

Myocardial transcription factors in diastolic dysfunction: clues for model systems and disease

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Abstract

There are multiple intrinsic mechanisms for diastolic dysfunction ranging from molecular to structural derangements in ventricular myocardium. The molecular mechanisms regulating the progression from normal diastolic function to severe dysfunction still remain poorly understood. Recent studies suggest a potentially important role of core cardio-enriched transcription factors (TFs) in the control of cardiac diastolic function in health and disease through their ability to regulate the expression of target genes involved in the process of adaptive and maladaptive cardiac remodeling. The current relevant findings on the role of a variety of such TFs (TBX5, GATA-4/6, SRF, MYOCD, NRF2, and PITX2) in cardiac diastolic dysfunction and failure are updated, emphasizing their potential as promising targets for novel treatment strategies. In turn, the new animal models described here will be key tools in determining the underlying molecular mechanisms of disease. Since diastolic dysfunction is regulated by various TFs, which are also involved in cross talk with each other, there is a need for more in-depth research from a biomedical perspective in order to establish efficient therapeutic strategies.

Keywords

Diastolic dysfunction; Transcription factors; MicroRNAs

Introduction

Heart failure with preserved ejection fraction (HFpEF), termed in the past “diastolic HF,” is a heterogeneous clinical syndrome of impaired diastolic function with normal or near normal left ventricular EF (LVEF \geq 50 %). Patients with HFpEF have a normal-sized LV, often with signs of a mild hypertrophy, with impaired filling, to different degrees, due to abnormal LV relaxation and increased LV stiffness. While extra-cardiac pathophysiological conditions, such as hypertension, obesity, diabetes mellitus, renal dysfunction, and aging, can contribute to HFpEF development, a maladaptive concentric LV remodeling appears to be the main underlying substrate of impaired diastolic function in patients (recently reviewed in [1–7]).

Studies of gene expression in human HFpEF settings are limited by the low availability of tissue samples from diseased and non-failing control hearts. These limitations with human cardiac tissue are overcome, with varying degrees of success, by the use of patient-mimicking animal models [8, 9] in which HFpEF is developed as a consequence of spontaneous and experimentally induced hypertension or metabolic syndromes (obesity and diabetes). The results of these and other cross-sectional studies showed that LV diastolic dysfunction conditions can be associated with: (1) fluctuation in cardiac calcium-handling protein levels [10], (2) alterations in proteins, which play an important role in maintaining the sarcomeric structure and functionality [11–13], and (3) aberrant extracellular matrix protein turnover [14–17]. Although these data reflect some underlying expression features in HFpEF settings, the molecular mechanisms regulating the progression from normal diastolic function to severe dysfunction and then to HFpEF still remain poorly understood. Only a few recent studies point to the cardiomyocyte circadian clock [18] and mineralocorticoid receptor signaling [19] as potentially important mediators in triggering and in the progression of diastolic dysfunction in mice.

Transcription factors (TFs) are essential players in the control of gene expression by influencing RNA polymerase activity in a gene-selective manner. One distinct feature of TFs is that they have DNA-binding domains which recognize specific sequences in the promoters or enhancers of target genes. The other characteristic feature of their structure is an activation/repression domain that interacts with various cofactors, which either promote or impair the transactivation of target genes. Normal heart development is orchestrated by a suite of highly conserved TFs that includes (among others) TBX5, GATA-4, GATA-6, SRF/MYOC, NRF2, and PITX2 (Fig. 1a). These multifaceted cardio-enriched TFs are responsible for the tight regulation of expression of a broad array of myocardial-related genes during heart development, and the perturbation of expression and regulation of these TFs disrupts normal heart structure and function (recently reviewed in [20, 21]). Of note, these core TFs can physically interact with each other and co-occupy the promoters of target genes (Fig. 1b). These TFs are co-expressed in the adult human myocardium (Fig. 1c), suggesting that interactions between them could be physiologically relevant in heart.

It has become increasingly apparent that fetal cardiac-enriched TFs play critical roles in the regulation of expression of many myocardial genes in the adult normal and diseased heart (reviewed in [22–24]). Regarding heart disease, there is recent, mounting evidence suggesting a causal and specific role of several cardiac-enriched TFs in development and progression of HFpEF. In this review, we provide a research status update of the expression and function of the fetal cardiac TFs in adult diastolic dysfunction. Targeting TFs could be a promising therapeutic approach to modulate gene expression in the HFpEF in a specific fashion. Although TFs have traditionally been considered as “undruggable” targets for therapeutics, targeting them, due to recent technological advances, is again becoming a realistic therapeutic perspective (recently reviewed in [25]).

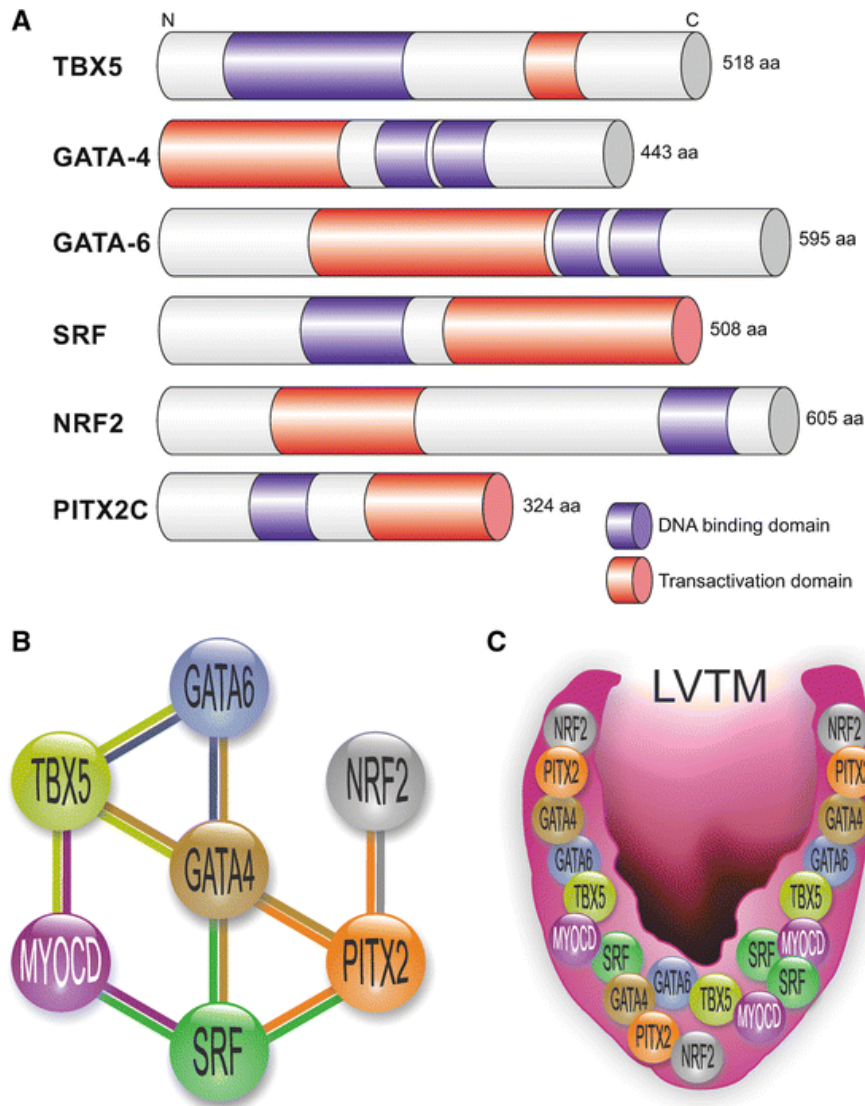


Fig. 1. Schematic overview of cardiac-enriched transcription factors governing diastolic function. **a** DNA-binding and transactivation domains of the human TBX5, GATA-4, GATA-6, SRF, NRF2, and PITX2 transcription factors (TFs) are shown [129]. N/C: amino/carboxyl terminus; aa—amino acids. **b** Combinatorial TF interactions. Schematic illustrates TBX5::GATA4 [130], TBX5::GATA-6 [131], TBX5::MYOCD [132], GATA-4::GATA-6 [54], SRF::MYOCD [68], SRF::PITX2 [133], PITX2::GATA-4 [134], and PITX2::NRF2 [82] protein–protein interactions that lead to cooperative regulation of target gene expression in vitro. **c** TBX5 [29], GATA-4 [135], SRF [136], MYOCD [137], NRF2 [138], and PITX2 [97] are co-expressed in the adult human left ventricle (LV), suggesting that the interplay of these TFs in vitro (shown in **b**) also takes place in vivo (data for GATA-6 equivalent to those for GATA-4 are currently lacking). LVTF—left ventricular TF machinery which can be involved in molecular regulation of diastolic function

TBX5 transcription factor

The *TBX5* gene belongs to a family of genes that share the so-called T-box DNA-binding domain of about 180 amino acid residues (reviewed in [26–28]). *TBX5* is expressed in human embryonic and adult heart [29] and transcriptionally activates multiple cardiomyocyte lineage-associated genes encoding, among others, NPPA (natriuretic peptide precursor A or ANP), CX40 (connexin 40), MYH7 (myosin, heavy chain 7, cardiac muscle, beta), TNNT2 (troponin I type 2 skeletal), TNNT2 (troponin T type 2 cardiac), and SCN5A (sodium channel, voltage-gated, type V alpha subunit) [30, 31]. It should be noted that several splicing variants of *TBX5* mRNA have been identified in the adult mouse [32] and human heart [33], and the resulting protein isoforms are characterized by diverse transcriptional and functional activities. This production of different *TBX5* isoforms seems to be a mechanism which can play an important role in *TBX5* dosage regulation.

TBX5 function in the heart appears to be exquisitely sensitive to gene dosage. Both over- and under-expression of the *TBX5* gene have equally deleterious effects on the heart. In transgenic mouse embryos, cardiac *TBX5* overexpression results in inhibition of ventricular-specific gene expression and impaired ventricular trabeculation [34]. Similarly, in humans, *TBX5* overexpression due to gene duplication leads to cardiac abnormalities [35]. In the mouse, systemic *TBX5* gene ablation (*TBX5*^{-/-} mice) causes decreased expression of myocardial genes associated with extreme underdevelopment of the heart, whereas heterozygous *TBX5*^{+/-} mice mimic heart and limb abnormalities observed in Holt–Oram (“heart-hand”) syndrome (HOS) in humans [36]. Of note, *TBX5* was the first T-box gene where loss-of-function mutations (mainly located within the highly conserved T-box domain) were found to cause a HOS (recently reviewed in [37]); diastolic dysfunction is detected in a cohort of HOS patients [38]. Patients with low diastolic blood pressure show substantially increased ventricular–arterial stiffness and a tendency for diastolic dysfunction (reviewed in [39]). In this regard, large-scale genome-wide association studies have identified an association of *TBX5* with diastolic blood pressure [40].

Mice with heterozygous conditional deletion of *TBX5* (*TBX5*^{del/+} mice) manifested a clear LV diastolic dysfunction (attributed to a disturbance of LV isovolumic relaxation) with preserved LV systolic function. In addition, a significant correlation was found between decreased *TBX5* gene expression and increased LV filling pressure [41]. However, the possibility that deterioration of LV relaxation is secondary to right heart overloads due to atrial or ventricular septal defects in these mice could not be excluded. The latter issue has been addressed by Zhu et al. [38] who generated mice (*TBX5*^{Vdel/+} mice) with haploinsufficiency of *TBX5* in only ventricular cardiomyocytes. These mice did not have septal or any other defects in cardiac structure, but did manifest impaired ventricular relaxation and diastolic dysfunction, whereas the systolic function remained normal. In the LV of *TBX5*^{Vdel/+} mice, the decrease in *TBX5* expression was paralleled by a comparable reduction in transcript and protein levels of *SERCA2a* (sarcoplasmic reticulum Ca²⁺ ATPase) which was found to be a dose-dependent target of *TBX5* in cardiomyocytes. This model suggests that the molecular pathogenesis of isolated diastolic dysfunction is due to downregulation of the *TBX5*–*SERCA2a* pathway in ventricular cardiomyocytes that may have implications for clinical management. Intriguingly, in this regard, a significant downregulation of cardiac *SERCA2a* levels has been observed in the diabetic (mRen-2)27 rat model of HFpEF [10].

GATA-4 and GATA-6 transcription factors

GATA-binding protein 4 (GATA-4) and GATA-binding protein 6 (GATA-6) are the members of the GATA family of zinc finger transcription factors which recognize the GATA motif in the promoters of most cardiac muscle-specific genes, especially those that are altered by the hypertrophic response (reviewed in [24, 42]). *GATA-4* directly regulates expression of MYH6 (myosin, heavy chain 6, cardiac muscle, alpha), MYL1 (myosin, light chain 1, alkali, skeletal, fast), TNNT1 (cardiac troponin C type 1), NPPA (natriuretic peptide precursor A), NPPB (natriuretic peptide precursor B), ANKRD1 (cardiac-restricted ankyrin repeat protein 1), SLC8A1 (solute carrier family 8 sodium/calcium exchanger, member 1), and CDK2 and 4 (cyclin-

dependent kinase 2 and 4) [42–44]. *GATA-6* seems to regulate the expression of *NPPA*, *EDN1* (endothelin-1), *AT1A* (angiotensin II receptor isoform A), and *SEMA3C* (semaphorin 3C) [45]. In addition, either *GATA-4* or *GATA-6* is essential for expression of other cardio-associated TFs that regulate the onset of cardiomyocyte gene expression in early cardiogenesis. Not surprisingly, when both *GATA-4* and *GATA-6* were simultaneously disrupted in mouse embryos, the onset of cardiac development was completely blocked [46].

GATA-4 and *GATA-6* are expressed in both fetal and adult cardiomyocytes and required for physiological hypertrophic remodeling during postnatal heart growth as suggested by controlled *GATA-4* [47] and *GATA-6* overexpression [48, 49] in the heart. There is a good reverse correlation between these results and the data from conditional *GATA-4*/*GATA-6*-knockout models: Mutant mice with mid-to-late fetal cardio-specific deletion of *GATA-4* [50] or combined deletion of *GATA-4* and *GATA-6* [49] develop dilated cardiomyopathy with severe systolic dysfunction in adulthood. Similarly, the simultaneous loss of both *GATA-4* and *GATA-6* in perinatal cardiomyocytes causes progressive systolic dysfunction and ventricular dilatation [51].

GATA-4 and *GATA-6* regulate cardiac morphogenesis, cardiomyocyte differentiation, and gene expression in a dosage-dependent manner. Mice homozygous for a hypomorphic *GATA-4* mutation (*GATA-4^{H/H}* mice), expressing 70 % less *GATA-4* protein in the atria and LV, displayed a common atrioventricular canal, double outlet right ventricle, and hypoplasia of the LV compact myocardium. Altered diastolic function was suspected in mutants because ventricular active relaxation was found to be correlated with the compact myocardium development in normal mouse embryos [52]. In fact, in vivo hemodynamics in *GATA-4^{H/H}* mice did reveal signs of severe diastolic dysfunction, in the absence of changes in systolic function. The diastolic dysfunction phenotype of these mutants did not result from downregulation of putative *GATA-4* target genes in the heart; *GATA-6* expression was not altered in *GATA-4^{H/H}* mutant embryos [53]. This fetal heart model was suggestive of a possible involvement of *GATA-4* in the regulation of diastolic function of fetal heart. However, the possibility of a direct causative role for *GATA-4* in regulating the diastolic function remained in question, since mutant mice lacking 70 % of the *GATA-4* protein displayed complex cardiac structural and functional abnormalities.

Recently, the functions of *GATA-4* and *GATA-6* in adult heart have been highlighted using mouse models of temporally controlled, cardiomyocyte-specific gene inactivation [51]. In the adult heart, simultaneous cardiomyocyte-specific deletion of *GATA-4* and *GATA-6* leads to dramatically attenuated diastolic function, whereas systolic performance is only slightly impaired. Molecular characterization of these mutant mice demonstrated that expression of the *GATA-4* preferential target, *MYH6* (myosin, heavy chain 6, cardiac muscle, alpha), is decreased in the diastolic heart. However, the expression of the other *GATA-4*-dependent gene, *MYH7* (myosin, heavy chain 7, cardiac muscle, beta) as well as the *NPPA* gene (a bona fide target for both *GATA-4* y *GATA-6* factors [54]) is increased in mutant myocardium, suggesting that the diastolic phenotype could not result from a general downregulation of *GATA-4/6* target genes.

Overall, therefore, the results of *GATA-4* and *GATA-6* knockdown in the adult heart strongly suggest that stress-induced downregulation of these TFs in myocardium might play a critical role in or contribute to the development of diastolic dysfunction. It should be noted in this sense that either *GATA-4* or *GATA-6* physically interact with *FOG-2* (friend of *GATA-2*), a multi-zinc finger transcription cofactor, which is co-expressed with *GATA-4/6* in the heart and is known to potently repress *GATA-4* activation of target promoters (reviewed in [55]). Adult mice with fetal cardiomyocyte-restricted loss of *FOG-2* developed a dilated cardiac phenotype, indicating that *FOG-2* is required for normal adult heart function [56]. *FOG-2* upregulation is a feature of human cardiomyopathic heart. Mice with cardiac-specific overexpression of *FOG-2* display normal-sized ventricles with enlarged atria; a significant downregulation of *MYH6* and *SERCA2a* was found in ventricular myocardium of *FOG-2* transgenic mice [57]. Although unproven as yet, it is tempting to speculate that a severe decrease in *GATA-4* and *GATA-6* transcriptional activity, which could lead to diastolic dysfunction [51], might be associated, at least in part, with overexpression of *FOG-2* in the stressed heart.

SRF and myocardin transcriptional regulators

Serum response factor (SRF) is a founding member of the MADS (MCM1, agamous, deficiens, and SRF)-box superfamily of TFs. This ubiquitously expressed TF binds DNA as a dimer (through the highly conserved DNA-binding and dimerization MADS-box domain) and regulates many target genes through serum response elements in their promoters (reviewed in [58, 59]). More than 200 SRF-dependent genes that are important for metabolism, cytoskeleton, extracellular matrix, ion transport, stress response, transcription, and translational regulation have been identified in the ventricular myocardium [24, 60]. Alternative splicing generates several isoforms of SRF, with full-length SRF being the predominant cardiac isoform. *SRF* is highly expressed in the heart during embryonic, fetal, and postnatal development. Gene-targeting studies in mouse models provided insights into potentially pathological consequences of both *SRF* redundancy and deficiency in the adult heart. Augmented *SRF* expression leads to hypertrophic cardiomyopathy, while inhibition of SRF activity results in development of dilated cardiomyopathy (reviewed in [61, 62]).

During aging, the heart undergoes, even in the absence of evident cardiovascular pathology, subtle remodeling changes that include moderate LV hypertrophy and altered LV diastolic function. *SRF* expression is increased from adulthood to senescence. In this context, in transgenic mouse models, in which upregulation of the *SRF* gene was low-to-mild, young adult animals displayed accelerated cardiac aging and developed diastolic dysfunction [63]. A subsequent report showed that a low-forced *SRF* expression did not affect either cardiac gene expression or cellular structure [64]. Whether low-amplitude increases in cardiac *SRF* levels are causative for triggering diastolic dysfunction or merely reflective of aging heart remodeling remained unclear.

SRF is characterized by a relatively low intrinsic transcriptional activity itself, but its association with a wide array of cofactors that possess potent transactivation domains leads to a strong enhancement of *SRF* transactivation capacity in a cell context-dependent manner. One of such cofactors is myocardin (*MYOCD*) that regulates the expression of multiple smooth muscle (SM) and cardiac contractile genes, including *CNN1* (calponin 1, basic, smooth muscle), *MYH11* (myosin, heavy chain 11, smooth muscle), *ACTA2* (actin, alpha 2, smooth muscle, aorta), *ACTG2* (actin, gamma 2, smooth muscle), *TAGLN* (transgelin), and *MYH6* (myosin, heavy chain 6, cardiac muscle, alpha) (reviewed in [23, 65–68]).

Loss- and gain-of-function experiments demonstrated that *MYOCD* is absolutely required for maintenance of adult heart function. Postnatal cardio-restricted *MYOCD* knockdown induces dilated cardiomyopathy and fatal HF in mice [69], while forced expression of *MYOCD* in ventricular myocardium impairs LV systolic function and cardiac ECG activity in pigs [70]. In light of these results, it comes as no surprise that a strong association between the altered expression of *MYOCD* and cardiac pathological conditions has been established in different animal models as well as patients with end-stage HF (reviewed in [23, 67, 68]).

In the porcine model of doxorubicin (Dox)-induced HFpEF, gene expression profiling revealed a significant upregulation of *MYOCD*- and *MYOCD*-dependent SM genes in failing LV myocardium, with the *SRF* levels unchanged as compared to controls. *ACTG2* was the most remarkably upregulated *MYOCD* target gene in Dox-injected piglets. Of note, the ectopic expression of *ACTG2* in the mouse heart significantly reduces the rates of ventricular relaxation [71]. *In vivo* silencing of endogenous upregulated *MYOCD* via intramyocardial delivery of short-hairpin RNAs at mid-advanced stages of HFpEF resulted in downregulation of *MYOCD*-dependent SM gene expression in the failing porcine myocardium. Such adjusting of *MYOCD* and SM-target expression levels to the range of physiological variation led to restoring diastolic function and extending the survival of failing animals without compromising the physiological functions of *MYOCD* signaling as part of the adaptive response of the heart to stress [72]. These findings demonstrate that the normalization of altered *MYOCD* signaling could represent a strategic operative tool for the prevention of the development of diastolic dysfunction.

NRF2 transcription factor

Nuclear factor erythroid 2-related factor 2 (NRF2) is a redox-sensitive basic leucine zipper TF which controls the gene expression of several hundred of detoxification and antioxidant enzymes bearing a target sequence, known as the antioxidant-responsive element (ARE), in their promoters [73]. *NRF2* transcriptionally activates ARE-bearing genes in response to oxidative stress-induced injury (reviewed in [74, 75]). *NRF2* ubiquitously expressed in the cardiovascular system is an essential endogenous suppressor of oxidative stress in both cardiomyocytes and cardiac fibroblasts, and *NRF2* deficiency appears to be a condition for the early onset of HF in humans [76, 77]. In addition, functional *NRF2* polymorphisms are associated with risk of human cardiovascular disorders [78].

The results from loss- and gain-of-function experiments in animal models have provided clues to the understanding of the role/impact of *NRF2* in cardiac function. Several lines of mice devoid of *NRF2* have been generated by homologous recombination in embryonic stem cells, using various strategies. In all cases, disruption of the targeted *NRF2* gene did not lead to any apparent structural and functional abnormalities in the neonatal and early postnatal heart under non-stressed physiological conditions [79]. However, *NRF2* deficiency resulted in a rapid onset of cardiac dysfunction during experimental pressure overload (due to transverse aortic constriction [80]) or regional ischemic injury (due to cardiac artery occlusion [81, 82] in young adult (2-month-old) mice. These results indicated that *NRF2* inhibition can increase sensitivity of the young heart to pathological stress and thus exaggerate susceptibility to cardiac dysfunction. Recently, it was found that *NRF2* loss-of-function leads to suppression and distortion of regenerative processes in the apex resection mouse model [82].

Further studies focused on adult *NRF2*-knockout mice (5–6 months of age) demonstrated that a *NRF2* gene deficiency leads to the development of LV diastolic dysfunction even in non-stressed heart. Impaired diastolic function in these *NRF2* knockouts was associated with mild cardiac hypertrophy but preserved systolic function. In addition, a significant decline in cardiac *SERCA2a* and total glutathione levels were found in the myocardium of these animals [83]. The results suggest that *NRF2* is an essential regulator of cardiac diastolic function upon non-stressed physiological conditions and that its downregulation might cause severe maladaptive reactions.

NRF2 expression is upregulated during early stages of physiological cardiac hypertrophy but decreased at mild pathological hypertrophy (a condition often associated with HFpEF development). In various models of hypertrophic cardiomyopathy, forced expression of *NRF2* and its target genes, such as *HO-1* (heme oxygenase-1), *GPX* (glutathione peroxidase), *TXNRD1* (thioredoxin reductase 1), *NQO1* (NADPH:oxioreductase 1), and *SOD2* (superoxide dismutase 2, mitochondrial), significantly reverses LV remodeling and fibrosis [84, 85]. Similarly, an increase in *NRF2* expression/activity (due to phytochemical intake) attenuates diastolic dysfunction in hypertensive Dahl salt-sensitive rats with HFpEF [86].

In patients, diabetic cardiomyopathy is characterized by signs of clinically significant diastolic dysfunction independent of coronary disease or hypertension [87]. Expression of *NRF2* is downregulated in cardiomyocyte nuclei in cardiac samples from patients with diabetes. Promisingly, emerging evidence revealed that induction of *NRF2* expression can protect from diabetes-associated cardiac dysfunction by decreasing the oxidative stress and preventing oxidative DNA damage of myocardium (reviewed in [88, 89]).

PITX2 transcription factor

The PITX2, a paired-like homeodomain transcription factor 2, was originally identified as the candidate gene for the human Axenfeld-Rieger syndrome [90] associated, although not frequently, with congenital heart defects [91, 92]. Aggregating evidence demonstrates that *PITX2* is expressed, as three distinct variants/isoforms (A, B, and C), in the mammalian and human heart, with *PITX2C* being the predominant or the only variant detected in the adult LV myocardium [93–98]. A fourth isoform, PITX2D described, to date, only in humans acts as a dominant-negative factor [99]. *PITX2* regulates the expression of cyclin D2 (*CCND2*) [100], forkhead box J1 (*FOXJ1*) TF [101], lymphoid enhancer factor (*LEF-1*) [102], natriuretic peptide precursor A (*NPPA*) [103, 104], myogenic factor 5 (*MYF5*) [105], and antioxidant scavenger genes [82]. Other target genes of *PITX2* include channel and calcium-handling genes, and genes are expressed in intercalated disks of cardiac myocytes (recently reviewed in [106]).

Selective *PITX2* deletion in the developing myocardium resulted in delayed differentiation of ventricular (but not atrial) cardiomyocytes and enlargement of right heart chambers associated with severely impaired ventricular systolic function [107]. The roles played by *PITX2* within the four chambered adult heart are still poorly understood. Genes involved in cell junction assembly, ion transport, and proliferation pathways were found to be activated in mouse mutants with conditionally inactivated *PITX2* in the postnatal atrial myocardium [108], but it is unclear whether these are direct or indirect *PITX2C* target genes in a cardiomyocyte background. Recently, a total of 505 direct *PITX2* target genes were identified in the mouse postnatal ventricular myocardium, including genes encoding transport chain components and reactive oxygen species scavengers [82].

While several lines of evidence from animal models as well as clinical studies strongly support that *PITX2C* has a role in susceptibility to atrial fibrillation [94, 109–114], the relevance of this TF to LV diastolic dysfunction remained unsuspected and was therefore not tested till recently. LV levels of *PITX2C* mRNA and protein were first shown to be elevated in the Dox-induced porcine model of HFpEF [97]. In particular, it was found that the expression of *PITX2C* is significantly re-activated in HFpEF myocardium which, in turn, is associated with increased expression of a restrictive set of *PITX2* target genes. Among these, *MYF5* (myogenic factor 5) was identified as the top upregulated gene. In vitro, forced expression of *PITX2C* in cardiomyocytes activates *MYF5* in dose-dependent manner. Of note, ectopic overexpression of *MYF5* in the heart activates a skeletal muscle gene expression that results in progressive cardiomyopathy [115, 116]. The latter suggests that aberrant *PITX2*–*MYF5* co-activation seen in the porcine model of HFpEF might negatively impact on diastolic cardiac function.

Expression of both *PITX2* and *MYF5* was detected in LV myocardium from HFpEF piglets [97] as well as cardiomyocyte-like (CML) cells from patients with dilated cardiomyopathy. Forced expression of *SERCA2a* improved contractility of CML cells that, in turn, was associated with downregulation of *PITX2* and *MYF5* in *SERCA2a*-transduced cardiomyocytes [117]. Myocardial hypertrophy and subsequent diastolic dysfunction are prominent features of diabetic cardiomyopathy. In this sense, *PITX2C* is significantly upregulated in human cardiac myocytes in response to high glucose treatment [118].

In spite of the results discussed above, it is not known whether upregulation of *PITX2C* can cause HFpEF or if its activation is merely a secondary manifestation of impaired LV diastolic function. Notably, *PITX2* expression is induced in ischemically injured ventricular myocardium and is required for neonatal cardiac regeneration in mice [82].

Conclusions and perspectives

HFpEF can be considered as the new epidemic of the twenty-first century, since the prevalence of this complex clinical syndrome caused by a variety of diseases has remained high or has even increased throughout the last two decades (reviewed in [6]). Investigation into molecular mechanisms underlying HFpEF has been hampered by lack of relevant and tractable models in animals which could recapitulate phenotypic features of diastolic dysfunction in humans. Nevertheless, some studies which focused on single specific endpoints have revealed concordant changes in increased calcium and sodium levels and elevated expression of the stiff titin isoform with myocardial diastolic dysfunction (reviewed in [3]). Despite these efforts, advancement has been made over the past several years, proving essential for the identification of the transcriptional regulation of diastolic function with potential implications for HFpEF. Surprisingly, the study of the molecular regulatory background of diastolic dysfunction and HFpEF does not show a great diversity of responsible TFs and downstream gene pathways (Fig. 2). All of these TFs play important roles in cardiac development and regulate inducible gene expression in cardiac myocytes in the adult heart. It should be pointed out that there is an experimental evidence to suggest that diastolic dysfunction could result from altered expression of more than one of these TFs in the LV myocardium. In the porcine model of HFpEF, upregulation of *MYOCD* and its SM-target genes is associated with augmented *PITX2* and *MYF5* expression, suggesting an additive negative effect on diastolic function [72, 97].

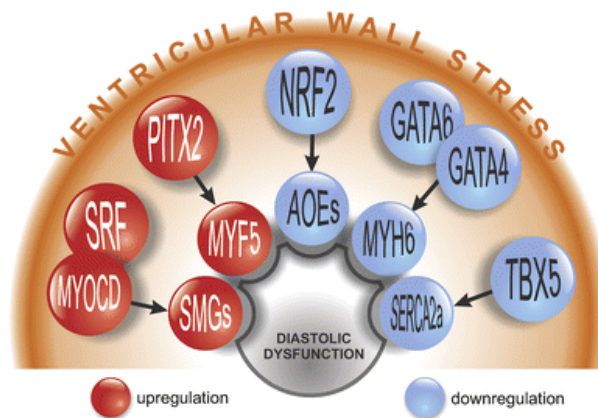


Fig. 2. Dissecting the roles of cardiac TFs in development of diastolic dysfunction. Cardiac transcription factors (TFs) are members of gene regulatory circuits, each of which can play a role in the control of a branch of terminal effector genes in ventricular myocardium. The results from loss- and gain-of-function experiments in animal models provide clues toward the understanding the roles of *TBX5*, *GATA-4/6*, *SRF/MYOCD*, *NRF2*, and *PITX2* in development of cardiac diastolic dysfunction. Severe inhibition of *TBX5*, *GATA-4/6*, and *NRF2* as well as augmented expression of *SRF/MYOCD* and *PITX2* leads to molecular changes that may contribute to ventricular diastolic impairment. These changes include, respectively, downregulation of *SERCA2a*, *MYH6*, and antioxidative enzyme (AOE) genes, on the one hand, and activation of smooth muscle (SM) and skeletal muscle (*MYF5*) gene expression in ventricular myocardium, on the other hand. AOE—*NRF2* target genes encoding HO-1, GPX, TXNRD1, NQO1 (NAD(P)), SOD2, and SOD3. SM—*MYOCD* target genes encoding CNN1, MYH11, ACTA2, ACTG2, and TAGLN. See text for further details

The discovery that deficiency in core cardiac TFs, such as *TBX5*, *GATA-4/6* and *NRF2*, causes diastolic dysfunction will prompt an evaluation or re-evaluation of the expression of these TFs in other animal models of HFpEF as well as patients with diastolic dysfunction. Perhaps, the best example of this is the identification of a *TBX5*-dependent pathway in the regulation of diastolic function that has direct relevance to patients with HOS [38]. Collectively, the results suggest that mutant mice with cardio-specific knockdown of *TBX5*, *GATA-4/6*, or *NRF2* are promising models for investigating the molecular mechanisms underpinning the development of diastolic dysfunction.

Through the use of other animal models that mimic of HFpEF, upregulation of *SRF*, *MYOCD*, and *PITX2* is linked to the development of diastolic dysfunction, whereas restoration of their altered expression to the range of physiological variation can potentially reduce or even eliminate diseases, as demonstrated through silencing *MYOCD* overexpression in the porcine model of HFpEF [72]. Of note, detection of elevated levels of *MYOCD* in circulating blood cells has been shown to have a certain biomarker utility in patients (reviewed in [68]). Thus, it appears to be reasonable to test whether adjusting a given TF expression level to the range of its physiological variation may lead to restoring diastolic function in preclinical settings.

In sum, the above studies clearly illustrate how inappropriately high or low expression of a set of cardiac TFs can lead to diastolic dysfunction (see Fig. 2). Therefore, manipulating their levels to either increase the expression of stress-downregulated TFs or suppress the expression of stress-upregulated TFs may represent therapeutic tools to ameliorate HFpEF progression. Mechanistically viewed, modulation of TFs in myocardium in vivo can be achieved by direct targeting (through gene therapy) their expression levels as well as by affecting the combinatory TF interactions (see Fig. 1b) or altering their DNA-binding activity (reviewed in [25]). In this context, a number of delivery platforms have been described that are translatable to the clinical setting (reviewed in [119]). However, it is necessary to develop more efficient delivery systems for cardio-selective (not systemic) targeting of TFs in the diastolic heart. An alternative and complementary approach (as demonstrated in the case of *NRF2*; see [89]) may be pharmacological activation/inhibition of the candidate TF or its critical cofactors for improving diastolic dysfunction in certain cardiovascular settings.

In addition to the points raised above, it is essential to pinpoint a possible involvement of microRNAs (miRNAs) in pathophysiological mechanisms underlying HFpEF (recently reviewed in [120]). Compared to the cardiac-enriched TFs (cardiac TF-ome system) which control gene expression at the level of transcriptional regulation, cardiac-expressed microRNAs (cardiac miRNA-ome system) regulate gene expression at posttranscriptional levels. Rather than acting independently of one another, these systems act in an integrated fashion to regulate cardiac gene expression in a coordinated manner. In fact, several cardiac-expressed miRNAs were found to be involved in the *TBX5* [114, 121], *GATA-4* [122–126], *SRF/MYOCD* [124, 126, 127], and *PITX2* [97] regulatory network in settings of cardiovascular disease. There is evidence that circulating miRNAs may be used as biomarkers for patients with HFpEF, but their expression has not yet been assessed in the diastolic heart [128]. Nonetheless, each advance discussed above highlights the importance of multiple layers of molecular control in regulating cardiac gene expression at HFpEF.

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Compliance with ethical standards

The manuscript does not contain clinical studies or patient data.

Conflict of interest

The authors have declared that no competing interests exist.

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