# Aging, MicroRNAs, and Heart Failure

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**Abstract: Aging is a major risk factor for heart failure, one of the leading causes of death in Western society. The mechanisms that underlie the different forms of heart failure have been elucidated only in part and the role of noncoding RNAs is still poorly characterized. Specifically, microRNAs (miRNAs), a class of small noncoding RNAs that can modulate gene expression at the posttranscriptional level in all cells, including myocardial and vascular cells, have been shown to play a role in heart failure with reduced ejection fraction. In contrast, miRNAs role in heart failure with preserved ejection fraction, the predominant form of heart failure in the elderly, is still unknown. In this review, we will focus on age-dependent miRNAs in heart failure and on some other conditions that are prevalent in the elderly and are frequently associated with heart failure with preserved ejection fraction. (Curr Probl Cardiol 2020;45:100406.)**

## Introduction

### *Aging and Heart Failure*

Aging is a major risk factor for cardiovascular diseases (CVD), which represent more than 30% of all deaths worldwide. As the average human

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lifespan continues to increase, the prevalence of age-associated diseases including heart failure (HF), hypertension, and diabetes is assuming epidemic proportions.

Aging is characterized by the decline of the physiological functions necessary for survival and reproduction. During aging, a progressive deterioration of cellular processes occurs in all tissues and all cell functions are involved in association with distinct biologic characteristics, including genomic stability due to the accumulation of DNA damage and impairment of DNA repair[1]; continuous shortening of telomeres[2]; variations in control of gene expression, such as epigenetic and alternative splicing[3,4]; cellular senescence[5]; dysfunctions in mitochondria metabolism[6] and protein homeostasis[7]; and enhanced oxidative stress.[8]

Aging per se modifies myocardial and vascular function even in the absence of clinical disease. The aging heart exhibits progressive structural and functional changes such as left ventricular hypertrophy, which is associated with a delay in ventricular relaxation, and changes in diastolic intracellular calcium dynamics (Fig 1A). Both heart failure with reduced ejection fraction (HFrEF) and with preserved ejection fraction (HFpEF) are very common in older persons and frequently coexist in the same patient. Specifically, HFpEF affects approximately 70% of elderly individuals with heart failure[9]; in fact, at the time of diagnosis, most of the patients with HFpEF are more than 60 years old[10] and is more common in women than in men with a 2:1 ratio.[9]

It is noteworthy that the presence of diastolic dysfunction associated to a left ventricular ejection fraction >50% is not diagnostic for HFpEF and that clinical evidence of HF is also required. Mechanisms responsible for HFpEF are poorly characterized, although there is some evidence to support a role for low-grade inflammation,[11] microvascular endothelial dysfunction,[12,13] cardiomyocyte hypertrophy,[14] and enhanced fibrosis,[15] ultimately leading to an increase in cardiac stiffness and impaired relaxation.[16] Further, certain conditions, including hypertension,[17] obesity,[18] and diabetes mellitus,[19] are frequent comorbidities associated with HFpEF. Elucidation of the mechanisms responsible for HFpEF is a major health priority, not only because of the large number of patients involved but also because therapeutic strategies are limited to diuretics and optimal fluid balance. This is in stark contrast to the large array of pharmacologic and nonpharmacologic treatment options in patients with HFrEF.

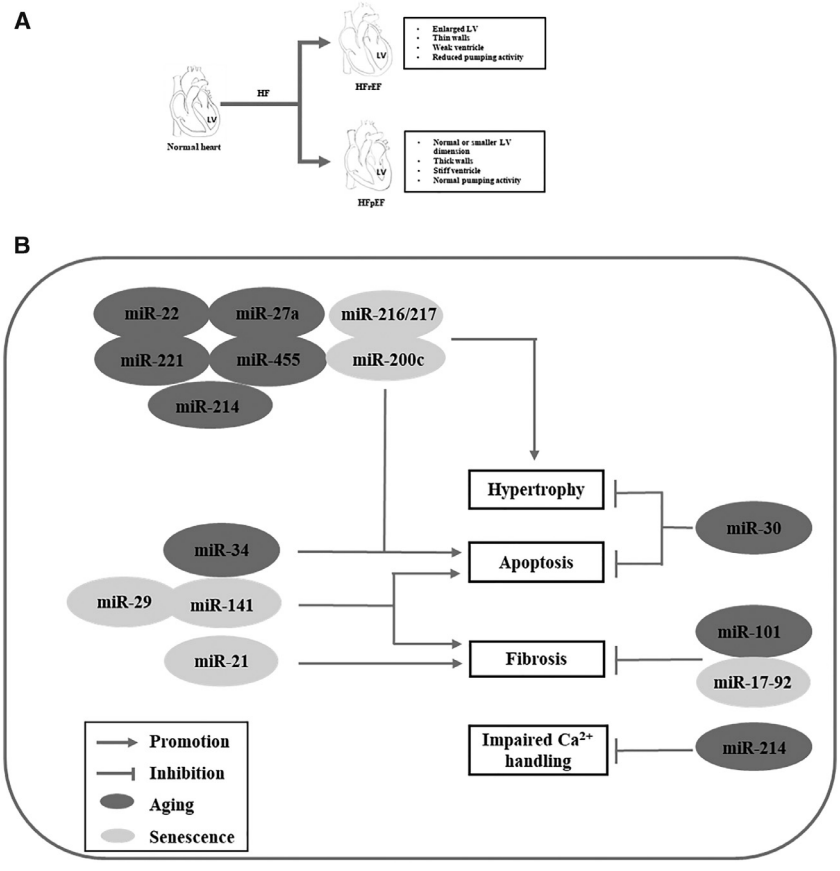


**FIG 1.** Effect of miRNAs modulated by aging or senescence on cardiac remodeling. (A) main characteristics of heart failure (HF) with preserved (HFpEF) and reduced ejection fraction (HFrEF) (LV: Left Ventricle); (B) effects of age-dependent miRNAs on hypertrophy, apoptosis, fibrosis, impaired $Ca^{2+}$ handling, ie, events implicated in cardiac remodeling.

# microRNAs

## *microRNAs' Function and CVD*

microRNAs (miRNAs) are short noncoding RNAs, approximately 22 nucleotides long, that regulate the expression of miRNA targets in a sequence specific manner, through the induction of miRNA degradation or translational repression.[20] miRNAs modulate a variety of important cell functions including stem cell self-renewal, cell proliferation, apoptosis, metabolism, and oxidative stress,[21] and have been recently considered

relevant regulators of cellular senescence and aging.[22] Numerous studies have shown the fundamental role of some miRNAs in cardiac and vascular homeostasis, and several CVD and cardiac remodeling (Fig 1B) have been related to miRNA dysregulation.[23-26] Finally, circulating miRNAs are already used as diagnostic and/or prognostic biomarkers, and modulation of miRNA expression has been proposed as a potential therapeutic strategy.

### *miRNAs As Biomarkers*

miRNAs are detectable in different body fluids including plasma, serum, saliva, and urine, as well as in some circulating blood cells, and represent promising biomarkers of several diseases including some CVD.[27,28] It is notable that extracellular miRNAs are relatively stable and protected, at least in part, from RNAse-mediated degradation.[29] It is estimated that 80%-99% of total circulating miRNAs are linked to RNA-binding proteins, eg, Ago2-family proteins,[30,31] or to lipoproteins such as high-density lipoproteins.[32] Approximately 10% of circulating miRNAs occupy extracellular vesicles, ie, exosomes, microvesicles, and apoptotic bodies.[32] Exosomes develop from endosomal membranes, that blend with the plasma membrane and then emit their content as vesicles into the extracellular environment[32-34]; microvesicles originate from the cell surface are involved in the cell-cell communication through transfer of genetic information; and apoptotic bodies are derived from cells during the late stages of apoptosis.[35,36]

## miRNAs in Aging and HF

In this review we will discuss miRNAs that may be involved in HF and examine separately miRNAs that exhibit an age-dependent modulation in the heart, in tissues other than the heart and miRNAs that may help differentiate HFpEF vs HFrEF.

### *Age-Modulated miRNAs in the Heart and Their Potential Involvement in HF*

The vast majority of the studies on age-associated miRNAs and HF relate to HFrEF and very little is known on miRNAs and HFpEF.

***miR-17-92.*** The miR-17−92 cluster is composed of six individual miRNAs (miR-17, miR-18a, miR-19a, miR-20a, miR-19b, and miR-92a) and is down-regulated in human aging cell models including endothelial cells

and skin fibroblasts.[37] Further, the expression of miR-18a, miR-19a, and miR-19b also decreases in the cardiac tissue of old mice and in isolated neonatal cardiomyocytes aged in culture (CMs)[38] and miR-19b is downregulated in both serum and myocardial tissue of patients with HF.[39] These miRNAs are considered the most strongly repressed miRNAs in aged CMs and hearts of old and failure-prone mice. Members of this cluster are involved in the regulation of extracellular matrix (ECM) production and experimental studies suggest that this may occur via different mechanisms. miR-18a, miR-19a, and miR-19b target connective tissue growth factor and thrombospondin 1.[38] Connective tissue growth factor and thrombospondin 1 enhance transforming growth factor beta (TGF$\beta$) expression, cardiac fibrosis, and left ventricular stiffening.[40-42] Thus, miR-19a and -19b act as negative regulators of TGF$\beta$ signaling by targeting directly TGF$\beta$R2 and this mechanism contributes to downmodulate fibrosis and reduce cardiac remodeling.[43] The overexpression of miR-17 and miR-20a, in a murine model of age-related HF, reduces p21Waf1/Cip1/Sdi1 (p21) activation and suppresses primary human fibroblasts senescence.[38] Taken together these studies indicate that the age-associated decrease in miR-17-92 cluster in the heart enhances cardiac fibrosis and stiffness.

***miR-21.*** miR-21 expression is increased in old mouse hearts,[44] and this miRNA has been widely studied in relation to its involvement in age-associated fibrosis and augmented expression both in murine cardiac fibroblasts and cardiac tissue[45] and in the myocardium of patients with aortic stenosis.[46]

In a mouse model of acute cardiac allograft transplantation,[47] miR-21 has been associated to myocardial disease because of the activation of mitogen-activated protein kinase (MAPK) signaling in fibroblasts and the induction of cardiac fibrosis.[48]

miR-21 levels are increased in fibroblasts within the failing mouse heart, and can downregulate Sprouty homolog 1, a negative regulator of the extracellular signal-regulated kinase/mitogen-activated protein kinase (extracellular-signal-regulated kinase ERK) and MAPK signaling pathway; via this mechanism miR-21 elicits a profibrotic effect[48] and modifies cardiomyocyte morphology.[49] More recently, it has been demonstrated that the senescence induced by doxorubicin (DOX) in cardiomyocytes is promoted by miR-21, possibly by targeting phosphatase and tensin homolog and is prevented by miR-21 inhibition.[50] Notably, circulating miR-21 levels are higher in serum obtained from peripheral vein and coronary sinus of patients with HF,[51] in plasma, and in myocardial cells in patients

with aortic stenosis.[46] These studies suggest that the age-dependent increase in miR-21 expression may contribute to the development of cardiac fibrosis.

***miR-22.*** miR-22 is upregulated during cardiac aging; its overexpression induces senescence and promotes the migratory activity of cardiac fibroblasts.[52] Interestingly, miR-22 increases during cardiac hypertrophy, inducing cardiac hypertrophy and remodeling.[53,54] It is essential to the development of hypertrophic cardiac growth in response to stress in a mouse model in which is either globally or cardiac-specifically deleted.[54] Moreover, miR-22 loss favors the development of dilated cardiomyopathy and fibrosis under stress conditions.[54,55]

Different miR-22 targets are involved in cardiac hypertrophy and remodeling.

Two histone deacetylases, Sirtuin 1 (Sirt1) and Histone Deacetylase 4, are miR-22 targets in the heart, implying that miR-22 plays a role in the epigenetic regulation of gene expression during cardiac hypertrophy.[54] Purine rich element binding protein B, a repressor of transactivation of serum response factor is another miR-22 target and miR-22 knockout represses the expression of serum response factor-depended hypertrophic genes by purine rich element binding protein B re-expression.[56] Phosphatase and tensin homolog, a phosphatase involved in the regulation of the cell cycle, is an miR-22 target and its inactivation in cardiomyocytes causes cardiac hypertrophy.[57] Peroxisome proliferator-activated receptor gamma (PPAR$\alpha$) and peroxisome proliferator-activated receptor $\gamma$ coactivator 1 (PGC-1$\alpha$) are also targets of miR-22.[54,55] PPAR$\alpha$ activity depends on PGC-1$\alpha$, that is positively induced by SIRT1, thus PPAR$\alpha$, PGC-1$\alpha$ and SIRT1 form a master cluster transcriptional factors/cofactors, that controls fatty acid and lipid metabolism which is down-regulated in hearts of miR-22 transgenic mice.[55]

Therefore, it is tempting to speculate that miR-22 plays a key role in the shift of the metabolic program during the development of cardiomyopathies.

Further, miR-22 targets acetyltransferase CBP/p300 (cAMP-response element-binding protein/p300). In hearts exposed to ischemia-reperfusion, miR-22 reduces the protein levels of CBP/p300 and acetylates p53, protecting cardiomyocytes from apoptosis.[58]

Notably, in the Bio-SHiFT study, a prospective observational study of 263 chronic heart failure patients, the expression of circulating miR-22 was associated with prognosis, in fact lower miR-22 levels are associated with worsening of chronic heart failure.[59]

In summary, the age-dependent increase in cardiac miR-22 expression appears to have a cardioprotective role by promoting hypertrophy and preventing cell death.

***miR-29.*** The miR-29 family consists of 3 different members, miR-29a, miR-29b, and miR-29c that differ for 2 or 3 bases and share a common seed sequence.[60] miR-29 is upregulated in the aging heart[60,61] and during senescence in human HeLa cells and primary human foreskin fibroblasts.[62] miR-29 inhibits B-Myb oncogene expression and DNA synthesis, regulating the expression of several genes involved in cell proliferation.[62] miR-29 is mainly expressed in cardiac fibroblasts and its repression leads to activation of TGF$\beta$, contributing to cardiac fibrosis in mice, both in vivo and in vitro.[61,63] Further, miR-29b is repressed during the final stages of dilated cardiomyopathy, leading to an increase in the profibrotic activity of matrix metalloproteinase 2.[64,65] In hypertensive heart disease induced by angiotensin II miR-29b administration inhibits TGF$\beta$/Smad3 signaling.[66]

Higher levels of miR-29a have been found in the plasma of patients with cardiac hypertrophy and are inversely associated with cardiac fibrosis.[67]

Therefore, miR-29a in HF appears to have an antifibrotic action, possibly due to inhibition of angiotensin II-mediated cardiac remodeling by targeting TGF-$\beta$/Smad3 signaling.

***miR-34a.*** miR-34 family consists of 3 microRNAs, miR-34a, b, and c. miR-34a has been shown to increase with aging in several mice tissues including the heart, liver, and brain,[21] and in rat endothelial progenitor cells.[68] In these cells, miR-34a is induced by the tumor suppressor p53 and inhibits the deacetylase SIRT1,[69] a major regulator of longevity and metabolic disorders, that is progressively reduced in multiple tissues during aging. This results in increased levels of acetylation and activation of p53 and forkhead box O1 (FOXO1), with the consequent induction of cell senescence.[69] Consistent with these data, miR-34a inhibition induces proliferation of human cardiac progenitor cells.[70] miR-34a has also been associated to HF: miR-34a upregulation in cardiomyocytes directly targets and inhibits proproliferative and prosurvival proteins CyclinD1 and Bcl2.[71] Recently, phosphatase 1 nuclear targeting subunit, which is downregulated in aged mice hearts, has been proposed as a novel miR-34a target.[72] Phosphatase 1 nuclear targeting subunit overexpression enhances cardiac contractile function in vivo.[72] Moreover, within several days post-MI, older mice exhibit a significant increase of

miR-34a expression compared to young mice, resulting in a decline of cardiac performances and cardiac remodeling. Efficacy of the miRNA inhibition has been tested in adult mice and it has been observed that either miR-34a inhibition or inhibition of the entire miR-34 family in mice led to an improvement of post-MI cardiac function, and a significant decrease of fibrosis, cardiac remodeling and cardiomyocyte death.[71]

Finally, higher miR-34a levels have been found in diabetic human plasma, in diabetic cardiac progenitor cells and in cardiomyocytes exposed to high glucose. In agreement with this SIRT1 intracellular levels are reduced in both cardiac progenitor cells and cardiomyocytes, suggesting that miR-34 may play a role in the accelerated senescence of diabetic hearts.[73] In summary, the age-dependent increase of miR-34 can induce fibrosis and cardiac remodeling during HF.

## *Age-Modulated miRNAs in Tissues Other Than the Heart and Their Potential Involvement in HF*

An age-associated change in the expression of a given miRNA needs not be a generalized phenomenon and may be limited to a specific tissue. Some miRNAs that are involved in HF, exhibit age-dependent modulation in tissues and organs other than the heart and their expression in the heart as a function of age has not been examined.

***miR-27a.*** miR-27a exhibits an age-associated increase in the mouse liver and delays the onset of liver aging,[74] it is unknown, however, whether a similar increase occurs in the heart. The studies on miR-27a and CVD are limited to the following reports. miR-27 upregulation occurs in the mouse heart during cardiac hypertrophy; under this condition, it induces the downregulation of the thyroid receptor beta 1 gene and, consequently, the upregulation of beta-myosin heavy chain expression in cardiac myocytes,[75] thus suggesting that it may enhance LV contractility.[76] miR-27a is significantly upregulated in cardiac tissue of patients who underwent cardiac transplantation after a period of support with left ventricular assist devices, when compared to the group that received a heart transplant without prior left ventricular assist device treatment.[77,78] Further, circulating plasma levels of miR-27a are lower in HF patients with peripheral arterial disease compared to patients without peripheral arterial disease and are linked to elevated levels of molecules that induce angiogenesis, inflammation, and endothelial dysfunction in atherosclerosis.[79]

***miR-101a.*** miR-101 is upregulated in murine brain with aging.[80] It targets and downregulates the expression of enhancer of zeste homolog 2, a major histone methyltransferase of polycomb repressor complex 2 involved in cell proliferation and differentiation.[81] miR-101 is an important regulator of cardiac fibrosis and hypertrophy: low levels of miR-101 are detected in hypertrophic and postinfarct rat hearts.[82] Its overexpression inhibits fibrosis and the deterioration of cardiac activity in postinfarct rats, by targeting c-FOS, a modulator of TGF$-\beta$ expression. The expression of miR-101 is downregulated in rat cardiac fibroblasts and cardiac myocytes, following coronary artery ligation, but if overexpressed it can attenuate the LV deterioration, by inhibiting collagen production and ECM deposition, thus ameliorating myocardial stiffness.[82] The overexpression of miR-101 in a rat model of cardiac hypertrophy inhibits CMs hypertrophy by decreasing its target protein Rab1a, a small GTPase that regulates vesicular protein transport[83] and a positive modulator of cardiac hypertrophy[84] that causes HF.

***miR-141.*** miR-141 is a member of miR-200 family that increases in human liver with aging.[85] In contrast, its expression decreases during replicative senescence in human fibroblasts contributing to the increase of its target Mitogen-Activated Protein Kinase 4.[86] miR-141 is involved in mitochondrial dysfunction that plays a key role in cardiac abnormalities associated with type 1 diabetes.[87] miR-141 targets the solute carrier family 25 member 3, an inner mitochondrial membrane phosphate transporter, which provides inorganic phosphate to the mitochondrial matrix and is essential for ATP production. Accordingly, solute carrier family 25 member 3 expression is significantly decreased in type 1 diabetes and has a deleterious effect on ATP production and cell viability.[87]

***miR-200c.*** miR-200c expression increases with aging in skeletal muscle from rhesus monkeys,[88] in human liver,[85] in human fibroblasts,[89] and in the mouse femoral artery.[89] To-date it is still unknown whether aging modulates the expression of miR-200c in the heart. Neither it is known the functional role of miR-200c in myocardial cells. Oxidative stress has been shown to increase miR-200c expression in endothelial cells. This increase is associated with the disruption of the molecular loop among 3 proteins that are directly targeted by miR-200c: SIRT1, endothelial nitric oxide synthase, and FOXO1.[89] The downmodulation of these proteins leads to reactive oxygen production and a reduction in nitric oxide, that contribute to endothelial dysfunction under conditions of increased oxidative stress such as aging and ischemia.[89,90] Moreover, miR-200c has been

linked to cellular senescence induced by oxidative stress[91] by a p53- and a pRb-dependent mechanism, and its upregulation causes apoptosis and cellular senescence through the downregulation of ZEB1 protein, a miR-200c target, in endothelial cells.[91] miR-200c plays also an important role in CVD.[92] It has been shown that miR-200c is highly induced in mouse skeletal muscle following acute hindlimb ischemia[91] and it is also induced in diabetes[90] and by high glucose-induced cardiac hypertrophy.[93] In fact, miR-200c targets dual-specific phosphatase-1 (DUSP-1), an important regulator of the MAPKs (ERK1/2, JNK, and p38): an increase in miR-200c downregulates DUSP1, causing cardiac hypertrophy via an increase in the expression of phosphorylated ERK, p38, and JNK. Inhibition of miR-200c restores DUSP-1 expression and attenuates hypertrophy in high glucose-treated cardiomyocytes.[93] miR-200c has been also shown to increase in HF induced by DOX treatment in mice[94] and in human cardiac mesenchymal progenitor cells exposed to DOX. Administration of stromal cell-derived factor-1, partially inhibits DOX-induced miR-200c and p53 protein upregulation in mouse hearts, and reverts both the adverse remodeling and the impaired LV function (−dP/dt).[94]

Interestingly, circulating miR-200c is increased also in children with Familial Hypercholesterolemia[95] and in adult patients with carotid artery plaques.[96] Moreover, miR-200c increases in atherosclerotic carotid plaques in human patients and it is further enhanced in unstable vs stable plaques.[96]

***miR-214.*** miR-214 is upregulated in the liver of extremely old (33-month) mice[97] but it is still unknown whether it exhibits an age-dependent increase in the heart. Studies of miR-214 in the heart have focused on cardiac hypertrophy, ischemia/reperfusion injury and coronary artery disease (CAD). Several lines of evidence support an important role of miR-214 in cardiac hypertrophy. First, miR-214 is significantly upregulated in mouse models of cardiac hypertrophy induced by thoracic aorta constriction and calcineurin-A and in the final stages of human HF[45,98]; then, miR-214 contributes to cardiac hypertrophy caused by pressure overload directly targeting enhancer of zeste homolog 2.[99]

Further, the long noncoding RNA Plscr4 acts as a sponge for miR-214 and inhibits pressure overload-induced cardiac hypertrophy in mice by interfering with the miR-214-Mitofusin 2 pathway.[100] miR-214 can also act on XBP-1, a key transcription regulator of endothelial cell proliferation, thus inhibiting cellular proliferation angiogenesis in mice with cardiac hypertrophy, possibly contributing to the transition from compensatory cardiac hypertrophy to heart failure. In addition, high

levels of miR-214 have been detected in failing human heart tissue and also in blood, indicating that miR-214 may be a circulating biomarker and a potential diagnostic predictor for HF and during ischemia/reperfusion injury.[101] Moreover, miR-214 has a cardioprotective effect due to the repression of the Sodium-Calcium exchanger 1, $Ca^{2+}$/calmodulin-dependent protein kinase II, cyclophilin D and BIM, proapoptotic Bcl2 family member.[102]

Furthermore, low plasmatic levels of circulating miR-214 are downregulated in patients with CAD, showing an inverse correlation with the severity of the CAD, thus suggesting miR-214 as a promising biomarker for this particular condition.[103]

***miR-216/217.*** miR-216 and miR-217 operate as clusters[104] and are both upregulated in replicative senescence in endothelial cells.[105] No in vivo studies have examined whether there is an age-dependent change in miR-216/217 expression in any tissue.

miR-216 has been shown to play an important role in autophagic impairment associated with endothelial and cardiovascular dysfunction, and atherosclerosis.[106] It directly targets 2 autophagy related proteins, Beclin1 and autophagy related 5. Moreover, miR-216a is induced in unstable plaques compared to stable, and there is an inverse correlation between miR-216a and Beclin1 and autophagy related 5 both in atherosclerotic plaques and in myocardial biopsies from HF patients.[106] Interestingly, miR-216a strongly increased in cardiac biopsies of both diabetic-HF and nondiabetic-HF patients compared with control subjects, and negatively correlated with LV ejection fraction.[107] TGF$\beta$ can induce miR-216a expression in endothelial cells, therefore, the release of TGF$\beta$ from myocytes after infarction and/or during transition to HF may be implicated in the increase in miR-216a expression in HF patients.[107] miR-216a is strongly upregulated both in heart tissue and in plasma samples obtained from patients with advanced HF.[78]

miR-217 targets SIRT1 and via this mechanism it increases FoxO1 and endothelial nitric oxide synthase acetylation, reducing their activities. In endothelial cells miR-217 overexpression induces a senescence-like phenotype and impairs angiogenesis, whereas its inhibition reduces senescence and increases angiogenic activity via SirT1 increase. miR-217 expression also negatively correlates with SirT1 expression and with FoxO1 acetylation status in human atherosclerotic lesions.[105]

Interestingly, miR-217 increases in pathologic hypertrophy and reduces the expression of its targets H3K9 dimethyltransferases EHMT1 and EHMT2,[108] by triggering a pathologic hypertrophic response

characterized by cardiac phenotypic remodeling and re-expression of the fetal gene program.[108] Particularly, EHMT1/2 levels increase during CM maturation from the fetal to adult stage, and miR-217 levels become reduced. Thus, suppression of this pathway protects against pathologic hypertrophy both in vitro and in mice in vivo.[108]

***miR-221.*** miR-221 plays a major role in the proliferation and terminal differentiation of myogenic precursors; it has been shown to decrease in skeletal muscle aging in mice[109] and in Peripheral Blood Mononuclear cells (PBMCs) of old compared to young humans.[110] miR-221 is significantly upregulated in patients with hypertrophic cardiomyopathy, and also in a mouse model of cardiac hypertrophy and HF induced by pressure overload. In vitro miR-221 promotes cardiac hypertrophy, and cardiac-specific overexpression of miR-221 in mice regulates autophagy and promotes HF by regulating the p27/CDK2/mTOR axis.[111,112] Notably, circulating serum miR-221 levels are lower in patients with HFpEF and HFrEF than in healthy controls (see below).[113]

***miR-455.*** miR-455 expression decreases with aging in murine skeletal muscles[109] and in the skeletal muscle of a mouse model of disuse-induced atrophy.[114] Interestingly, miR-455 induces a hypertrophic phenotype in myotubes in vitro.[114] In mouse heart, miR-455 is repressed during pressure overload and its restoration by gene overexpression in vivo can worsen hypertrophy and reduce myocardial fibrosis. miR-455 overexpression inhibits apoptosis by targeting Calreticulin, a protein relevant in cardiac development and in cardiac calcium regulation. Therefore, targeting miR-455 may represent a potential therapeutic strategy to ameliorate the maladaptive cardiac remodeling typical of HF.[115]

## *miRNAs in HFrEF Vs HFpEF*

Although several studies have examined a mechanistic role of miRNAs in HFrEF, no study has convincingly addressed the role of miRNAs in HFpEF, including age-associated HFpEF. Most reports in this field have evaluated miRNAs in comorbidities that are associated with, and often implicated in HFpEF, such as aging,[116] hypertension,[117] diabetes,[118] obesity,[119] and low-grade inflammation.[120] However, these studies did not establish whether HFpEF was actually present. Further, the rodent models of HFpEF fail to fully reproduce the human clinical phenotype of the disease, which is characterized by diastolic dysfunction without LV dilation, preserved EF and

pulmonary congestion.[121] In contrast, there are 2 clinical studies that have evaluated, respectively, circulating miRNAs and PBMC miRNAs in HFpEF vs HFrEF.

miRNA expression in serum has been studied in 3 cohorts of patients approximately 70 years old: (i) 90 asymptomatic patients without HF but with cardiovascular risk factors for future HF insurgence, ie, LV dysfunction, hypertension, obesity, type 2 diabetes and hypercholesterolemia; (2) 90 patients with HFrEF, with a prior hospitalization for HF associated with LVEF <50%; (3) 90 patients with HFpEF, with a prior hospitalization for HF associated with LVEF >50% with diastolic dysfunction, and without valvular heart diseases.[113] miR-30c, -146a, -221, -328, and -375 were differentially regulated; their expression was reduced in patients with HF, however miR-375 was reduced only in patients with HFrEF. Further, both miR-328 and miR-375 were lower in patients with HFrEF than with HFpEF. It was suggested, based upon these results that in a community setting, when echocardiography is not readily available, different combinations of the above miRNAs, in conjunction with natriuretic peptide levels, strengthen the diagnostic power of natriuretic peptide and may also help differentiate HFpEF from HFrEF.[113]

A miRNA screening in plasma identified 12 dysregulated miRNAs in HF patients approximately 65 years old: (1) 30 subjects without coronary disease or HF history; (2) 30 patients with HF and LVEF <40% diagnosed with HFrEF; (3) 30 patients with HF and LVEF >50% diagnosed with HFpEF.[122] Among the miRNAs selected for analysis and validation after an initial screening, miR-1233, -183-3p, -190a, -193b-3p, -193b-5p, -211-5p, -494, and-671-5p distinguished HF patients from controls. miR-125a-5p, -183-3p, -193b-3p, -211-5p, -494, -638, and-671-5p were potential HFrEF markers, whereas miR-1233, -183-3p, -190a, -193b-3p, -193b-5p, and-545-5p were potential HFpEF markers. Specifically, miR-125a-5p, -190a, -550a-5p, and-638 discriminated between HFrEF and HFpEF.[122]

The analysis of the expression levels of some miRNAs in PBMC isolated from hypertensive patients, approximately 66 years old, with and without HFpEF showed that miR-26b, -208b, and -499 exhibited higher expression in hypertensive patients with HFpEF and a positive correlation with peak $VO_2$.[123]

Finally, an initial genome-wide microarray followed by a validation on plasma of 20 healthy controls in 14 hospitalized HF patients with LVEF ≥50% (HFpEF) and 18 hospitalized HF patients with LVEF <40% (HFrEF) identified miR-3135b, miR-3908, and miR-5571-5p as candidate

circulating miRNA markers of HF. Specifically, miR-3135b and miR-3908 expression levels were higher in HFpEF rather than HFrEF.[124]

It is noteworthy that, except for a study[123] that focused on hypertensive patients, the above clinical studies enrolled patients of similar ages and did not link HFpEF to a specific possible etiology such as aging, diabetes, obesity; patients with all these conditions were included in the HFpEF group. Further, the 4 clinical studies reported above failed to identify even a single miRNA that could distinguish

**TABLE 1.** Effect on cardiac remodeling of miRNAs modulated by aging and senescence.

| miRNA | Aged tissue | Target | Effect on cardiac remodeling | References |
|---|---|---|---|---|
| miR-17-92 | Heart, endothelial cells, skin fibroblasts | CTGF, TSP-1, TGF$\beta$R2 | Antifibrotic | 38,43 |
| miR-21 | Heart | MAPK, SPRY1 | Profibrotic | 48 |
| miR-22 | Heart, brain | PURB, PTEN, SIRT1, HDAC4 | Prohypertrophic | 54,56,57 |
| miR-27a | Heart, brain | Angiogenin, neutropilin, galectin3 | Prohypertrophic | 75,125 |
| miR-29 | Heart, endothelial cells, skin fibroblasts | Extracellular matrix genes | Profibrotic | 61 |
| miR-34a | Heart, brain | CyclinD1, Bcl2, PNUTS, SIRT1 | Proapoptotic | 71,72 |
| miR-101a | Heart, brain | Rab1a, TGF$\beta$ | Antihypertrophic antifibrotic | 82 |
| miR-141 | Liver, skin fibroblasts | MKK4, Slc25a3 | Profibrotic proapoptotic | 86,87 |
| miR-200c | Liver, femoral artery, skeletal muscles | DUSP1, ZEB1, eNOS, SIRT1, FOXO1 | Prohypertrophic apoptosis, senescence, oxidative stress | 85,88,89,91,93,96 |
| miR-214 | Liver, heart | XBP-1, NCX, EZH2 | Prohypertrophic positive effect on $Ca^{2+}$ handling | 101,102 |
| miR-216/217 | Endothelial cells | EHMT1, EHMT2, SIRT1 | Prohypertrophic | 108 |
| miR-221 | Skeletal muscle, heart | p27 | Prohypertrophic | 112 |
| miR-455 | Skeletal muscle, heart | Calreticulin | Prohypertrophic | 115 |

HFpEF from HFrEF, which was common to at least 2 of the studies. Possible reasons for this failure to achieve a consensus on any given miRNA include (1) sources of body liquid differed (plasma, serum, or tissue); (2) the methods to detect and microarray screenings performed before or after the qRT-PCR could have conditioned the comparison; (2) different storage conditions; (4) different efficiencies of miRNA extraction; and (5) different methods of standardization. It is evident that additional studies that take the above factors into account and, of foremost importance, select the likely specific miRNAs involved in HFpEF, are needed.

## Conclusions

Literature discussed in this review shows that some age-dependent miRNAs play a key role in HFrEF and suggest that they may be important therapeutic targets to improve heart function, either via their downmodulation or overexpression. Further, there are several reports that have examined age-modulated miRNAs in HF but have not established whether age-dependent changes in expression of these miRNAs occurred also in the heart. We summarized the effect of miRNAs modulated by aging and senescence on cardiac remodeling and heart failure (Table 1). In Table 2 we summarized the effect on HF of circulating miRNAs associated with aging.

Studies on miRNAs and HFpEF have been limited to the evaluation of miRNAs in plasma and in blood cells as biomarkers. No studies have addressed whether any miRNA plays a mechanistic role in HFpEF, the most common form of HF in the elderly population.

**TABLE 2.** Circulating miRNAs.

| Circulating miRNA | Modulation | Source | Diseases | References |
|---|---|---|---|---|
| miR-19b | Downregulation | Serum | Aortic stenosis and HF | 39 |
| miR-21 | Upregulation | Serum | HF | 51 |
| miR-22 | Downregulation | Serum | HF | 59 |
| miR-34a | Upregulation | Plasma | Diabetes | 73 |
| miR-29a | Upregulation | Plasma | Cardiac Hypertrophy | 67 |
| miR-200c | Upregulation | Plasma | Familial Hypercholesterolemia in children Atherosclerosis | 95,96 |
| miR-214 | Downregulation | Serum | Coronary Artery Disease | 103 |
| miR-216 | Upregulation | Plasma | HF | 78 |
| miR-221 | Downregulation | Plasma | HF | 113 |

This vacuum in our knowledge is largely due to the absence of an animal model that, in addition to diastolic dysfunction, also develops HFpEF. Nevertheless, miRNAs represent an exciting and potentially clinically relevant area of investigation to understand the mechanism that underlie the different form of HF and provide novel therapeutic options.

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