Published in final edited form as:

J Cardiovasc Transl Res. 2017 June ; 10(3): 295–304. doi:10.1007/s12265-016-9723-z.

Molecular Approaches in HFpEF: MicroRNAs and iPSC-Derived Cardiomyocytes

Alison J. Kriegel¹, Melanie Gartz², Muhammad Z. Afzal², Willem J. de Lange³, J. Carter Ralphe³, and Jennifer L. Strande²

¹Department of Physiology, Medical College of Wisconsin, Milwaukee, WI, USA

²Department of Medicine, Medical College of Wisconsin, Milwaukee, WI, USA

³Department of Pediatrics, University of Wisconsin School of Medicine and Public Health, Madison, WI, USA

Abstract

Heart failure with preserved left ventricular ejection fraction (HFpEF) has emerged as one of the largest unmet needs in cardiovascular medicine. HFpEF is increasing in prevalence and causes significant morbidity, mortality, and health care resource utilization. Patients have multiple co-morbidities which contribute to the disease complexity. To date, no effective treatment for HFpEF has been identified. The paucity of cardiac biopsies from this patient population and the absence of well-accepted animal models limit our understanding of the underlying molecular mechanisms of HFpEF. In this review, we discuss combining state-of-the-art technologies of microRNA profiling and human induced plu-ri-potent cell-derived cardiomyocytes (iPSC-CMs) in order to uncover novel molecular pathways that may contribute to the development of HFpEF. Here, we focus the advantages and limitations of microRNA profiling and iPSC-CMs as a disease model system to discover molecular mechanisms in HFpEF.

Keywords

Heart failure with preserved ejection fraction; MicroRNA; Human induced pluripotent stem cells; Cardiomyocytes; Engineered heart tissues

Introduction

No improvement in heart failure with preserved ejection fraction (HFpEF) patient outcomes has been realized over the past two decades [1], and treatment remains challenging. Pharmacologic strategies as used in patients with HF with reduced EF, such as afterload reducing agents and beta-blockers, do not alter the outcomes of patients with HFpEF [1, 2].

Correspondence to: Jennifer L. Strande.

Associate Editor Sanjiv Shah oversaw the review of this article

Compliance with Ethical Standards

Conflict of Interest: The authors declare that they have no conflict of interest.

Ethical Approval: No human or animal studies were carried out by the authors for this article.

This suggests that different mechanisms are responsible for the two types of heart failure. However, a major limitation to conducting mechanistic studies is the lack of appropriate in vitro approaches or representative animal models [3]. Further research into the biochemical and structural mechanisms that underlie HFpEF is needed to clarify the pathobiology and develop effective therapies.

Our current understanding of HFpEF is derived from limited patient-based clinical studies. Patients with HFpEF are more often elderly, female, and have associated co-morbidities such as hypertension, chronic obstructive pulmonary disease (COPD), diabetes mellitus (DM), and chronic kidney disease (CKD) [4]. Multiple circulating inflammatory biomarkers have also been associated with the syndrome [5]; however, it is poorly understood how these co-morbidities or biomarkers contribute to the development of HFpEF. Some investigators believe that common co-morbidities associated with low-grade systemic inflammation cause endothelial cell dysfunction with increased reactive oxygen species and decreased nitric oxide production. These mediators diminish the activity of soluble guanylate cyclase and protein kinase G eventually leading to myocardial remodeling, impaired relaxation, and increased myocardial stiffness [6]. Unfortunately, clinical trials targeting the cyclic GMP pathway with phosphodiesterase-5 inhibitors did not result in any benefit except in HFpEF patients in which pulmonary hypertension was predominant [7]. This suggests that the complex molecular mechanisms of HFpEF may differ depending on the associated co-morbidities.

The large number of associated co-morbidities and various combinations of these co-morbidities that exist between individuals gives rise to a largely heterogeneous HFpEF population. It has been speculated that this heterogeneity has contributed to the repeated failure of clinical trials. Therefore, the identification and classification of clinical phenotypes is of utmost importance for understanding the disease mechanisms and predicting treatment responses. The exact method of clinical phenotyping remains a matter of debate on whether patients are grouped primarily based on co-morbidities, cardiac structure and function, biomarker profiles including microRNA (miRNA) based bio-markers, or a combination of variables [8, 9].

Ultimately, the complexity and heterogeneity of this condition make a systematic approach to classifying HFpEF necessary not only to understand disease mechanisms but also to improve outcomes. In this review, we will discuss miRNAs as both biomarkers and contributors to disease mechanisms along with patient-specific induced pluripotent stem cells (iPSCs) as a model system to determine miRNA-mediated mechanisms towards the disease phenotype.

MicroRNA as Biomarkers and Disease Modifiers

Introduction to MicroRNA

MicroRNA is a class of endogenously produced small RNAs (approximately 18 to 22 nucleotides). After transcription, the primary transcript, or miRNA precursor, is processed to produce a duplex of two complementary mature miRNAs distinguished by a “-5p” or “-3p” suffix. Each miRNA strand may have unique regulatory interactions and become part of a

miRNA-induced silencing complex (miRISC), which consists of various proteins, the most notable being the argonaute proteins (AGO 1–4) [10].

Mechanisms of miRNA regulation vary, but they generally act to reduce the expression of targeted protein coding mRNAs primarily through complimentary base pairing with nucleotides in the 3'-untranslated region of target mRNAs and promote either translational repression or degradation of the mRNA target [10] (Fig. 1). Since a single miRNA may suppress multiple mRNA targets simultaneously, dysregulation of miRNA can alter expression of target genes in an entire network and result in diverse disease states [11, 12]. Even though the actions of miRNAs occur within the cell, miRNAs are also secreted from cells into the circulation.

Circulating MicroRNAs as Biomarkers

Circulating miRNA profiling has been performed under numerous disease processes with the goal of identifying bio-markers of disease. Secreted miRNAs within the plasma and serum are either encapsulated within membrane-bound extracellular vesicles or bound with Argonaute proteins or lipoproteins (Fig. 1) where they are protected from nuclease-mediated degradation [13, 14]. MicroRNAs within vesicles have significantly extended longevity and are stable for several hours compared to miRNA bound to argonautes or lipoproteins [13, 14]; therefore, miRNAs within extracellular vesicles may provide more stable expression patterns in clinical biofluid samples and thus may serve as more reliable biomarkers.

Circulating MicroRNAs as Diagnostic Biomarkers in HFpEF

Recently, two independent studies have identified differences in circulating miRNA profiles in subject heart failure with preserved vs. reduced ejection fraction (HFpEF) [15, 16]. Both studies showed that either individual or multiple miRNAs in combination with B-type natriuretic peptide (BNP) was superior to using BNP alone to distinguish HFpEF from HFrEF. However, the studies identified different miRNA profiles in HFpEF populations. This highlights the limitations of miRNA biomarker discovery. The fundamental technical challenge of miRNA quantity normalization between samples is frequently overlooked in the analysis of data sets. Since there are no reliably expressed “housekeeping” RNAs or miRNAs for normalization, “spike-in” controls are often used. Careful consideration of variables, such as extraction efficiency, biofluid selection, and volume, should be accounted for when designing studies of circulating miRNA.

In addition, the differences between the two studies could also be explained by either the cross-sectional nature of the studies or differences introduced by sample pooling and by varied exclusion criteria. The study by Wong et al. excluded patients with coronary artery disease (CAD), COPD, and atrial fibrillation, whereas the study by Watson et al. included these patients. Of note, the miRNA candidates identified in the Watson study did not have any relationship with echocardiographic parameters of ejection fraction or left atrial volume index [15].

This brings up another important point when studying HFpEF; the different co-morbidities of each subject are likely to dictate their circulating miRNA profile. The circulating miRNA

profiles have shown to differ between patients with CAD [17], COPD [18], DM [19], and CKD [20]. Profiles change with the addition of a second co-morbidity. For instance, in patients with DM, miRNA-9, miRNA-126, and miRNA-370 levels increase with the presence of CAD [19]. As disease develops in a continuous fashion, miRNA profiles are also shifting based on the progression of disease. One example is the dynamic miRNA profiles detected depending on the advancing stages of COPD [18]. In addition, the medications used to treat co-morbidities may also influence miRNA profiles. This suggests that the alterations in circulating miRNAs seen in HFpEF may be independent of the myocardial disease mechanisms and rather are more associated with the underlying co-morbidities and treatments.

To remedy these limitations, a high-throughput miRNA sequence approach should be performed with samples where repeated assessments are performed in patients whose clinical phenotype has been rigorously verified (Fig. 2). A matched case-control study design is necessary to explore miRNA profile changes between successive stages of disease progression. For example, subjects with asymptomatic diastolic dysfunction could be followed until after they develop heart failure. Patients should be matched for age, gender, and one or more of the following co-morbidities: DM, hypertension, obesity, hyperlipidemia, and atrial fibrillation. Ideally, the severity or stage of the co-morbidity along with the medications needs to be carefully considered and matched as these variables will also influence microRNA profiles. Matching will not eliminate but should improve the balance of these important covariates that likely alter miRNA profiles between cases and controls. It is also important that the covariates are adjusted for in the statistical analysis to account for the clinical context within which miRNA changes are interpreted. Using this rigorous design, the study would generate a number of findings which underscore several unique characteristics of miRNAs as disease biomarkers.

Circulating MicroRNAs as Disease Modifiers Through Paracrine and Endocrine Signaling

Many investigators have focused on the potential of circulating miRNAs as disease biomarkers for the reasons described above; however, it is also widely believed that circulating miRNAs may have a role in cell-to-cell communication in distant organs. MicroRNAs encapsulated in membrane-bound vesicles such as exosomes, microvesicles, and apoptotic bodies can participate in paracrine or endocrine-like effects to nearby or distant cells. Exosomes and microvesicles are relatively small (40–120 and <50–1000 nm, respectively), while apoptotic bodies are quite large (ranging in size from 500 to 2000 nm) [21]. The extracellular transfer of miRNAs occur through membrane fusion and endocytosis of the extracellular vesicle [22–24]. This type of communication between cell types can have dramatic regulatory effects within several physiology systems including the cardiovascular system.

Influence of MicroRNA on Cardiac Function

In recent years, a great deal of work has been done to understand the role of miRNAs in cardiac function. MicroRNAs can have a role in both acute and chronic pathology; however,

their regulatory actions are complex and incompletely understood, even within a given organ or tissue type [25]. For example, the short-term upregulation of miRNA-21-5p, a highly studied miRNA, can be either protective or damaging depending on the disease model, while chronic upregulation of miRNA-21-5p has been associated with pathological fibrosis, inflammation, cancer, and autoimmune disease [25–28].

It has been found that miRNA regulation mediates a dynamic adaptation of the structure and functionality of a wide spectrum of cells including cardiomyocytes, fibroblasts, and endothelial cells. MicroRNAs can play an important role in the tonic or “physiological” regulation of gene expression [12]. They can also increase or decrease in abundance in response to environmental challenges and biochemical and biomechanical stress [22, 29, 30]. This suggests that deregulation of miRNA and its transcriptional targets may provide a clue for the pathological remodeling of the heart and vessels.

In conclusion, the net effects of miRNAs may relate to the various co-morbidities and other risk factors associated with HFpEF. The miRNA pattern along with the pattern of specific classes of target transcripts, which regulate the phenotypic response of a failing heart, may give insight into the pathophysiological mechanisms that underlie HFpEF. However, the intrinsic and extrinsic mechanisms regarding the causal pathway to HFpEF are not fully understood and require more investigation using disease models.

Induced Pluripotent Stem Cells as a Cardiac Disease Model to Decipher Disease Mechanisms of HFpEF

The hallmark cardiac function abnormality of HFpEF is left ventricle (LV) diastolic dysfunction resulting in increased filling pressures. LV hypertrophy and interstitial fibrosis contribute to increase LV stiffness [31] and impair calcium cycling [32], or abnormalities in phosphorylation of key regulatory proteins such as cardiac myosin binding protein C (cMyBP-C) [33, 34] contribute to active relaxation abnormalities. However, whether these intrinsic changes within cardiomyocytes occur in HFpEF has been difficult to elucidate due to the paucity of cardiac biopsies from this disease population. Hence, patient-specific iPSCs have become a powerful model system to explore human disease pathobiology in vitro.

The ability to generate iPSCs from a variety of cell types collected from any individual combined with the development of gene-editing tools, defined protocols for cardiac differentiation, and the ability to generate three-dimensional tissues have enabled the application of iPSCs for in vitro HFpEF disease modeling. The field of iPSCs has advanced rapidly since Takahashi and Yamanaka discovered that mouse fibroblasts can be reprogrammed into an embryonic stem cell-like state by introducing the four transcription factors, OCT4, SOX2, KLF4, and cMYC, into the cell [35]. Shortly afterwards, Yu and colleagues showed that human somatic cells can be reprogrammed by introducing OCT4, SOX2, LIN28, and NANOG transcription factors [36]. Early reprogramming efforts focused on introducing the four reprogramming factors into cells using integrating vectors such as lentivirus or retroviruses. The current state of the field now relies on the use of non-integrating vectors such as episomal plasmids [37] or the Sendai virus [38]. Additionally,

iPSCs can be generated from somatic cell sources that are collected non-invasively such as peripheral blood polymononuclear cells [39] and urine-derived cells [40].

iPSCs can then be differentiated into cardiomyocytes (iPSC-CMs) using protocols which rely on single cell mono-layer cultures combined with either a matrix sandwich method with the addition of activin A and bone morphogenetic protein 4 [41] or small molecule modulators of the Wnt/ β -catenin signaling pathway to yield >90% cardiac troponin T-positive cells [42]. These advancements in cardiac differentiation have made it possible for automation and large-scale preparations which greatly enhance their potential for molecular mechanism screening applications.

Patient-Specific iPSC-Derived Cardiomyocytes to Study Genetic Modifiers

The use of iPSC-CMs to model cardiomyopathic disease has more often been restricted to genetically inherited or spontaneous cardiomyopathies such as hypertrophic cardiomyopathy [43], Pompe's disease [44], or Duchenne muscular dystrophy [40, 45]. Less commonly, iPSC-CMs are used to model acquired cardiomyopathies such as HFpEF.

Patient-specific iPSC-CMs can also be used to decipher the potential role of genetic contributions towards cardiovascular disorders without a clearly defined gene mutation [46–49]. For instance, iPSC-CMs generated from patients with hypo-plastic left heart syndrome display disorganized myofilaments, induction of fetal gene expression profiles, abnormalities in calcium handling due to ryanodine receptor dysfunction, and diminished ability to respond to β -adrenergic stimulation [46]; iPSC-derived cardiomyocytes can also be used to explore the contributions of polymorphisms towards the disease phenotype. The E487K polymorphism in the aldehyde dehydrogenase 2 gene is a good example of this application. The polymorphism itself is not disease causing but is associated with increased susceptibility to ischemic heart damage. iPSC-derived cardiomyocytes containing this polymorphism have increased levels of reactive oxygen species and toxic aldehydes which activate the apoptosis signaling genes. Hypoxia and ischemic mimics exaggerated these findings, and reactive oxygen species scavengers reversed the phenotypes [48]. In this case, iPSC-CM disease modeling led to an increased understanding of the molecular mechanisms resulting from the polymorphism and gave powerful insight into the pathways amenable to therapeutic intervention.

The role of genomic variance has not been clearly defined in HFpEF, although there is recent evidence that gene polymorphisms may increase individual risk of developing HFpEF [50]. With the advent of gene-editing tools, iPSCs can now be used to either confirm or refute disease causality for genetic variants. The CRISPR/Cas9 system can be utilized to create either single nucleotide base pair changes or large deletions or insertions in order to engineer the disease-associated genetic variants within the genome of a non-affected iPSC line. Editing can also be applied to correct specific mutations or polymorphisms in affected iPSC lines. In either approach, the isogenic paired lines will differ only at the disease-linked mutations. Having genetically matched pairs that differ only at the disease-linked mutation will limit the functional and biochemical variation typically seen between non-related iPSC lines to allow for rigorous genotype–phenotype functional correlation studies.

Patient-Specific iPSC-Derived Cardiomyocytes to Study Environmental Modifiers

Beyond genetic predisposition, several secondary factors including co-morbidities or environmental factors can contribute to the development or progression of HFpEF. The different combinations of these underlying factors which may seemingly cause HFpEF in one individual but not in another suggest that HFpEF may be particularly suited to a precision medicine approach. The lack of mechanistic studies which establishes the link between the complex effects of the co-morbidities and environmental factors on HFpEF remains an important knowledge gap. Human iPSC-CMs without known mutations can be exposed to various conditions to precipitate metabolic, oxidative, or endoplasmic reticular stress or subjected to hypoxia, growth factors, or neurohormones to mimic certain disease states. For instance, a diabetic environment can be mimicked by supplementing the culture media with glucose, endothelin-1, and cortisol which allows iPSC-CMs to be used as a diabetic cardiomyopathy model system [47]. Exposure of iPSC-CMs to hypoxic conditions during their differentiation recapitulated similar phenotypic characteristics of fetal cardiomyocytes derived from hypoplastic left heart syndrome tissues, including oncogene-mediated senescence and upregulation of the TGF- β 1 pathway [49].

Disease Phenotyping of iPSC-Derived Cardiomyocytes

Once patient-specific iPSC-CMs are derived, there are several different biochemical and functional assays available to define in vitro phenotypes. Phenotypes can be assessed in either single or clusters of cardiomyocytes and scaled up for high throughput in either 96 or 384 well formats. These include mitochondrial bioenergetic measurements of oxidative phosphorylation and glycolysis using the XF-96 Extracellular Flux Analyzer, electrophysiology by whole cell current clamping, voltage-sensitive fluorescent dyes or multi-electrode array (MEA) systems, calcium transients using a variety of calcium-sensitive fluorophores, B-type natrietic peptide measurements as a marker for hypertrophy, and susceptibility to injury or death by assaying for cardiac troponin I, or lactate dehydrogenase release, caspase activity, propidium iodide, or terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) staining.

Functional assessments including contractility, tissue strength, and excitation–contraction coupling can be modeled in engineered cardiac tissues (ECTs). These integrated tissue models, which can be formed in a variety of formats, produce well-aligned 3D tissue strips with a mixture of cardiac and non-cardiac cells [51, 52] (Fig. 3). Within a dish, the human ECT manifests contractile characteristics similar to in vivo myocardium, with intact Frank–Starling and force–frequency responses, calcium transients, and adrenergic responsiveness [51, 52] (Fig. 4a–c). The human ECT can be cultured under physiologic conditions or with the addition of mechanical, metabolic, or pharmacologic stress. ECT can be produced using cardiomyocytes from existing mouse models as depicted here in Fig. 4a, d–e, which shows the impact of loss of functional cMyBP-C on contractile kinetics, with a pronounced effect on relaxation. The functional readout of contractility permits a careful dissection of both contraction and relaxation, rendering ECTs suitable for investigations into mechanisms of

HFpEF (Fig. 4d–f). Disease modeling using either patient-derived iPSCs or introduced by CRISPR/Cas9 editing can be performed under tightly controlled and reproducible conditions using ECTs. Additionally, the effects of phenotypic modifier genes can be explored using gene-editing approaches to introduce specific variants on a disease background and tested within the ECT.

Limitations of iPSC-Derived Cardiomyocytes as a Model System

Not only are iPSC-CMs valuable in understanding disease mechanisms but also they allow for disease and patient-specific pharmacological screening. However, these advantages must be carefully weighed with the limitations of this model. iPSC-derived cardiomyocytes are more similar to embryonic or fetal heart-derived cardiomyocytes, so one can question their usefulness as a model system. Compared to mature LV cardiomyocytes, iPSC-derived cardiomyocytes express similar contractile proteins, including cardiac troponin T, α -actinin, and α -myosin and β -myosin heavy chains. However, the pattern of distribution by immunofluorescence (Fig. 5) suggests a more embryonic or fetal sarcomere organization which is not as structured as an adult cardiomyocyte [53]. This limitation can be overcome by using ECT which have improved structural alignment of the contractile proteins in the sarcomere (Fig. 3c). However, this needs to be balanced with the ability of iPSC-CMs to recapitulate functional aspects of endogenous human cardiomyocytes and allow for more robust in vitro transcription and proteome profiling for conditions such as LV hypertrophy and cardiac dysfunction [54, 55]. The expression of fundamental calcium handling proteins such as the ryanodine receptor, sarco/endoplasmic reticulum Ca^{2+} -ATPase, and the sodium/calcium exchanger is also present in iPSC-CMs [56]. iPSC-CMs derived from early cardiac differentiation protocols which relied first the formation of embryoid bodies displayed immature calcium-handling properties; however, with the existing monolayer cardiac differentiation protocols, iPSC-CMs display a mature calcium handling phenotype by 21 days [56]. The localization of connexin-43, a critical gap junction protein as well as ion channels (sodium, L-type calcium, and human ether-à-go-go-related gene (hERG)), is similar to that of adult cardiomyocytes (Fig. 5). Ion channel function and aggregated action potentials can be determined electrophysiologically either by patch clamping or using microelectrode arrays. Within an iPSC-CM culture, there will be a heterogeneous mixture of action potential morphologies that include nodal-like, atrial-like, and ventricular-like morphologies depending on the age and state of maturity of the iPSC-cardiomyocyte [57]. This heterogeneity of cardiomyocyte types and the inability to control the subtype with any precision are limitations of using iPSCs as a model system. As yet, no marker exists to enable the purification of ventricular cardiomyocytes from the rest of the cells. In terms of inotropic and chronotropic responses, human iPSC-CMs demonstrate appropriate dose-dependent responses to 40 cardioactive drugs (reviewed in [58]). In addition, iPSC-derived cardiomyocytes respond to neurohormonal stimuli such as endothelin-1 to develop hypertrophy [59]. Despite the limitations of iPSC-CMs as a model system, they remain extremely useful for systematic molecular approaches.

Bed-to-Bench Translational Model for a Precision Medicine Approach

A bed-to-bench translational system may be used to identify new potential mediators of HFpEF by combining “-omic” analysis and mechanistic studies in patient-specific iPSC-CMs [60]. The general concept of this strategy is to incorporate bioactivity studies into guiding the selection of biomarkers identified in -omic studies for further investigation and development. The overall rationale for this two-step screening process (Fig. 6) is to identify biomarkers that have biological effects in order to increase the likelihood that it is causally involved in the disease process [60]. After miRNA profiling, it can be determined whether a specific miRNA has detrimental or beneficial effects on iPSC-CMs. Depending on the in vitro phenotypic outcomes, the miRNA can then be used a research tool to further investigate the underlying mechanisms as a result of the deregulated molecular pathways (Fig. 6).

This two-step screening process has the potential to discover new miRNA disease modifiers or biomarker candidates in HFpEF. The identification of specific miRNAs associated with certain phenotypes of HFpEF as defined by the individuals’ co-morbidities along with further verification of miRNAs that alter cardiac function may suggest mechanisms that contribute for poorer outcomes in specific HFpEF phenotypes. This will allow the in-depth mechanistic investigation of miRNAs in HFpEF and make important progress towards identifying or developing therapeutic interventions that will improve cardiac outcomes in HFpEF patients. The discovery of a direct role for circulating miRNAs in HFpEF would also broaden the potential impact of miRNA-based therapies in disease treatment. Further understanding the pathways impacted by miRNA alterations in the myocardium could help identify more effective novel and “traditional” individually tailored therapies for HFpEF.

In conclusion, a systematic approach to delineating the role of deregulated miRNAs in modulating cardiac and vascular function from the cell to the patient can provide insight to the molecular mechanisms of HFpEF and may also identify future therapeutic targets.

Acknowledgments

Sources of Funding: This work was supported by the National Institutes of Health K08 HL111148 (J.L.S), R01 HL107367-05 (J.C.R.), and R01 HL128332 (A.J.K).

Abbreviations

BNP	B-type natriuretic peptide
CAD	Coronary artery disease
CM	Cardiomyocyte
cMyBP-C	Cardiac myosin binding protein C
COPD	Chronic obstructive pulmonary disease
CKD	Chronic kidney disease
DM	Diabetes mellitus

ECT	Engineered cardiac tissues
EF	Ejection fraction
HFpEF	Heart failure with preserved ejection fraction
HFREF	Heart failure with reduced ejection fraction
miRNA	MicroRNA
LV	Left ventricle
iPSC	Induced pluripotent stem cell
iPSC-	Induced pluripotent stem cell-derived
CMs	cardiomyocytes

References

1. Owan TE, et al. Trends in prevalence and outcome of heart failure with preserved ejection fraction. *The New England Journal of Medicine*. 2006; 355(3):251–259. [PubMed: 16855265]
2. Paulus WJ, van Ballegoij JJ. Treatment of heart failure with normal ejection fraction: an inconvenient truth! *Journal of the American College of Cardiology*. 2010; 55(6):526–537. [PubMed: 20152557]
3. Conceicao G, et al. Animal models of heart failure with preserved ejection fraction. *Neth Heart J*. 2016; 24(4):275–286. [PubMed: 26936157]
4. Ho JE, et al. Predictors of new-onset heart failure: differences in preserved versus reduced ejection fraction. *Circulation Heart Failure*. 2013; 6(2):279–286. [PubMed: 23271790]
5. Glezeva N, et al. Exaggerated inflammation and monocytosis associate with diastolic dysfunction in heart failure with preserved ejection fraction: evidence of M2 macrophage activation in disease pathogenesis. *Journal of Cardiac Failure*. 2015; 21(2):167–177. [PubMed: 25459685]
6. Paulus WJ, Tschope C. A novel paradigm for heart failure with preserved ejection fraction: comorbidities drive myocardial dysfunction and remodeling through coronary microvascular endothelial inflammation. *Journal of the American College of Cardiology*. 2013; 62(4):263–271. [PubMed: 23684677]
7. Guazzi M, et al. Pulmonary hypertension in heart failure with preserved ejection fraction: a target of phosphodiesterase-5 inhibition in a 1-year study. *Circulation*. 2011; 124(2):164–174. [PubMed: 21709061]
8. D'Elia E, et al. Role of biomarkers in cardiac structure phenotyping in heart failure with preserved ejection fraction: critical appraisal and practical use. *European Journal of Heart Failure*. 2015; 17(12):1231–1239. [PubMed: 26493383]
9. Shah SJ, et al. Phenomapping for novel classification of heart failure with preserved ejection fraction. *Circulation*. 2015; 131(3):269–279. [PubMed: 25398313]
10. Bartel DP. MicroRNAs: target recognition and regulatory functions. *Cell*. 2009; 136(2):215–233. [PubMed: 19167326]
11. Sayed D, Abdellatif M. MicroRNAs in development and disease. *Physiological Reviews*. 2011; 91(3):827–887. [PubMed: 21742789]
12. Kriegel AJ, et al. Endogenous microRNAs in human microvascular endothelial cells regulate mRNAs encoded by hypertension-related genes. *Hypertension*. 2015; 66(4):793–799. [PubMed: 26283043]
13. Koberle V, et al. Differential stability of cell-free circulating microRNAs: implications for their utilization as biomarkers. *PloS One*. 2013; 8(9):e75184. [PubMed: 24073250]

14. Ge Q, et al. miRNA in plasma exosome is stable under different storage conditions. *Molecules*. 2014; 19(2):1568–1575. [PubMed: 24473213]
15. Watson CJ, et al. MicroRNA signatures differentiate preserved from reduced ejection fraction heart failure. *European Journal of Heart Failure*. 2015; 17(4):405–415. [PubMed: 25739750]
16. Wong LL, et al. Circulating microRNAs in heart failure with reduced and preserved left ventricular ejection fraction. *European Journal of Heart Failure*. 2015; 17(4):393–404. [PubMed: 25619197]
17. Liu W, et al. Circulating microRNAs correlated with the level of coronary artery calcification in symptomatic patients. *Scientific Reports*. 2015; 5:16099. [PubMed: 26537670]
18. Kara M, Kirkil G, Kalemci S. Differential expression of microRNAs in chronic obstructive pulmonary disease. *Advances in Clinical and Experimental Medicine*. 2016; 25(1):21–26. [PubMed: 26935494]
19. Al-Kafaji G, Al-Mahroos G, Abdulla Al-Muhtaresh H, Sabry MA, Abdul Razzak R, Salem AH. Circulating endothelium-enriched microRNA-126 as a potential biomarker for coronary artery disease in type 2 diabetes mellitus patients. *Biomarkers*. 2016; :1–11. DOI: 10.1080/1354750X.2016.1204004
20. Nassirpour R, Raj D, Townsend R, Argyropoulos C. MicroRNA biomarkers in clinical renal disease: from diabetic nephropathy renal transplantation and beyond. *Food and Chemical Toxicology*. 2016; 98(Pt A):73–88. DOI: 10.1016/j.fct.2016.02.018 [PubMed: 26925770]
21. Mitchell PS, et al. Circulating microRNAs as stable blood-based markers for cancer detection. *Proceedings of the National Academy of Sciences of the United States of America*. 2008; 105
22. Zerneck A, et al. Delivery of microRNA-126 by apoptotic bodies induces CXCL12-dependent vascular protection. *Science Signaling*. 2009; 2(100):ra81. [PubMed: 19996457]
23. Wang X, et al. Cardiomyocytes mediate anti-angiogenesis in type 2 diabetic rats through the exosomal transfer of miR-320 into endothelial cells. *Journal of Molecular and Cellular Cardiology*. 2014; 74:139–150. [PubMed: 24825548]
24. Bang C, et al. Cardiac fibroblast-derived microRNA passenger strand-enriched exosomes mediate cardiomyocyte hypertrophy. *The Journal of Clinical Investigation*. 2014; 124(5):2136–2146. [PubMed: 24743145]
25. Xu X, et al. miR-21 in ischemia/reperfusion injury: a double-edged sword? *Physiological Genomics*. 2014; 46(21):789–797. [PubMed: 25159851]
26. Chau BN, et al. MicroRNA-21 promotes fibrosis of the kidney by silencing metabolic pathways. *Science Translational Medicine*. 2012; 4(121):121ra18.
27. Garchow BG, et al. Silencing of microRNA-21 in vivo ameliorates autoimmune splenomegaly in lupus mice. *EMBO Molecular Medicine*. 2011; 3(10):605–615. [PubMed: 21882343]
28. Jazbutyte V, Thum T. MicroRNA-21: from cancer to cardiovascular disease. *Current Drug Targets*. 2010; 11(8):926–935. [PubMed: 20415649]
29. Wang R, et al. Unique MicroRNA signatures associated with early coronary atherosclerotic plaques. *Biochemical and Biophysical Research Communications*. 2015; 464(2):574–579. [PubMed: 26159918]
30. Zhang Y, et al. Secreted monocytic miR-150 enhances targeted endothelial cell migration. *Molecular Cell*. 2010; 39(1):133–144. [PubMed: 20603081]
31. Douglas PS, Tallant B. Hypertrophy, fibrosis and diastolic dysfunction in early canine experimental hypertension. *Journal of the American College of Cardiology*. 1991; 17(2):530–536. [PubMed: 1825098]
32. Primessnig U, et al. Novel pathomechanisms of cardiomyocyte dysfunction in a model of heart failure with preserved ejection fraction. *European Journal of Heart Failure*. 2016; 18(8):987–997. [PubMed: 27135883]
33. Rosas PC, et al. Phosphorylation of cardiac myosin-binding protein-C is a critical mediator of diastolic function. *Circulation Heart Failure*. 2015; 8(3):582–594. [PubMed: 25740839]
34. Gresham KS, Stelzer JE. The contributions of cardiac myosin binding protein C and troponin I phosphorylation to beta-adrenergic enhancement of in vivo cardiac function. *The Journal of Physiology*. 2016; 594(3):669–686. [PubMed: 26635197]
35. Takahashi K, et al. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell*. 2007; 131(5):861–872. [PubMed: 18035408]

36. Yu J, et al. Induced pluripotent stem cell lines derived from human somatic cells. *Science*. 2007; 318(5858):1917–1920. [PubMed: 18029452]
37. Yu J, et al. Human induced pluripotent stem cells free of vector and transgene sequences. *Science*. 2009; 324(5928):797–801. [PubMed: 19325077]
38. Ban H, et al. Efficient generation of transgene-free human induced pluripotent stem cells (iPSCs) by temperature-sensitive Sendai virus vectors. *Proceedings of the National Academy of Sciences of the United States of America*. 2011; 108(34):14234–14239. [PubMed: 21821793]
39. Staerk J, et al. Reprogramming of human peripheral blood cells to induced pluripotent stem cells. *Cell Stem Cell*. 2010; 7(1):20–24. [PubMed: 20621045]
40. Guan X, et al. Dystrophin-deficient cardiomyocytes derived from human urine: new biologic reagents for drug discovery. *Stem Cell Research*. 2014; 12(2):467–480. [PubMed: 24434629]
41. Zhang J, et al. Extracellular matrix promotes highly efficient cardiac differentiation of human pluripotent stem cells: the matrix sandwich method. *Circulation Research*. 2012; 111(9):1125–1136. [PubMed: 22912385]
42. Lian X, et al. Robust cardiomyocyte differentiation from human pluripotent stem cells via temporal modulation of canonical Wnt signaling. *Proceedings of the National Academy of Sciences of the United States of America*. 2012; 109(27):E1848–E1857. [PubMed: 22645348]
43. Han L, et al. Study familial hypertrophic cardiomyopathy using patient-specific induced pluripotent stem cells. *Cardiovascular Research*. 2014; 104(2):258–269. [PubMed: 25209314]
44. Raval KK, et al. Pompe disease results in a Golgi-based glycosylation deficit in human induced pluripotent stem cell-derived cardiomyocytes. *The Journal of Biological Chemistry*. 2015; 290(5):3121–3136. [PubMed: 25488666]
45. Afzal MZ, et al. Nicorandil, a nitric oxide donor and ATP-sensitive potassium channel opener, protects against dystrophin-deficient cardiomyopathy. *Journal of Cardiovascular Pharmacology and Therapeutics*. 2016; 21(6):549–562. [PubMed: 26940570]
46. Jiang Y, et al. An induced pluripotent stem cell model of hypoplastic left heart syndrome (HLHS) reveals multiple expression and functional differences in HLHS-derived cardiac myocytes. *Stem Cells Transl Med*. 2014; 3(4):416–423. [PubMed: 24591732]
47. Drawnel FM, et al. Disease modeling and phenotypic drug screening for diabetic cardiomyopathy using human induced pluripotent stem cells. *Cell Reports*. 2014; 9(3):810–821. [PubMed: 25437537]
48. Ebert AD, et al. Characterization of the molecular mechanisms underlying increased ischemic damage in the aldehyde dehydrogenase 2 genetic polymorphism using a human induced pluripotent stem cell model system. *Science Translational Medicine*. 2014; 6(255):255ra130.
49. Gaber N, et al. Fetal reprogramming and senescence in hypoplastic left heart syndrome and in human pluripotent stem cells during cardiac differentiation. *The American Journal of Pathology*. 2013; 183(3):720–734. [PubMed: 23871585]
50. Bahramali E, et al. Association of ACE gene D polymorphism with left ventricular hypertrophy in patients with diastolic heart failure: a case-control study. *BMJ Open*. 2016; 6(2):e010282.
51. de Lange WJ, et al. Neonatal mouse-derived engineered cardiac tissue: a novel model system for studying genetic heart disease. *Circulation Research*. 2011; 109(1):8–19. [PubMed: 21566213]
52. Bielawski KS, Leonard A, Bhandari S, Murry CE, Sniadecki NJ. *Tissue Engineering Part C: Methods*. 2016; 22(10):932–940. DOI: 10.1089/ten.tec.2016.0257 [PubMed: 27600722]
53. Germanguz I, et al. Molecular characterization and functional properties of cardiomyocytes derived from human inducible pluripotent stem cells. *Journal of Cellular and Molecular Medicine*. 2011; 15(1):38–51. [PubMed: 20041972]
54. Aggarwal P, et al. RNA expression profiling of human iPSC-derived cardiomyocytes in a cardiac hypertrophy model. *PloS One*. 2014; 9(9):e108051. [PubMed: 25255322]
55. Zhi D, et al. Whole-exome sequencing and an iPSC-derived cardiomyocyte model provides a powerful platform for gene discovery in left ventricular hypertrophy. *Frontiers in Genetics*. 2012; 3:92. [PubMed: 22654895]
56. Hwang HS, et al. Comparable calcium handling of human iPSC-derived cardiomyocytes generated by multiple laboratories. *Journal of Molecular and Cellular Cardiology*. 2015; 85:79–88. [PubMed: 25982839]

57. Ben-Ari M, Naor S, Zeevi-Levin N, Schick R, Ben Jehuda R, Reiter I, Raveh A, Grijnevitch I, Barak O, Rosen MR, Weissman A, Binah O. Developmental changes in elec-trophysiological characteristics of human-induced pluripotent stem cell-derived cardiomyocytes. *Heart Rhythm*. 2016; 13(12):2379–2387. DOI: 10.1016/j.hrthm.2016.08.045 [PubMed: 27639456]
58. Dick E, et al. Evaluating the utility of cardiomyocytes from human pluripotent stem cells for drug screening. *Biochemical Society Transactions*. 2010; 38(4):1037–1045. [PubMed: 20659000]
59. Carlson C, et al. Phenotypic screening with human iPS cell-derived cardiomyocytes: HTS-compatible assays for interrogating cardiac hypertrophy. *Journal of Biomolecular Screening*. 2013; 18(10):1203–1211. [PubMed: 24071917]
60. Raphael R, et al. Combining patient proteomics and in vitro cardiomyocyte phenotype testing to identify potential mediators of heart failure with preserved ejection fraction. *Journal of Translational Medicine*. 2016; 14(1):18. [PubMed: 26792056]

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Fig. 1.

MicroRNAs as biomarkers and disease modifiers. MicroRNAs can be encapsulated into microvesicles and secreted by donor cells into the circulation and taken up by recipient cells. MicroRNAs can affect gene expression in either donor or recipient cells by binding mRNA targets to trigger translational repression or degradation as demonstrated in the donor and recipient cells. Encapsulated miRNAs are protected from nucleases in the circulation and are resistant to degradation. Freely circulating microRNAs are less stable but can be provided some protection through binding to circulating proteins and lipoproteins

Fig. 2.

Essential features of a precision medicine approach towards defining molecular mechanisms in HFpEF. Considering the heterogeneity of the HFpEF population, subjects should be rigorously classified based on clinical phenotype prior to biomarker profiling. Blood can be collected for microRNA profiling at the same time; urine can be collected to generate induced pluripotent stem cells. The generation of iPSCs and their differentiation into specific cell types (i.e., cardiomyocytes) is essential for further scientific discovery by way of assessing in vitro phenotypes, testing disease mechanisms of specific microRNAs, identifying DNA variants as disease-causing, additional genomic, proteomic, transcriptomic profiling, and drug screening

Fig. 3. Human engineered cardiac tissue. **a** ECT in culture suspended between tabs within a 6-well FlexCell dish. **b–d** Immunohistochemistry of ECT labels with alpha-actinin (*green*) and cardiac myosin binding protein C (cMyBPC) (*red*) showing overall tissue organization and detailed sarcomere structure. Nuclei labels with DAPI (*blue*). **e** ECT mounted on force transducer within perfusion chamber for physiologic testing

Fig. 4.

Using engineered cardiac tissues to model diastolic dysfunction. **a** Representative twitch tracings depicting normalized force (mN) over time (s) from murine ECTs comparing contractile kinetics in wild-type (WT) cardiomyocytes to cardiomyocytes deficient in cMyBPC (knockout (KO)). **b** Human DF19-9-11T.H. iPSC cardiomyocytes grown in ECTs reveal intact Frank-Starling relationship shown by increasing force (μN) generation with increasing strain and positive force (μN)–frequency (Hz) response (**c**). **d–f** The first derivative of the twitch force shown in panel a vs. time ($dFdt/F_{Max}$) reveals the abnormally accelerated diastolic relaxation kinetics ($+dFdt_{Max}$ and $-dFdt_{Max}$) of the cMyBP-C KO ECT compared to WT, illustrating the utility of the ECT model to differentiate differences in diastolic function

Fig. 5. iPSC-derived cardiomyocytes grown in monolayer. This is a representative immunofluorescent image to show the irregular pattern of sarcomeres (*red*) and connexin-43 (*green*) localization within iPSC-derived cardiomyocytes. Nuclei label *blue* with DAPI

Fig. 6.

Bed-to-bench translational approach to identify molecular mechanisms in HFpEF. Blood samples are obtained to conduct extensive microRNA (miRNA) profiling by miRNA sequencing. Data is accumulated and analyzed to identify potential biomarkers that are associated with disease. To implicate causation in the disease process, specific miRNAs can be tested for bioactivity on patient-specific iPSC-derived cardiomyocytes. The miRNAs can either be downregulated or upregulated in the cells by the application of anti-miRNAs or miRNA mimics. Different in vitro phenotyping assays can be conducted to assess for bioactivity including electrophysiology, calcium handling, and mitochondrial bioenergetics among others. Once a miRNA is identified to have bioactivity in the iPSC-derived cardiomyocytes, additional profiling can be conducted. After upregulating or downregulating of the specific miRNA, the iPSC-derived cardiomyocytes can be subject to proteomics, transcriptomics, or metabolomics. This data can then be analyzed for specific molecular pathway identification. This may lead to new areas of research within HFpEF