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SIRTUIN 3, ENDOTHELIAL METABOLIC REPROGRAMMING AND HEART FAILURE WITH PRESERVED EJECTION FRACTION

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Abstract

The incidences of heart failure with preserved ejection fraction (HFpEF) are increased in the aged populations as well as diabetes and hypertension. Coronary microvascular dysfunction has been contributed to the development of HFpEF. Endothelial cells (ECs) depend on glycolysis rather than oxidative phosphorylation for generating ATP to maintain vascular homeostasis. Glycolytic metabolism has a critical role in the process of angiogenesis since endothelial cells rely on the energy produced predominantly from glycolysis for migration and proliferation. Sirtuin 3 (SIRT3) is found predominantly in mitochondria and its expression declines progressively with aging, diabetes, obesity, and hypertension. Emerging evidence indicates that endothelial SIRT33 regulates a metabolic switch between glycolysis and mitochondrial respiration. SIRT3 deficiency in EC resulted in a significant decrease in glycolysis, whereas, it exhibited higher mitochondrial respiration and more prominent production of reactive oxygen species (ROS). SIRT3 deficiency also displayed strikingly increases in acetylation of p53, EC apoptosis, and senescence. Impairment of SIRT3-mediated EC metabolism may lead to a disruption of EC/pericyte/cardiomyocyte communications and coronary microvascular rarefaction which promotes cardiomyocyte hypoxia, Titin-based cardiomyocyte stiffness, and myocardial fibrosis thus leading to a diastolic dysfunction and HFpEF. This review summarizes current knowledge of SIRT3 in EC metabolic reprograming, EC/pericyte interactions, coronary microvascular dysfunction, and HFpEF.

Keywords

SIRTUIN3 (SIRT3); endothelial cell; glycolysis; mitochondrial respiration; angiogenesis; diastolic function; Heart failure with preserve ejection fraction (HFpEF)

Introduction

Heart failure (HF) is a progressive disease with high incidence that develops with advanced age, hypertension, and diabetes [1–3], 4, 5]. Each year about 600,000 patients are newly diagnosed with HF in the United States; costs of care are estimated at $34.8 billion per year.

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More than half of these patients have heart failure with preserved ejection fraction (HFpEF) [6, 7]. Heart failure with reduced ejection fraction (HFrEF) is another phenotype of HF that is defined as reduced left ventricle ejection fraction. So far, standard-of-care of HFrEF medications have failed to show efficacy in large clinical trials in patients with HFpEF [8]. HFpEF is commonly seen in older patients who usually have cardiovascular, metabolic, and inflammatory comorbidities [4]. Paulus and colleagues suggest that these comorbidities lead to systematic inflammation that results in coronary endothelial dysfunction and thus decreased bioavailability of NO which ultimately causes diastolic dysfunction [9]. Despite its importance, our understanding of HFpEF in regards to either pathophysiology or molecular mechanism is very limited.

Compelling evidence indicates that epigenetic modification is involved in the regulation of cellular function and stresses as well as progression of cardiovascular disease [5, 10]. Recently, Sirtuins have been investigated intensively regarding to the ability to regulate energy metabolism, reactive oxygen species (ROS) production, and cell survival [5]. In particular, SIRT3, a NAD^+-dependent lysine residue deacetylase which was first identified in the heart mitochondria, has emerged as a novel regulator of mitochondrial function and cellular metabolism [5, 11–13]. As compared to younger individuals, SIRT3 levels were found to be decreased in old adults with sedentary lifestyles [14]. SIRT3 levels are also decreased in the heart of diabetic db/db mice which are associated with microvascular rarefaction and cardiac dysfunction in diabetes [15]. Our recent studies indicate that reduction of SIRT3 leads to a metabolic reprogramming in EC and diastolic dysfunction in mice that might be relevant to the HFpEF phenotypes such as aging, diabetes, obesity, and hypertension [16–19].

Endothelial glycolysis has a critical role in the regulation of angiogenesis since ECs rely on glycolysis-derived ATP for migration and proliferation [20, 21]. Among the many enzymes in the glycolytic metabolism of glucose, 6-phosphofructo-2-kinase/fructose-2, 6-bisphosphatase, isoform 3 (PFKFB3), is a key regulator of glycolysis in endothelial cells. PFKFB3 has been shown to promote endothelial proliferation and angiogenic sprouting [21–23]. Patients with HFpEF have coronary microvascular rarefaction and more cardiac hypertrophy [4]. At physiological conditions, myocardial growth and angiogenesis are well coordinated. However, with the presence of hypertension, coronary vascular disease, and myocardial infarction, cardiac hypertrophy and new vessel formation are imbalanced, known as pathological hypertrophy, leading to progression of eventual heart failure [24]. Coronary microvascular rarefaction with reduced coronary flow reserve (CFR) results in poor perfusion to the myocardium thus creating a hypoxic environment which would exaggerate ischemic injury and apoptosis in cardiomyocytes. Therefore, therapeutic myocardial angiogenesis and improvement of CFR are promising approaches for the prevention and treatment of heart failure [24, 25]. Previous study demonstrated that overexpression of angiogenic growth factor apelin increased myocardial vascular density and attenuated ischemia-induced heart failure (HF) in STZ-induced hyperglycemic mice, but these protective effects were abolished in STZ-SIRT3KO mice [26]. Although accumulating evidence reveals a regulatory role of SIRT3 in angiogenesis and HF [15, 26], there is still much to uncover about the precise mechanisms in the pathogenesis of HFpEF and HFrEF.

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This review discusses the emerging role of SIRT3 in reprograming cell metabolism, EC/pericyte interactions, and HFpEF.

Cell specific metabolic phenotypes of SIRT3

SIRT3 is a mitochondrial deacetylase and its expression is in the highest metabolically active organs including brain, heart, kidney, liver, and skeletal muscle [27]. SIRT3 has been shown to regulate almost every major aspect of mitochondrial function, including oxygen consumption, ATP generation, and ROS formation. Previous study revealed non-tissue-specific roles for SIRT3 in global metabolic homeostasis [28]. However, a recent study also indicated that SIRT3 regulates the acetyl-proteome in core mitochondrial processes common to brain, heart, kidney, liver, and skeletal muscle but regulates metabolic pathways differentially in fuel-producing and fuel-utilizing tissues [27]; this suggests SIRT3 regulates mitochondrial acetylation in a tissue-specific manner. Thus, functional role of SIRT3 is likely much more complex than initially appreciated, particularly involving tissue and cell specific. Some studies have been reported that deficiency of SIRT3 in myoblast and cancer cells decreases mitochondrial respiration and increases ROS formation [29, 30]. Moreover, respiratory capacity and ATP synthesis were decreased in cardiac mitochondria of SIRT3 KO mice [31]. Although the function of mitochondrial SIRT3 has been well investigated, the metabolic modifications of SIRT3 in EC has not been highlighted. We are particularly interested in the functional role of endothelial SIRT3 deficiency on the glucose metabolic switch and cardiac diastolic function. Our study demonstrated that loss of SIRT3 in EC reprogrammed cell metabolism by reduced glycolysis and increased oxidative phosphorylation [17]. Moreover, specific deletion of endothelial SIRT3 caused an impairment of coronary microvascular function and led to a diastolic dysfunction in mice. Our study indicated a cell specific role of SIRT3 on cell metabolism and diastolic function [17].

SIRT3, endothelial metabolic flexibility, and angiogenesis

ECs have high glycolytic activity that is comparable with that of tumor cells and much higher than that of cardiomyocytes and other oxidative cell types [23]. Recent study indicated that glycolytic flux in endothelial cells was over 200-fold higher than glucose oxidation, fatty acid oxidation, and glutamine oxidation; in addition, mitochondrial respiration was lower in ECs than in cardiomyocytes [21]. In the process of glycolysis, the conversion of fructose-6-phosphate (F6P) to fructose-1, 6-bisphosphate (F1,6P_2) is catalyzed by 6-Phosphofructo-1 kinase (PFK-1). PFK-1, a bottleneck enzyme in the glycolytic pathway, is activated by its allosteric activator, fructose-2,6-bisphosphate (F2,6P_2) [21, 23]. In endothelial cells, F2,6P_2 is mainly synthesized and regulated by PFKFB3 and TP53-induced glycolysis and apoptosis regulator (TIGAR). Despite immediate access to oxygen in the blood, healthy ECs depend on glycolysis rather than oxidative phosphorylation to maintain vascular barrier function, tissue homeostasis, and capacity to quickly respond to stress such as hypoxia, nutrient deprivation, or tissue damage [21, 32]. ECs possess the ability to switch from oxidative phosphorylation to glycolysis as the primary energy source, known as metabolic flexibility, to protect cells with increased energy demand from elevated oxidative stress [4]. Upon stimulation in pathological conditions, ECs increase glycolytic

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flux mediated by, at least in part, upregulation of PFKFB3 [21, 33]. Previous study showed that expression of PFKFB3 was significantly reduced in the culture ECs isolated from SIRT3 KO mice. Moreover, the glycolytic function of SIRT3 KO-ECs was impaired, as evidenced by the decreased glycolysis, glycolytic capacity, and glycolytic reserve [17]. Intriguingly, loss of SIRT3 resulted in elevated basal oxygen consumption rate in ECs. These findings indicated SIRT3 deficiency in ECs resulted in a metabolic reprogramming that ECs were more depended on oxygen-dependent oxidative phosphorylation thus impaired metabolic flexibility. Moreover, the production of ROS was significantly increased in SIRT3 KO ECs [17]. Recent studies have shown that PFKFB3-mediated glycolysis is required for angiogenesis, and pharmacological inhibition of PFKFB3 blocks pathological angiogenesis [21–23]. Study also showed that inhibition of PFKFB3 suppressed endothelial glycolysis. This led to a significant reduction of angiogenic factors and increase in pro-inflammatory factors. These results support the possible mechanism of which SIRT3 deletion or inhibition of PFKFB3 triggers endothelial dysfunction via promoting pro-inflammatory state [17]. Impaired angiogenesis has been described in many cardiac disease associated with aging, diabetes, and obesity. Loss of SIRT3 impaired hypoxic signaling which altered glycolytic metabolism and oxygen consumption and, in turn, contributed to reduced angiogenic capabilities of ECs [16, 17, 19]. ECs are quiescent and rarely sprout, migrate, or proliferate under normal conditions. Under ischemic conditions, EC senses and responds to signals released from hypoxic tissue resulting in a metabolic shift to favoring glycolytic metabolism and leading to a highly migratory and proliferative phenotype to stimulate angiogenesis [12, 34]. This phenotype change is mainly mediated by the mechanisms involving HIFs activation, VEGF/VEGFR2, or FGF stimulation-induced glycolysis via PFKFB3 [21, 23, 35, 36]. Increasing the levels of HIFs via the inhibition of PHDs by a PHD inhibitor DMOG rescued the glycolysis in the SIRT3 KO ECs suggesting that HIFs are involved in the regulation of glycolytic metabolism [18]. The HIFs are the transcriptional factors that regulate oxygen-related energy metabolism and angiogenesis [37]. The level of HIFs has been shown to decrease with aging that is correlated to an increased expression of PHDs [37]. Treatment with PHD inhibitor DMOG dose-dependently upregulated the expression of HIF-2α and PFKFB3 in WT and SIRT3 ECs suggesting that deficiency of SIRT3 may damage HIF-2α signaling in endothelial cells during hypoxia; this may lead to impaired hypoxia-induced angiogenesis [16, 18].

Microvascular rarefaction, CFR, and HFpEF

Coronary flow reserve (CFR) is defined as the ratio of maximal flow velocity in hyperemia to baseline flow velocity and usually represents functional change of coronary microvasculature. In clinic, coronary microvascular function is mainly evaluated by determination of CFR. Reduction of CFR is strongly associated with cardiac mortality in patients with heart failure, especially in HFpEF. Diabetic patients have higher prevalence of reduced CFR [38]. Preexisting reduction of CFR in diabetic patients may contribute to the microvascular dysfunction and obstruction thus leading to no-reflow after percutaneous coronary intervention. Myocardial capillary density is the main determinant of CFR [39]. Impairment of angiogenesis and reduced myocardial capillary density (microvascular rarefaction) are considered a major feature of HFpEF [40]. Microvascular rarefaction

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decreased eNOS and NO bioavailability, impaired cGS/cGMP signaling pathway, and resulted in cardiomyocyte stiffness, cardiac hypertrophy, and HFpEF [40–43]. Microvascular rarefaction also led to an impairment of CFR which sensitized the heart to hypoxia that resulted in an increased ROS formation, cardiomyocyte death, and heart failure with reduced ejection fraction (HFrEF).

SIRT3 ECKO mice have been reported to develop a diastolic dysfunction without having abnormalities in systolic function [17]. HFpEF patients are usually presented with abnormalities in coronary microcirculation due to endothelial dysfunction and coronary microvascular rarefaction [4]. Endothelial dysfunction and decreased microvascular density may limit coronary blood flow during reactive hyperemia [4]. CFR was decreased in SIRT3 ECKO mice [17, 18] indicating impaired myocardial perfusion and possible LV remodeling such as dilated cardiomyopathy [44]. Indeed, LV end-diastolic dimension and end-diastolic volume were found to be significantly increased in SIRT3 ECKO mice [17, 18]. Increasing ROS formation has been shown to diminish the nitric oxide bioavailability in cardiomyocytes [45]. Deficiency of SIRT3 KO in ECs resulted in a dramatic increase in ROS formation suggesting a possible impaired NO-cGMP-dependent mechanism that contributes to decreased CFR and impaired cardiomyocytes relaxation leading to a subsequent prolonged IVRT [16–19]. The heart is one of the most oxygen- and energy-demanding organs which mainly depends on oxidative phosphorylation in mitochondria [46]. In addition, decreased glycolysis and higher oxygen demand in ECs mean less oxygen is delivered to the cardiomyocytes. Therefore, advanced microvascular dysfunction may contribute to chronic hypoxia in the heart. Normal tissue counters hypoxia by upregulation of expression of HIFα system and its downstream signaling angiogenic factors such as VEGF and Ang-1. It also promotes angiogenesis to preserve oxygen supply and alleviates hypoxia-induced injury. In contrast, hypoxia-induced expression of HIF-1α, HIF-2α, Ang-1, and VEGF was impaired in SIRT3 KO-ECs as well as endothelial angiogenic capabilities indicating a defective hypoxia response in SIRT3 ECKO mice [17]. Study has shown that knockout of HIF-2α has been shown to impair homeostasis of ROS and cause cardiac hypertrophy which is one of the key risk factors for diastolic dysfunction [47]. In addition, microRNA profile analysis of the isolated ECs projected a putative highly interconnected subnetwork that was linked to fibrosis involving Smad2/3, TGF-β1, and Notch 2 (Figure 1A and B). Taken together, all these changes could result in diastolic dysfunction and HFpEF phenotype.

EC/pericyte interaction and coronary microvascular function

Pericytes are vascular mural cells of mesenchymal origin, embedded in the basement membrane of microvasculature, where they make specific local contacts with endothelium [50, 51]. The microvasculature of human hearts contains an abundance of pericytes. Each pericyte is associated with two or three ECs in the heart [52]. Loss of pericytes, known to lead to diabetic retinopathy [53–56], has been shown to also cause microvascular dysfunction in other vascular beds [57]. For example, recent studies have highlighted the critical role of capillary pericytes in the regulation of cerebral blood flow in ischemia/reperfusion (I/R)-injury [58, 59]. Pericytes are the second most frequent cell type in the coronary vasculature after endothelial cells (ECs) [52, 57, 58, 60–62]; surprisingly, almost

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nothing is known about cardiac pericyte in the regulation of coronary microvascular function and heart failure. A study showed that treatment of mice with sunitinib malate (FDA approved for the treatment of metastatic renal cell carcinoma) disrupted EC/pericyte interactions and led to impaired coronary blood flow (CBF) and cardiac dysfunction [57]. Notch3, a receptor expressed in pericytes, has a critical role in the regulation of pericyte differentiation [63, 64]. Notch3 has been reported to regulate pericyte number and maintain vascular integrity [65, 66]. Studies showed that Notch3KO mice challenged with Angiotensin II caused coronary microvascular dysfunction and heart failure [67, 68]. Deficiency of Notch3 also led to a significant reduction of pericytes in the mouse heart which resulted in an impairment of pericyte/capillary coverage and reduced CFR in mice. Furthermore, deficiency of Notch3 sensitized the heart to ischemic injury with larger infarcted size and higher rates of mortality [69]. In addition, deficiency of Notch3 decreased the numbers of $NG2^+$ (pericyte)/$Sca1^+$/c-kit^+ progenitor cells and impaired microvascular stabilization thus promoting ischemia-induced microvascular leakage and inflammation [69]. This study strongly suggests that cardiac pericyte is necessary to maintain both the maturation and the integrity of coronary microvasculature in response to myocardial ischemia. Therefore, cardiac pericyte is a promising therapeutic target for coronary no-reflow and progression of heart failure after myocardial infarction or I/R injury [69, 70].

In our previous study, we have demonstrated that pericyte/EC coverage was severely impaired in the heart of obese mice [71]. SIRT3 KO mice had a reduced pericyte/EC coverage in the heart together with a significant reduction of CFR [16]. Although the mechanism of decreased pericyte/EC coverage was not clear, our study indicated that impaired angiopoietins/Tie-2 and HIF-2α/Notch3 signaling pathways may be involved in the pericyte loss in SIRT3 KO mice [72]. Although the exact mechanism of loss of pericyte was not investigated, recent studies have shown that loss of pericyte or detachment of pericyte from capillary has the potential to differentiate into myofibroblasts. This may contribute to deposition of excessive fibrosis and myocardial stiffness which may contribute to HFpEF [48, 49]. Therefore, we reason that disruption of SIRT3 signaling may promote endothelial dysfunction and lead to cardiac diastolic dysfunction through blocking endothelial metabolism and disrupting endothelial cell/pericyte communications and pericyte detachment which leads to pericyte-fibroblast transition and results in myocardial and vascular stiffness (Figure 3).

SIRT3 and Titin-based cardiomyocyte stiffness

Cardiac stiffness (cardiomyocyte stiffness and fibrosis) is the center feature of HFpEF. The giant cytoskeletal protein Titin is the largest protein known and spans the entire half-sarcomere from the Z-disk to the M-band. It functions as a molecular spring that develops passive tension during diastole when sarcomeres elongate. The Titin-based cardiomyocyte stiffness is the key determinant for HFpEF [73–76]. Titin-based cardiomyocyte stiffness is mediated mainly through isoform shifts (N2BA/N2B) or alterations of phosphorylation of its elastic regions N2-Bus (decreases passive tension, PT) and PEVK (increases PT) [77, 78]. ECs have been reported to support cardiomyocyte function by increasing angiogenesis and releasing regulatory proteins. Surprisingly, little is known about EC/cardiomyocyte interactions on cardiac stiffness and HFpEF. Our recent study revealed that infusion of

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SIRT3KO mice with angiotensin II (Ang-II) significantly increased myocardial stiffness and accentuated Ang II-induced diastolic dysfunction (unpublished data). Although knockout of SIRT3 or specific knockout of endothelial SIRT3 ($SIRT3^{EC}KO$) in mice displayed an HFpEF phenotype with impaired CFR and cardiomyocyte stiffness, the regulatory role of SIRT3 on Titin-based cardiomyocyte stiffness has not been explored. Similar as phosphorylation, SIRT3 may also alter acetylation of Titin elastic regions N2-Bus and PEVK which subsequently regulates Titin-based cardiomyocyte stiffness. This notion leads us to further investigate in the future. We, therefore, propose that disruption of SIRT3 in EC reprograms endothelial glycolytic metabolism that leads to derangement of EC/ cardiomyocyte/pericyte interactions and microvascular rarefaction. These alterations may promote cardiomyocyte hypoxia and Titin-based cardiomyocyte stiffness and fibrosis thus leading to a diastolic dysfunction and HFpEF (Figure 3).

SIRT3, cardiomyocytes apoptosis, and HFrEF

SIRT3 is a mitochondrial lysine residue deacetylase that regulates energy metabolism and cellular functions in various tissues; the level of SIRT3 declines progressively with aging [79]. Cardiomyocytes are intense energy-utilizing cells that are highly dependent on the energy produced from mitochondrial oxidative phosphorylation [27]. Therefore, it is not surprising that SIRT3 has an important role in regulating cardiomyocyte function. Recent studies have shown that mice lacking SIRT3 have impaired mitochondrial fatty acid oxidation, reduced oxidative phosphorylation complex activity, and ATP production in the heart along with increased ROS production [79–83]. Thus, impaired mitochondrial dynamics seen in SIRT3 KO mice may be one of the most important factors that lead to cardiac hypertrophy and eventually heart failure. Oxygen conformance refers to the ability of cells to reduce energy expenditure which allows them to lower oxygen consumption in advance of an energetic crisis under insufficient oxygen such as ischemia. Such energy conservation induces a state of hypoxia tolerance and enhances survival at low oxygen tension. Recent study implicates PHD1 is the key regulator of oxygen conformance and induces hypoxia tolerance under hypoxic conditions. Deficiency of PHD1 lowers oxygen consumption and reduces mitochondrial oxidative stress and protects muscle against ischemic injury in a HIF-2α-dependent fashion [84]. This endogenous protection is mediated, at least in part, through a reprogramming of the basal glycolytic metabolism, in particular, by a reduction of oxidative glucose metabolism due to an up-regulation of pyruvate dehydrogenase kinase1 (PDK1) and PDK4 expression which correlates with a lower pyruvate dehydrogenase complex activity and restricts the entry of glycolytic intermediates in the tricarboxylic acid (TCA) cycle. Intriguingly, this protection is not attributable to an increase in oxygen supply through enhancing angiogenesis or vasodilatation but to reduction of reactive oxygen species (ROS) formation and enhancement of cells against deleterious effects of oxidative damage under hypoxic conditions. Intriguingly, even transient knockdown of PHD1 induces hypoxia tolerance and protects muscle against ischemic injury [84]. These findings suggest a novel role of PHD1 in the protection of ischemic muscle against oxidative damage by induction of hypoxia tolerance through a mechanism involving induction of a hibernating state and persevering mitochondrial integrity. Global SIRT3 KO mice had an increased PHD1 and decreased HIF-2 levels as well as developed systolic dysfunction at 12 months of age [18].

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Intriguingly, PHD inhibition by DMOG treatment improved systolic function further suggesting HIFαs in cardiomyocytes may be involved in the regulation of cardiac function [18]. These findings implicate that SIRT3 deficiency-induced upregulation of PHD1 may attenuate endogenous protection and exacerbate cardiac dysfunction via reducing myocardial tolerance to hypoxia.

To identify miRNA-mRNA regulatory network, we systematically evaluated miRNA-mRNA associations using expression profiles of ECs from WT and SIRT3 KO mice. The inferred putative target interactions formed two highly interconnected subnetworks which were linked to fibrosis, inflammation, cell senescence, and death. We used microRNA processing with a statistical cutoff of $p<0.02$ (Figure 1 A&B). Interestingly, network 1 is clustered around Smad2/3, TGF-β1, and TP53 (Figure 1A), while network 2 is associated with DICER1, AGO2, TNFSF12, and Notch2 signaling (Figure 1B). Among them, p53 is a pro-apoptotic protein which requires acetylation to be active for recruitment of transcription co-factors, such as PUMA and Bax [5, 85]. SIRT3 has been reported to interact with and directly deacetylate p53 in bladder carcinoma cells [5, 86]. Although the role of SIRT3 in regulation of p53-mediated apoptosis in cardiomyocyte remains unclear, our microRNA profile analysis indicated that loss of SIRT3 affected miRNAs expression that were associated with TNFSF12 and TP53 signaling which may provide a possible mechanism of SIRT3-mediated regulation of cell death and inflammation (Figure 1A and B). This was further confirmed by the data that the acetylation of p53 was significantly increased in the heart of SIRT3 KO mice (Figure 2). Cardiomyocyte death induced by increased oxidative stress and decreased survival signaling and fibrosis that replaces dead cardiomyocytes might count for another very important factor that contributes to heart failure.

P53, endothelial senescence, and HFpEF

HFpEF, which is one of the leading causes of mortality in aging, can be viewed as a systemic syndrome of aging-related phenotype. P53 is the master regulator of aging and has been shown to have a critical role in the pathogenesis of heart failure. The expression of p53 is elevated in the human failing heart [87, 88]. P53 and its transcriptional target gene TP53-induced glycolysis and apoptosis regulator (TIGAR) are activated in the cardiomyocyte under hypoxia [89, 90]. So far, two roles of p53 accumulation in the failing heart have been demonstrated: (1) induction of cell cycle arrest that causes proliferative cells arrest in the G1 stage of the cell cycle to induce senescence and (2) suppression of angiogenesis [91, 92]. Endothelial senescence has been reported to contribute to the development of HFpEF by promoting myocardial endothelial inflammation and peripheral vascular endothelial inflammation via the senescence-associated secretory mechanism. Endothelial senescence led to an impairment of systemic vasodilator reserve and coronary microvascular dysfunction in mice [93]. Elevation of p53 reduced capillary formation by inhibition of HIF-1α in the hypertrophic hearts [94]. In contrast, global knockout of p53 in mice attenuates doxorubicin-induced cardiac dysfunction [95, 96]. Specific knockout of p53 in EC reduces EC apoptosis and increases capillary density which attenuates pressure-overload induced heart failure [97]. Our data also showed that p53 acetylation was significantly increased whereas total p53 remains unaltered in the hearts of SIRT3 KO mice (Figure 2).

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This indicates p53 acetylation may cause endothelial senescence and microvascular dysfunction that promotes a diastolic dysfunction.

In summary, recent studies uncover a molecular mechanism of SIRT3 in the regulation of angiogenesis by reprogramming EC glycolytic metabolism. Based upon these studies, we proposed two cardiac phenotypes of SIRT3: 1) endothelial deletion of SIRT3 developed HFpEF phenotype due to endothelial metabolic dysfunction which may lead to EC senescence, microvascular rarefaction, and myocardial stiffness (Figure 3); whereas, 2) global knockout of SIRT3 resulted in HFrEF phenotype that is contributed by mitochondrial dysfunction and oxidative stress induced cardiomyocyte death [18, 19]. In addition, the current available therapeutic interventions, such as ACE-inhibitors, beta-blockers, and mineralocorticoid receptor antagonists, benefit HFrEF patients but have not been proven to improve the outcomes in HFpEF patients [1, 98]. Pharmacological trials are not promising in HFpEF patients, either [99–102]. As humans age, cardiac function declines especially diastolic function. Although it is clear that SIRT3 deficiency causes cardiac dysfunction, unfortunately, there is no clinical trial to explore the therapeutic role of SIRT3. This is due to lack of SIRT3 specific activators so far. Due to its unique location, it is urgent to develop new agents which are specific to target SIRT3 in the mitochondria. Honokiol, a Chinese medicine, has been shown to bind and activate SIRT3 as well as provide protective action against cardiac injury [103, 104]. Our recent studies also revealed a critical role of endothelial SIRT3 in regulation of cardiac diastolic function during aging as well as hypertension. These results indicate not only a link between aging and HFpEF, but also a valuable target and mechanism for therapeutic intervention in cardiac pathologies and aging process. Further studies are warranted to address the intracellular molecular mechanisms mediated by endothelial SIRT3 as well as discover new SIRT3 specific agonists and potential therapeutic approaches for targeting EC metabolism such as glycolytic metabolism in the treatment of HFpEF.

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Abbreviations

HFpEF	Heart failure with preserved ejection fraction
HFrEF	Heart failure with reduced ejection fraction
NO	nitric oxide
ROS	reactive oxygen species
PFKFB3	phosphofructose kinase/fructose 2, 6 bisphosphatase 3
CFR	coronary flow reserve
TIGAR	TP53-induced glycolysis and apoptosis regulator
F-6P	fructose 6 phosphate

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PFK-1	Phosphofructose kinase 1
VEGF	vascular endothelia growth factor
FGF	fibroblast growth factor
LV	left ventricular
HIF	hypoxia-inducible factor
PDK1	pyruvated dehydrogenase kinase-1
TCA	tricarboxylic acid cycle

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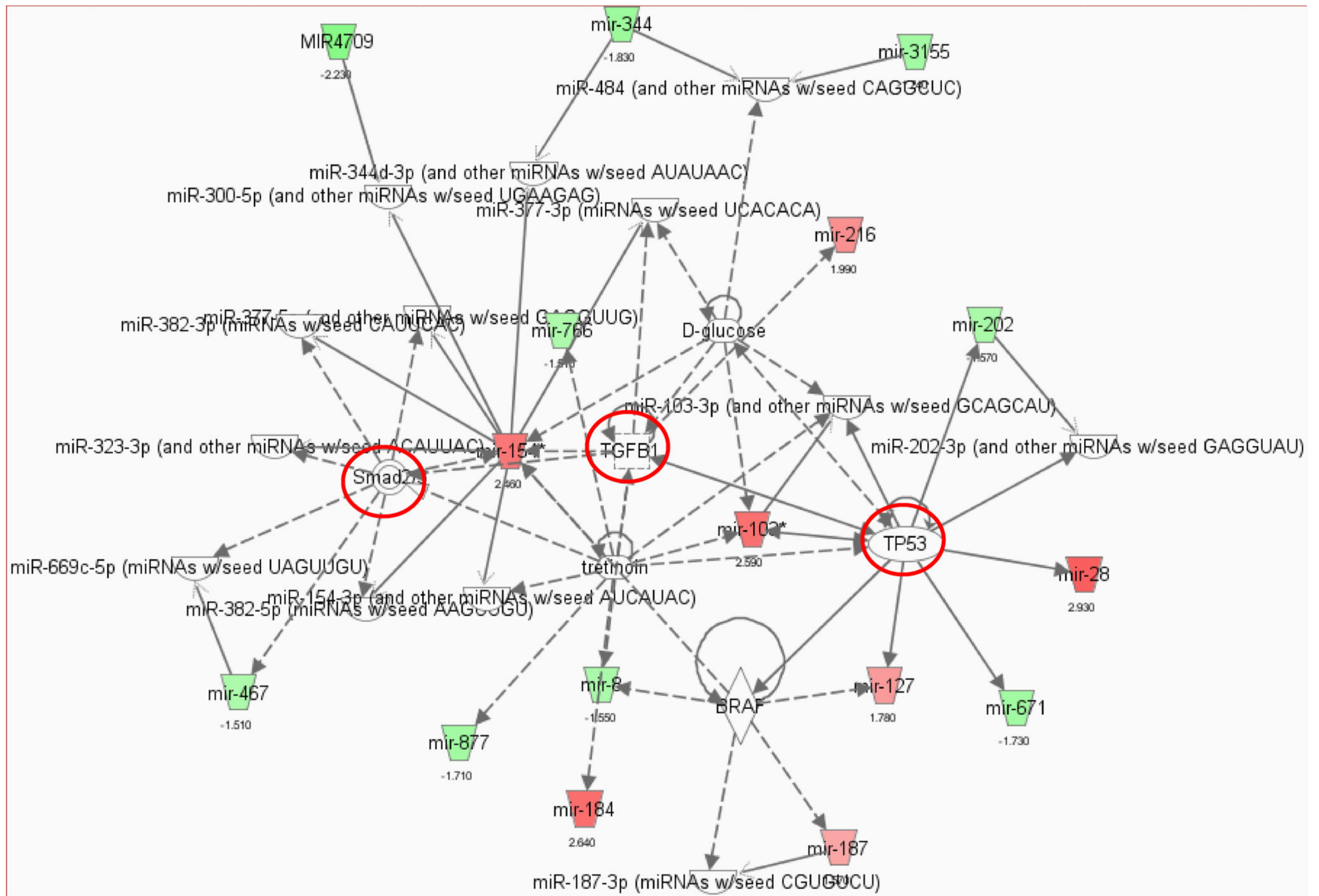

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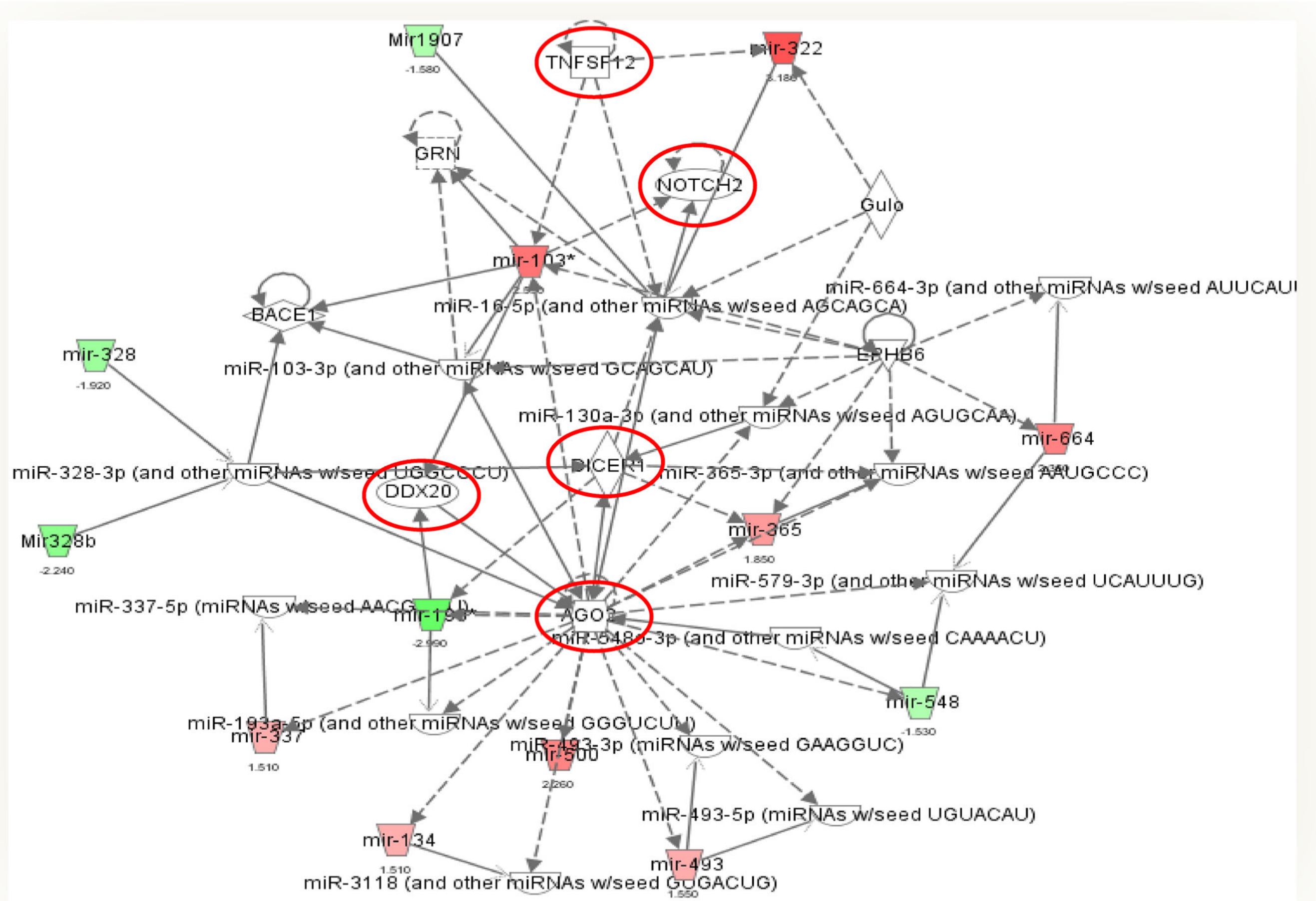

Figure 1.

miRNA-gene-network associated with SIRT3 ablation from the microarray data. Pathway analysis was performed with a statistical cutoff of $p < 0.02$. Green trapezoid represents downregulated miRNA, and red trapezoid represents upregulated miRNA. There were two highly interconnected networks associated with endothelial SIRT3 deletion.

A, Network 1 is involved in the regulation Smad2/3, TGF-β1, and TP53. The red circle represents upregulated genes.

B, Network 2 is associated with DICER1, AGO2, TNFSF12, and Notch signaling. The red circle represents upregulated genes.

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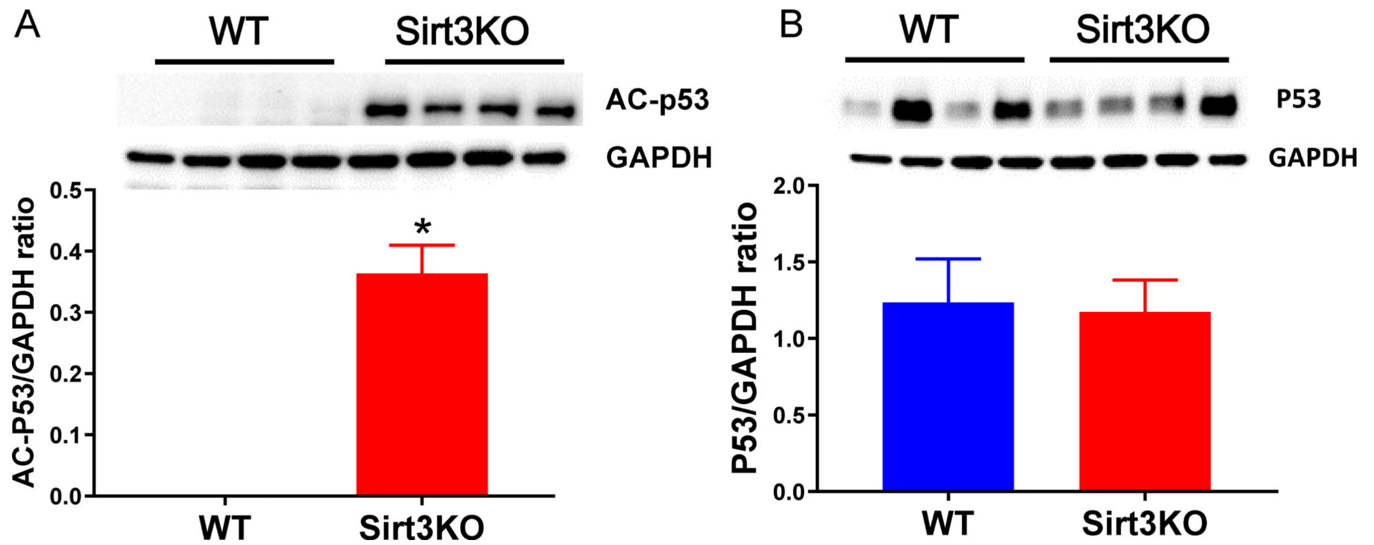

Figure 2.

Western blots analysis indicated that SIRT3 deletion altered cardiac expression of p53.

A, Expression of p53 acetylation was significantly increased in SIRT3 KO mice (n=4) vs. WT mice (n=4).

B, Expression of p53 was not significantly increased in SIRT3 KO mice. (n=4) vs. WT mice (n=4). *$p < 0.05$. p values were determined by Student's t test. Data are shown as the means ± s.e.m.

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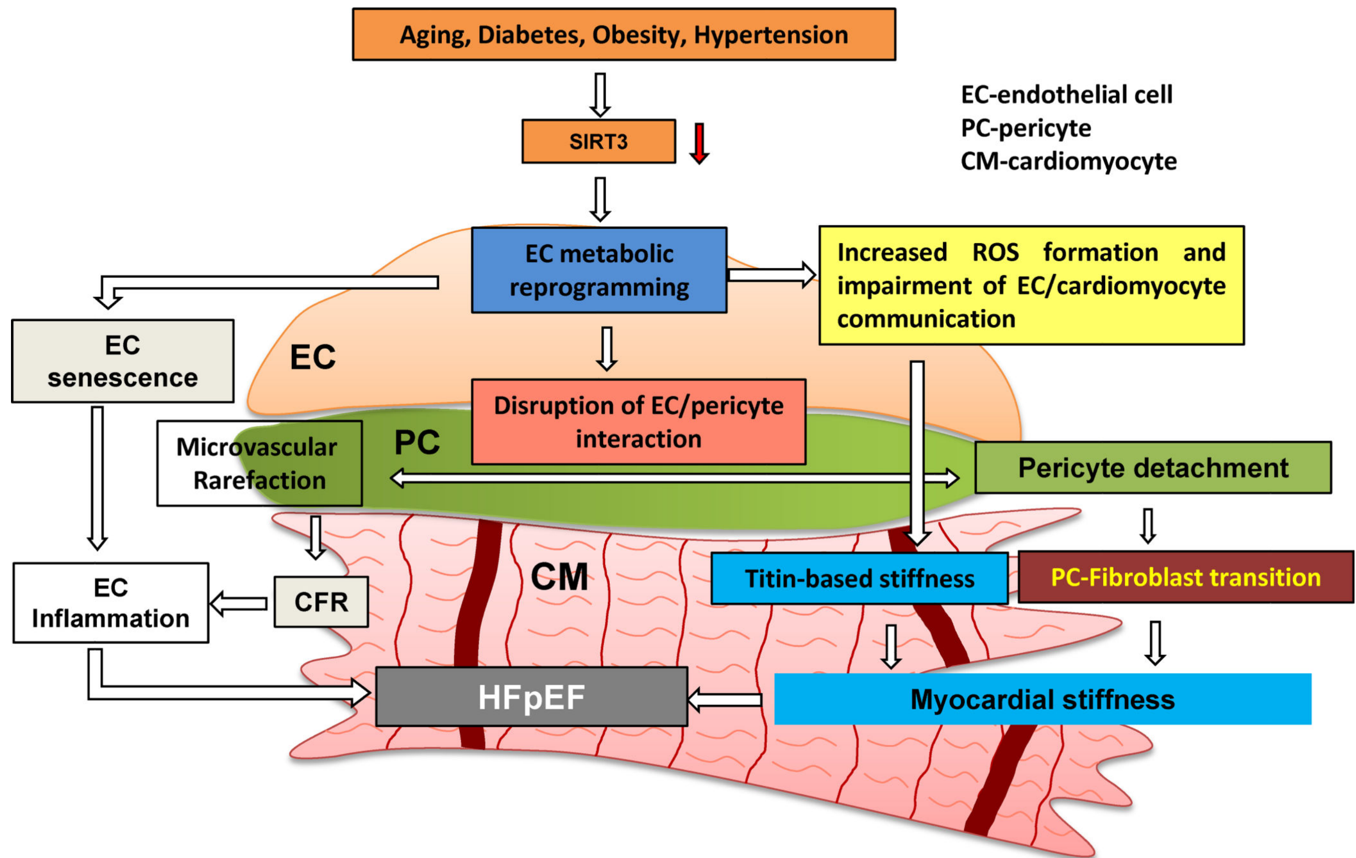

Figure 3.

Hypothesis regarding the role of endothelial metabolic reprogramming in the pathophysiology of HFpEF.

Risk factors, such as aging, diabetes, obesity, and hypertension, have been shown to reduce the expression of SIRT3 in EC. Myocardial stiffness (cardiomyocyte stiffness and myocardial fibrosis) is the center feature of HFpEF. In HFpEF, diastolic dysfunction and myocardial stiffness are driven by endothelial metabolic reprogramming. Loss of SIRT3 alters EC glycolytic metabolism and shifts ECs from oxygen-independent metabolism to highly oxygen-consuming metabolism. This metabolic reprogramming in EC increases oxygen demand and induces production of ROS, which diminishes EC/cardiomyocyte interaction, thus resulting in an increase in Titin-based cardiomyocyte stiffness. Disruption of endothelial glycolytic metabolism also leads to EC senescence and increases EC inflammation. Moreover, impairment of EC glycolysis decreases angiogenic capacity as well as promotes microvascular rarefaction and inflammatory response by disruption of EC/pericyte interaction. In addition, pericyte detachment promotes pericyte differentiation into myofibroblast and increase interstitial fibrosis deposition. All these critical steps may lead to microvascular dysfunction and diastolic dysfunction and HFpEF. SIRT3, Sirtuin 3, CFR, coronary flow reserve, EC, endothelial cell, PC, pericyte, CM, cardiomyocyte, HFpEF, heart failure with preserved ejection fraction.