Biochimica et Biophysica Acta xxx (2013) xxx–xxx

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 xxxx

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

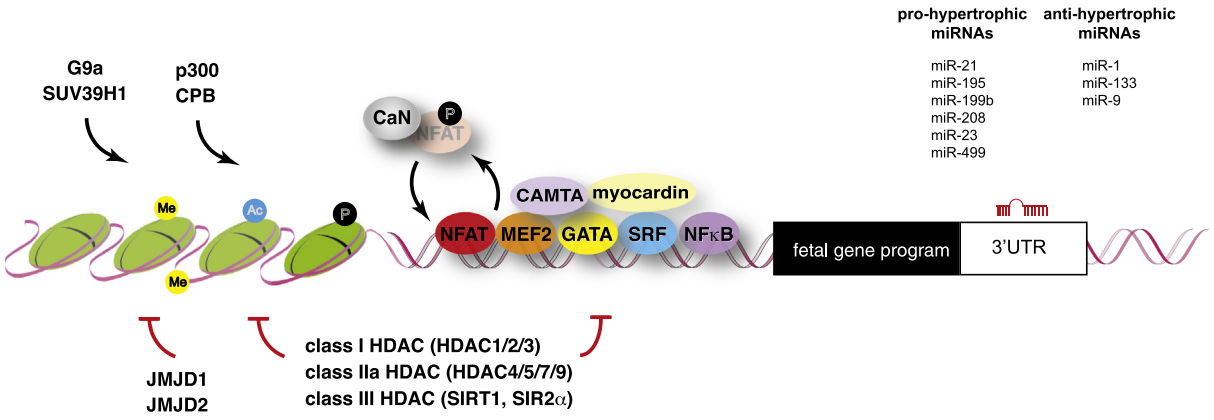

Fig. 1. Simplified overview of the gene regulatory mechanisms underlying re-expression of fetal gene programs in the adult myocardium. Epigenetic regulators influence chromatin condensation surrounding fetal genes, including the methylation status of chromosomal DNA and methylation, acetylation and phosphorylation 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

Please cite this article as: E. Dirkx, et al., Regulation of fetal gene expression in heart failure, Biochim. Biophys. Acta (2013), http://dx.doi.org/10.1016/j.bbadis.2013.07.023