# Chapter 1

Introduction

# INTRODUCTION

### *Cardiac hypertrophy and heart failure*

Heart failure, defined as the inability of the heart to cope with the metabolic demands of the peripheral tissues, represents the fastest growing subclass of cardiovascular diseases [1-3]. Because of the epidemic proportions it has taken in the past decades, medical and scientific interest in heart failure has been greatly stimulated [4]. Heart failure is the common end-stage for various forms of heart disease with heterogeneous etiologies. The single most powerful predictor of heart failure is sustained cardiac hypertrophy [5, 6]. For this reason, much effort is currently dedicated to elucidate the cellular and molecular determinants underlying hypertrophic remodeling of the heart.

Embryonic growth of the heart occurs primarily through proliferation of cardiac myocytes. Soon after birth, cardiomyocytes withdraw from the cell cycle and cardiac growth can only occur by an increase in size of the individual myocytes (hypertrophy). While this is part of normal postnatal development and thought to initially be a beneficial response of the adult heart in order to adapt to stress stimuli, it is often accompanied by reactivation of a “fetal” cardiac gene program and contributes to heart failure development [7-10]. Clinical conditions that induce cardiac hypertrophy include chronic hypertension, myocardial infarction, coronary artery disease, valvular defects, myocarditis, endocrine disorders and contractile dysfunction from inherited mutations in cardiac structural genes [4, 11-13]. Pathological hypertrophy frequently progresses into dilated cardiomyopathy, a chronic disorder of the heart muscle [6]. In humans, end-stage dilated cardiomyopathy is characterized by poorly contractile and dilated ventricles, accompanied by lengthening of individual cardiomyocytes, cardiomyocyte side-to-side slippage, loss of myofibrils, severe changes in cytoskeletal architecture and accumulation of collagen in perivascular and interstitial spaces (fibrosis) [14, 15].

The genetic underpinnings underlying these pathological myocardial alterations remain largely elusive. The initial hypertrophic response is evoked by activation of several intracellular signaling cascades [11, 16, 17]. These signaling pathways are interconnected and often culminate in the nucleus on only a few transcriptional regulators [18-20]. With the hope of revealing novel therapeutic targets in the treatment of heart failure, much effort is undertaken to unravel the role of these select transcription factors. One of the common downstream targets for several stress cascades in the heart is the transcription factor family known as myocyte enhancer factor-2 (MEF2).

***Myocyte enhancer factor-2***

MEF2 was originally identified as a DNA binding activity that recognizes conserved A/T-rich elements associated with numerous muscle-specific genes [21]. During the years, four MEF2 genes, *Mef2a*, *-b*, *-c*, and *-d*, have been characterized in vertebrates, with an expression pattern and function that extends beyond striated muscle. MEF2 transcription factors share high homology in an amino-terminal MADS (MCM1, Agamous, Deficiens, and Serum response factor) box and an adjacent MEF2-specific domain, which together mediate dimerization and binding to the consensus DNA sequence $CTA(A/T)_4TA(G/A)$ [22, 23]. Before recognition of the consensus site, MEF2 factors must homo- or hetero-dimerize, but given the extensive alternative splicing that occurs within the four MEF2 isoforms, many associations are possible among these factors [22].

By now, MEF2 factors have been implicated in the embryonic development of cardiac, skeletal, and smooth muscle [24, 25], and several lines of evidence suggest involvement in the progress to postnatal heart disease [18]. In general, *Mef2a-d* genes are widely expressed in the adult vertebrate organism, although a number of specific regulatory functions have been identified in immune, skeletal muscle, cardiac muscle, and neuronal cells [26-28].

MEF2 factors are related to another MADS-box containing transcription factor known as serum response factor (SRF) [29]. In the heart, myocytes undergo developmental and pathophysiological hypertrophy in response to neuro-endocrine-, mitogen- and stress-stimulation. These types of stimulation activate intracellular signal transduction cascades resulting in the modification of transcription factor activity and the reprogramming of cardiac gene expression. Similar to SRF, members of the MEF2 family have been implicated in regulating inducible gene expression in response to such stimuli [22, 29, 30]. A number of lines of evidence suggest that MEF2 factors might regulate gene expression in response to stimuli that underlie the cardiac hypertrophic response.

***MEF2 in cardiac hypertrophy***

In normal adult myocardium, MEF2 exhibits only basal activity, which is likely required for the maintenance of the cardiomyocyte contractile apparatus and energy metabolism [31, 32]. In cardiac disease, however, circumstantial evidence suggests that MEF2 factors serve as a transcriptional platform for prohypertrophic signaling cascades, including calcineurin, calcium/calmodulin-dependent protein kinase (CaMK), big mitogen-activated protein kinase (MAPK)-1 (BMK-1), and p38 MAPK [33, 34]. Additionally, stress signaling stimulates MEF2 transcriptional activity by causing the nuclear export of class II histone deacetylases (HDACs), which directly interact with MEF2 factors and suppress their activity [35]. Nuclear export of class II HDACs is mediated by stress-responsive protein kinases

including CaMK, protein kinase C (PKC) and protein kinase D (PKD), which promote the dissociation of class II HDACs from MEF2 and de-repress MEF2 activity [32, 36, 37]. In line, mice lacking HDAC9 and/or HDAC5 are hypersensitive to cardiac stress signaling and display excessive cardiac growth and fetal gene induction [38]. Additional evidence that MEF2 regulates stress-responsive signaling derives from the use of a MEF2 β-galactosidase reporter transgene [39], which showed enhanced staining in hypertrophic hearts from calcineurin and CaMK transgenic mice, and in HDAC9 knockout mice [32, 38].

Furthermore, MEF2 has been implicated in a more physiological model for hypertrophy as its DNA binding activity was shown to be increased in hearts of rats subjected to both pressure and volume overload [34, 40]. MEF2 DNA binding activity was also shown to be enhanced in hearts from mdx:Myod$^{-/-}$ mice, a murine model for muscle dystrophy [41].

Moreover, *Mef2c* null mice have altered cardiac gene expression and die during early embryonic development with arrested heart tube morphogenesis, suggesting a critical role in developmental heart growth [28]. Mice expressing a dominant negative mutant of MEF2C in the heart also die during postnatal development with attenuated ventricular growth [41]. Lastly, a portion of *Mef2a* null mice die suddenly during the perinatal period with dilated right ventricles, myofibrillar disorganization, and mitochondrial structural abnormalities [31].

Despite these circumstantial observations that suggest a role for MEF2 factors as transcriptional integrators for cardiac hypertrophic growth, a direct genetic assessment of MEF2 activation in adult cardiac muscle remains to be tested.

## OUTLINE OF THIS THESIS

In the present thesis, we report the existence of synergistic interactions between the NFAT and MEF2 transcription factors in the heart triggered by calcineurin (CnA) signaling. To circumvent the embryonic-lethality and mitochondrial deficiency associated with germline null mutations for MEF2C and MEF2A, respectively, we used conditional transgenesis to express a dominant-negative form of MEF2 (DNMEF2) in the murine postnatal heart and combined this with magnetic resonance imaging to investigate MEF2 transcriptional function in $Ca^{2+}$/CnA induced cardiac remodeling. Surprisingly, end-diastolic and end-systolic ventricular dimensions and contractility were normalized in the presence of severely hypertrophied LV walls upon MEF2 inhibition in calcineurin transgenic mice. In line, we generated lines of transgenic mice expressing MEF2A in the heart, which primarily displayed chamber dilation. Microarray profiling indicated that

MEF2 promotes a gene profile functioning primarily to or at the nucleus, the cytoskeletal and microtubular network, and mitochondria **(Chapter 2)**.

To test the mechanistic underpinnings how MEF2 transcriptional activity may promote features of dilated cardiomyopathy, we reverted to an *in vitro* cardiomyocyte culture system. Cultured ventricular myocytes subjected to adenoviral delivery of wildtype MEF2A or constitutively active MEF2 primarily underwent myocyte elongation, accompanied by sequential loss of myofibrils and redistribution of focal adhesion sites. Genome-wide gene chip analysis in a ventricular muscle cell line with inducible MEF2 activation provided further mechanistic insights into MEF2 function and target genes. Functional clustering of MEF2 target genes revealed an overrepresentation of genes involved in cytoskeletal remodeling and cell-matrix adhesion. We describe myotonic dystrophy protein kinase (DMPK) as a direct transcriptional target for MEF2. By siRNA-mediated knockdown of DMPK expression, we demonstrate the involvement of this gene in the pathological aspects of MEF2 induced cardiomyocyte remodeling **(Chapter 3)**.

Given the resemblance between MEF2 induced cardiac remodeling and the cardiac phenotype of mice lacking Muscle LIM Protein (MLP), an established mouse model for inherited dilated cardiomyopathy, we analyzed MEF2 transcription factor activity in MLP knockout mice by genetic crossbreeding with MEF2 reporter mice. Following confirmation that MEF2 activity was severely upregulated, we used conditional transgenesis to express a dominant-negative form of MEF2 (DNMEF2) in the murine MLP deficient heart and combined this with echocardiographic analysis to examine the effect on cardiac remodeling. Surprisingly, histological and functional analyses indicated that MEF2 inhibition in MLP knockout mice did not have any effect on the genesis of dilated cardiomyopathy **(Chapter 4)**.

Finally, the significance of MEF2 transcriptional activity was assessed in the setting of a more physiological model of biomechanical stress in the form of chronic murine pressure overload using conditional transgenesis to express a dominant-negative form of MEF2 (DNMEF2) in the postnatal heart. Surprisingly, echocardiography indicated that MEF2 inhibition did not improve end-diastolic and end-systolic ventricular dimensions and contractility as observed in calcineurin transgenic hearts with conditional MEF2 inhibition. In fact, cardiac function was significantly worsened in the setting of MEF2 inhibition, which could be correlated with specific defects in mitochondrial adaptation during pressure overload **(Chapter 5)**.

Taken together, in this thesis we demonstrate that MEF2 activation in the heart does not evoke the classic hypertrophic response, yet promotes a gene program primarily involved in processes associated with dilated cardiomyopathy.

Although MEF2 activation is required for maladaptive cardiac remodeling downstream of calcineurin signaling, its activity is not the sole trigger in certain forms of dilated cardiomyopathy, and is even necessary for the adaptive response of the heart during pressure overload.

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