhibitor in cardiac myocytes, Circ. Res. 95 (2004) 971–980.
[157] M. Hohl, M. Wagner, J.C. Reil, S.A. Muller, M. Tauchnitz, A.M. Zimmer, L.H. Lehmann, G. Thiel, M. Bohm, J. Backs, C. Maack, HDAC4 controls histone methylation in response to elevated cardiac load, J. Clin. Invest. 123 (2013) 1359–1370.
[158] H. Shirato, S. Ogawa, K. Nakajima, M. Inagawa, M. Kojima, M. Tachibana, Y. Shinkai, T. Takeuchi, A jumonji (Jarid2) protein complex represses cyclin D1 expression by methylation of histone H3-K9, J. Biol. Chem. 284 (2009) 733–739.

[159] Y. Lee, A.J. Song, R. Baker, B. Micales, S.J. Conway, G.E. Lyons, Jumonji, a nuclear protein that is necessary for normal heart development, Circ. Res. 86 (2000) 932–938.
[160] T. Takeuchi, M. Kojima, K. Nakajima, S. Kondo, jumonji gene is essential for the neurulation and cardiac development of mouse embryos with a C3H/He background, Mech. Dev. 86 (1999) 29–38.
[161] T.G. Kim, J. Chen, J. Sadoshima, Y. Lee, Jumonji represses atrial natriuretic factor gene expression by inhibiting transcriptional activities of cardiac transcription factors, Mol. Cell. Biol. 24 (2004) 10151–10160.
[162] T.G. Kim, J. Jung, M.R. Mysliwiec, S. Kang, Y. Lee, Jumonji represses alpha-cardiac myosin heavy chain expression via inhibiting MEF2 activity, Biochem. Biophys. Res. Commun. 329 (2005) 544–553.
[163] E. Bernstein, S.Y. Kim, M.A. Carmell, E.P. Murchison, H. Alcorn, M.Z. Li, A.A. Mills, S.J. Elledge, K.V. Anderson, G.J. Hannon, Dicer is essential for mouse development, Nat. Genet. 35 (2003) 215–217.
[164] Y. Wang, R. Medvid, C. Melton, R. Jaenisch, R. Blelloch, DGCR8 is essential for microRNA biogenesis and silencing of embryonic stem cell self-renewal, Nat. Genet. 39 (2007) 380–385.
[165] T. Fukuda, K. Yamagata, S. Fujiyama, T. Matsumoto, I. Koshida, K. Yoshimura, M. Mihara, M. Naitou, H. Endoh, T. Nakamura, C. Akimoto, Y. Yamamoto, T. Katagiri, C. Foulds, S. Takezawa, H. Kitagawa, K. Takeyama, B.W. O'Malley, S. Kato, DEAD-box RNA helicase subunits of the Drosha complex are required for processing of rRNA and a subset of microRNAs, Nat. Cell Biol. 9 (2007) 604–611.
[166] S. Morita, T. Horii, M. Kimura, Y. Goto, T. Ochiya, I. Hatada, One Argonaute family member, Eif2c2 (Ago2), is essential for development and appears not to be involved in DNA methylation, Genomics 89 (2007) 687–696.
[167] B.D. Harfe, M.T. McManus, J.H. Mansfield, E. Hornstein, C.J. Tabin, The RNaseIII enzyme Dicer is required for morphogenesis but not patterning of the vertebrate limb, Proc. Natl. Acad. Sci. U. S. A. 102 (2005) 10898–10903.
[168] P.A. da Costa Martins, M. Bourajjaj, M. Gladka, M. Kortland, R.J. van Oort, Y.M. Pinto, J.D. Molkentin, L.J. De Windt, Conditional dicer gene deletion in the postnatal myocardium provokes spontaneous cardiac remodeling, Circulation 118 (2008) 1567–1576.
[169] E. Chapnik, V. Sasson, R. Blelloch, E. Hornstein, Dgcr8 controls neural crest cells survival in cardiovascular development, Dev. Biol. 362 (2012) 50–56.
[170] T.E. Callis, D.Z. Wang, Taking microRNAs to heart, Trends Mol. Med. 14 (2008) 254–260.
[171] J.F. Chen, E.M. Mandel, J.M. Thomson, Q. Wu, T.E. Callis, S.M. Hammond, F.L. Conlon, D.Z. Wang, The role of microRNA-1 and microRNA-133 in skeletal muscle proliferation and differentiation, Nat. Genet. 38 (2006) 228–233.
[172] E. Wienholds, W.P. Kloosterman, E. Miska, E. Alvarez-Saavedra, E. Berezikov, E. de Bruijn, H.R. Horvitz, S. Kauppinen, R.H. Plasterk, MicroRNA expression in zebrafish embryonic development, Science 309 (2005) 310–311.
[173] C. Kwon, Z. Han, E.N. Olson, D. Srivastava, MicroRNA1 influences cardiac differentiation in *Drosophila* and regulates Notch signaling, Proc. Natl. Acad. Sci. U. S. A. 102 (2005) 18986–18991.
[174] Y. Zhao, E. Samal, D. Srivastava, Serum response factor regulates a muscle-specific microRNA that targets Hand2 during cardiogenesis, Nature 436 (2005) 214–220.
[175] D. Catalucci, M.V. Latronico, G. Condorelli, MicroRNAs control gene expression: importance for cardiac development and pathophysiology, Ann. N. Y. Acad. Sci. 1123 (2008) 20–29.
[176] Y. Zhao, J.F. Ransom, A. Li, V. Vedantham, M. von Drehle, A.N. Muth, T. Tsuchihashi, M.T. McManus, R.J. Schwartz, D. Srivastava, Dysregulation of cardiogenesis, cardiac conduction, and cell cycle in mice lacking miRNA-1–2, Cell 129 (2007) 303–317.
[177] D.G. McFadden, A.C. Barbosa, J.A. Richardson, M.D. Schneider, D. Srivastava, E.N. Olson, The Hand1 and Hand2 transcription factors regulate expansion of the embryonic cardiac ventricles in a gene dosage-dependent manner, Development 132 (2005) 189–201.
[178] V.P. Yin, A. Lepilina, A. Smith, K.D. Poss, Regulation of zebrafish heart regeneration by miR-133, Dev. Biol. 365 (2012) 319–327.
[179] Z. Niu, A. Li, S.X. Zhang, R.J. Schwartz, Serum response factor micromanaging cardiogenesis, Curr. Opin. Cell Biol. 19 (2007) 618–627.
[180] K.N. Ivey, A. Muth, J. Arnold, F.W. King, R.F. Yeh, J.E. Fish, E.C. Hsiao, R.J. Schwartz, B.R. Conklin, H.S. Bernstein, D. Srivastava, MicroRNA regulation of cell lineages in mouse and human embryonic stem cells, Cell Stem Cell 2 (2008) 219–229.
[181] D. Sayed, C. Hong, I.Y. Chen, J. Lypowy, M. Abdellatif, MicroRNAs play an essential role in the development of cardiac hypertrophy, Circ. Res. 100 (2007) 416–424.
[182] A. Care, D. Catalucci, F. Felicetti, D. Bonci, A. Addario, P. Gallo, M.L. Bang, P. Segnalini, Y. Gu, N.D. Dalton, L. Elia, M.V. Latronico, M. Hoydal, C. Autore, M.A. Russo, G.W. Dorn II, O. Ellingsen, P. Ruiz-Lozano, K.L. Peterson, C.M. Croce, C. Peschle, G. Condorelli, MicroRNA-133 controls cardiac hypertrophy, Nat. Med. 13 (2007) 613–618.
[183] S. Ikeda, A. He, S.W. Kong, J. Lu, R. Bejar, N. Bodyak, K.H. Lee, Q. Ma, P.M. Kang, T.R. Golub, W.T. Pu, MicroRNA-1 negatively regulates expression of the hypertrophy-associated calmodulin and Mef2a genes, Mol. Cell. Biol. 29 (2009) 2193–2204.
[184] S.V. Naga Prasad, Z.H. Duan, M.K. Gupta, V.S. Surampudi, S. Volinia, G.A. Calin, C.G. Liu, A. Kotwal, C.S. Moravec, R.C. Starling, D.M. Perez, S. Sen, Q. Wu, E.F. Plow, C.M. Croce, S. Karnik, Unique microRNA profile in end-stage heart failure indicates alterations in specific cardiovascular signaling networks, J. Biol. Chem. 284 (2009) 27487–27499.
[185] S.J. Matkovich, D.J. Van Booven, K.A. Youker, G. Torre-Amione, A. Diwan, W.H. Eschenbacher, L.E. Dorn, M.A. Watson, K.B. Margulies, G.W. Dorn II, Reciprocal regulation of myocardial microRNAs and messenger RNA in human cardiomyopathy and reversal of the microRNA signature by biomechanical support, Circulation 119 (2009) 1263–1271.
[186] R. Kumarswamy, A.R. Lyon, I. Volkmann, A.M. Mills, J. Bretthauer, A. Pahuja, C. Geers-Knorr, T. Kraft, R.J. Hajjar, K.T. Macleod, S.E. Harding, T. Thum, SERCA2a gene therapy restores microRNA-1 expression in heart failure via an Akt/FoxO3A-dependent pathway, Eur. Heart J. 33 (2012) 1067–1075.
[187] Q. Li, X. Lin, X. Yang, J. Chang, NFATc4 is negatively regulated in miR-133a-mediated cardiomyocyte hypertrophic repression, Am. J. Physiol. Heart Circ. Physiol. 298 (2010) H1340–H1347.
[188] T.E. Callis, K. Pandya, H.Y. Seok, R.H. Tang, M. Tatsuguchi, Z.P. Huang, J.F. Chen, Z. Deng, B. Gunn, J. Shumate, M.S. Willis, C.H. Selzman, D.Z. Wang, MicroRNA-208a is a regulator of cardiac hypertrophy and conduction in mice, J. Clin. Invest. 119 (2009) 2772–2786.
[189] E. van Rooij, E.N. Olson, MicroRNAs: powerful new regulators of heart disease and provocative therapeutic targets, J. Clin. Invest. 117 (2007) 2369–2376.
[190] P. Landgraf, M. Rusu, R. Sheridan, A. Sewer, N. Iovino, A. Aravin, S. Pfeffer, A. Rice, A.O. Kamphorst, M. Landthaler, C. Lin, N.D. Socci, L. Hermida, V. Fulci, S. Chiaretti, R. Foa, J. Schliwka, U. Fuchs, A. Novosel, R.U. Muller, B. Schermer, U. Bissels, J. Inman, Q. Phan, M. Chien, D.B. Weir, R. Choksi, G. De Vita, D. Frezzetti, H.I. Trompeter, V. Hornung, G. Teng, G. Hartmann, M. Palkovits, R. Di Lauro, P. Wernet, G. Macino, C.E. Rogler, J.W. Nagle, J. Ju, F.N. Papavasiliou, T. Benzing, P. Lichter, W. Tam, M.J. Brownstein, A. Bosio, A. Borkhardt, J.J. Russo, C. Sander, M. Zavolan, T. Tuschl, A mammalian microRNA expression atlas based on small RNA library sequencing, Cell 129 (2007) 1401–1414.
[191] E. van Rooij, D. Quiat, B.A. Johnson, L.B. Sutherland, X. Qi, J.A. Richardson, R.J. Kelm Jr., E.N. Olson, A family of microRNAs encoded by myosin genes governs myosin expression and muscle performance, Dev. Cell 17 (2009) 662–673.