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Emerging role of SIRT3 in endothelial metabolism, angiogenesis and cardiovascular disease

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Summary

Sirtuin 3 (SIRT3) a mitochondrial enzyme that plays an important role in energy homeostasis, cardiac remodeling, and heart failure. The expression of SIRT3 declines with advanced age, cardiovascular, and metabolic diseases. Accumulating evidence suggests that SIRT3 plays a critical role in protecting the heart from cardiac hypertrophy, cardiac dysfunction associated with heart failure, and in the protection of cardiac cells from stress-mediated cell death. Clinical studies have demonstrated that heart failure with preserved ejection fraction (HFpEF) in patients present with abnormalities in coronary microcirculation related to endothelial dysfunction and coronary microvascular rarefaction. Although SIRT3-mediated regulation of mitochondrial homeostasis and heart function has been intensively investigated, the effect of SIRT3 on endothelial cell glycolytic metabolism and microvascular function has not been well studied. Endothelial cells utilize glycolysis for generating ATP rather than oxidative phosphorylation to maintain their normal functions and promote angiogenesis and EC/cardiomyocyte interactions. Emerging evidence indicates that SIRT3 is involved in the regulation of endothelial metabolism and angiogenesis and thus affects the development of cardiovascular diseases associated with aging. This review will discuss the current knowledge on SIRT3 and its functional role on endothelial metabolism, cardiac function and cardiovascular diseases.

Keywords

SIRT3; endothelial cell metabolism; diastolic dysfunction; coronary microvascular dysfunction; induction of hypoxia tolerance; cardiovascular disease

Introduction

Sirtuins are a family of seven NAD⁺-dependent histone deacetylases (SIRT1-SIRT7), which are homologs of silent information regulation-2 gene encoded proteins known to slow aging in a species of yeast-*Saccharomyces cerevisiae* and highly conserved across species from bacteria to humans. (Frye, 2000; Imai et al, 2000; Lin et al, 2002) SIRT1 is the most studied member of this family, which has been shown to be associated with angiogenesis, cell cycle

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Conflict of Interest

The authors have no conflicts of interest to declare.

regulation and energy homeostasis. (Alhazzazi et al, 2013;Nogueiras et al, 2012;Potente et al, 2007) SIRT1 and SIRT2 are localized in both the nucleus and the cytoplasm in which SIRT1 may have a cell type dependent subcellular localization. (Kida and Goligorsky, 2016;Potente et al, 2007) SIRT3, SIRT4, and SIRT5 are mitochondrial proteins, while SIRT6 and SIRT7 are observed in the nucleus. (Kida and Goligorsky, 2016;de Moura et al, 2014) Beside their deacetylase activity, SIRT2 possesses demyristoylase activity (Teng et al, 2015); SIRT4 and SIRT6 may function as a lysine ADP-ribosyltransferase (Haigis et al, 2006;Liszt et al, 2005;Ahuja et al, 2007); and SIRT5 has demalonylase and desuccinylase activities in addition to its deacetylase activity. (Du et al, 2011)

Sirtuin 3 (SIRT3) is recognized as a mitochondrial enzyme that plays an important role in energy homeostasis, cardiac remodeling, and heart failure. SIRT3, primarily localized in mitochondria of organs with high metabolic activity, including liver, adipose and heart, has emerged as a novel regulator of mitochondrial energy metabolism and function.(Nogueiras et al, 2012;Tanno et al, 2012) SIRT3 decreases as much as 40% in old adults with sedentary lifestyles compared to younger individuals, but can be activated by calorie restriction.(Lanza et al, 2008) Studies over the last decade suggest that SIRT3 plays an important role in protecting the heart from oxidative stress.(Halestrap et al, 2007;Schwarz et al, 2014;Stride et al, 2013;Tsutsui et al, 2011) In addition, patients with failing hearts were reported to have reduced level of SIRT3 and excessive cardiac fibrosis.(Sundaresan et al, 2015) Although partially related to the regulation of mitochondrial function, energy homeostasis, and oxidative stress, a comprehensive understanding of how SIRT3 provides protection to the heart is still missing. Meanwhile, there could be cell-type specific actions mediated by SIRT3 since different types of cells rely on different types of “fuel”, such as cardiomyocytes mainly using fatty acids for oxidative phosphorylation or endothelial cells (ECs) and some types of cancer cells mainly using glucose through glycolysis for ATP production.

Cardiovascular disease leads to cardiac dysfunction and eventually heart failure, which is the leading cause of death in the United States (Go et al, 2014). Heart failure (HF) is a high prevalent, progressive disease that develops with advanced age, hypertension and diabetes. (Brouwers et al, 2013;Lam et al, 2011;Mohammed et al, 2015) and is typically divided into two phenotypes: heart failure with reduced ejection fraction (HFrEF) and heart failure with preserved ejection fraction (HFpEF). More than half of newly diagnosed HF patients present with preserved ejection fraction.(Dhingra et al, 2014;Upadhyaya et al, 2015) Diastolic dysfunction is one of the major characteristics of HFpEF, as well as in some population of asymptomatic patients and patients with HFrEF.(Dhingra et al, 2014;LeWinter and Meyer, 2013;Upadhyaya et al, 2015) Although intensive effort has been invested in exploring the mechanisms of HFpEF, the underlying pathophysiology and molecular mechanisms of HFpEF are poorly understood. Thus, the treatment options for HFpEF are also limited.

ECs play an important role in cardiovascular health. In fact, ECs line the entire circulatory system from the heart to the capillary, and the ratio of cardiac ECs to cardiomyocytes is about 3:1.(Brutsaert, 2003) Endothelium is a direct target of all the major risk factors for heart disease, including but not limited to ageing, diabetes, hypertension, and inflammation. (Davidson, 2010) However, ECs are not given enough attention in regards treating complications in cardiovascular diseases. Endothelial mitochondria are believed to be one of

the major sources of reactive oxygen species (ROS) in ECs, which have been shown to regulate hypoxia-inducible factor (HIF)-1 α and HIF-2 α stabilization.(Davidson, 2010) ROS is considered an important signaling molecule that reflects mitochondrial homeostasis and modulates endothelial function including proliferation, inflammation, integrity, and vascular remodeling.(Davidson, 2010) HIF-2 α has been shown to regulate angiogenesis and endothelial mitochondrial homeostasis via its function as a gene transcription factor. (Davidson, 2010) Reports also suggested that the interaction between ECs and cardiomyocytes regulate cardiac function via endothelial paracrine factors that promote cardiomyocyte organization and survival.(Hsieh et al, 2006;Leucker et al, 2011) This paracrine regulation includes platelet-derived growth factor-B (PDGF-B), VEGF-A, Angiopoietin-1 and endothelin-1, as well as nitric oxide (NO).(Leucker et al, 2011) Although the endothelium is well studied, there was not much attention given to its potential role in mediating cardiac protective actions. One of the most important functions mediated by ECs is angiogenesis which is the formation of new blood vessel. The growth and development of the heart is dependent on coordinated angiogenesis thus supplying the growing cardiomyocytes with essential nutrients, macromolecules, and oxygen.(Pillai et al, 2014;Oka et al, 2014) Importantly, this function of ECs has been found to be dampened in obesity, diabetes, and aging process.(Zeng et al, 2014a;Maizel et al, 2014;Porter et al, 2014) Our previous study has shown that the level of SIRT3 is decreased in the heart of db/db diabetic mice and associated with impaired angiogenesis and cardiac dysfunction.(Zeng et al, 2014a) In addition, apelin gene therapy in STZ-induced diabetic mice improved myocardial vascular density in the heart of wild-type (WT) mice, but failed to do so in SIRT3 knockout (KO) mice.(Hou et al, 2015) Together, these studies implicate an important role of SIRT3 in the process of endothelial cell-mediated angiogenesis and cardiac homeostasis. However, the direct role for SIRT3 in angiogenesis has not been well studied. This review will discuss the current knowledge on SIRT3 and its functional role on endothelial metabolism, cardiac function, and cardiovascular diseases.

Structural properties of SIRT3

The full length human SIRT3 protein consists of 399 amino acid residues with an N-terminal 101-residual mitochondrial targeting sequence that is cleaved by mitochondrial matrix processing peptidase (MPP) during its translocation into the mitochondria, resulting in a shorter and active form. Study of the crystal structure of human SIRT3 reveals a large domain containing a Roschmann fold and NAD⁺ binding site, and a small domain containing a helical complex and a zinc binding motif. (Jin et al, 2009b) The acetylated substrate is believed to insert into the cleft between the two domains. (Jin et al, 2009b) This study provides a structural basis that helps in understanding the deacetylation mechanism of SIRT3 in the regulation of cellular function.

Function of SIRT3 and fuel sensing by SIRT3

SIRT3 and other sirtuins catalyzes a NAD⁺-dependent chemical reaction that deacetylates the acetyl-lysine residues of target proteins. (Nogueiras et al, 2012) Biochemical studies demonstrate that the deacetylation reaction begins with the binding of NAD⁺ to the catalytic site with the assistance of catalytic phenylalanine and histidine residues. (Hawse et al,

2008;Hoff et al, 2006;Min et al, 2001;Sauve et al, 2001;Smith and Denu, 2006) However, another study suggests that the binding of acetylated substrate occurs before NAD⁺ binding. (Jin et al, 2009b) The binding and cleavage of NAD⁺ produce O-acetyl-ADP-ribose (O-AADPR) and nicotinamide which can act as a feedback inhibitor to reverse the reaction. (Nogueiras et al, 2012)

Regulation of SIRT3

Surprisingly, the knowledge of endogenous regulators of SIRT3 is still poor. Caloric restriction is known to activate SIRT3 in which the molecular mechanism may involve NAD⁺ and peroxisome proliferator activated receptor γ coactivator-1 α (PGC-1 α). (Finley et al, 2012a) NAD⁺ is a co-substrate in SIRT3-mediated deacetylation and is essential for the enzymatic activity of SIRT3. NAD⁺ is reduced to NADH during glycolysis, tricarboxylic acid (TCA) cycle, β -oxidation, and catabolic reactions. Increased ratio of NAD⁺ to NADH indicates energy depletion and activates SIRT3, whereas reduced ratio of NAD⁺ to NADH suggests plenty of energy and limits SIRT3 activity. NADH from cytosolic metabolism is transported into mitochondria and converges with NADH from TCA cycle to be oxidized into NAD⁺ by the electron transport chain. Therefore, utilization of NAD⁺ enables SIRT3 to be the sensor of cellular energy metabolism and to regulate the function of its down-stream targets. PGC-1 promotes transcription of a variety of genes controlling gluconeogenesis in liver, mitochondrial biogenesis and respiration in skeletal muscle cells, heart development, and other processes. (Lin et al, 2005;Finck and Kelly, 2006) PGC-1 α potently induces the expression of genes implicated in energy homeostasis in almost every cell type through interacting directly with and coactivating known mitochondrial regulators, such as the estrogen related receptors (ERRs), peroxisome proliferator-activated receptors (PPARs), or nuclear respiratory factors (NRFs). (Lin et al, 2005;Finck and Kelly, 2006;Wang et al, 2003;Giguere, 2002)

In addition to NAD⁺, a transcription factor, ERR α and its co-activator, PGC-1 α , have been shown to play an important role in regulation of SIRT3 expression. (Kong et al, 2010;Giralt et al, 2011) ERR α mediates the expression of medium-chain acyl coenzyme A dehydrogenase that is a key enzyme in the mitochondrial oxidation of fatty acids (Sladek et al, 1997), and of several components of the TCA cycle and mitochondrial electron transport chain. (Giguere, 2008) In the past few years, Giralt and colleagues reported that ERR α can bind the SIRT3 promoter directly when it is co-activated by PGC-1 α . (Giralt et al, 2011) Overexpression of ERR α upregulates target genes associated with mitochondrial respiration and fatty acid oxidation (Huss et al, 2004), whereas knockdown of PGC-1 α or ERR α decreases SIRT3 expression and several SIRT3-related ROS-detoxifying enzymes. (Kong et al, 2010) Kong and colleagues further demonstrated that SIRT3 is essential for mitochondrial biogenesis in myotubes since knockdown of SIRT3 decreases PGC-1 α -dependent induction of glutathione peroxidase-1 (GPx1), manganese superoxide dismutase-2 (SOD2 or MnSOD), ATP synthase 5c, and cytochrome c which are components of ROS-detoxifying pathway and electron transport chain. (Kong et al, 2010) These studies also suggest potential mitochondrial targets of SIRT3.

Besides NAD⁺, PGC-1 α , and ERR α mentioned above that have been shown to regulate the function of and the expression of SIRT3, little is known about the posttranslational regulation of SIRT3. Schlicker *et al.* report that structural relationship between N-terminal and C-terminal modulates SIRT3 activity, in which the N-terminal may function as an auto-inhibitory region, and cleavage of N-terminal at residue 113 results in higher enzymatic activity than when cleaved at residue 101. (Schlicker et al, 2008) Recently, by using a high-resolution mass spectrometry-based phosphoproteome analysis, Olsen and colleagues identified six phosphorylated serine residues between amino acids 101 and 118 which are close to the mitochondrial cleavage site. Their biological relevance or influence on SIRT3 function has not been analyzed yet. (Olsen et al, 2010) We can speculate that this phosphorylation site may play a role in regulating proteolytic cleavage by MPP and the interaction between N-terminal and C-terminal that modulates catalytic activity of SIRT3.

Distribution of SIRT3

In human and mouse, SIRT3 is highly expressed in tissue with high metabolic activity, such as kidney, heart, brain, brown adipose tissue and liver, and lower expression is also detected in lung, spleen and thymus. (Palacios et al, 2009; Jin et al, 2009a; Su et al, 2004) The full length SIRT3 (44 kDa) was initially reported to be enzymatically inactive until it is translocated into mitochondria where it is processed and activated by MPP. (Jin et al, 2009a; Bao et al, 2010) MPP removes 142 amino acids of the N-terminal segment that is the mitochondrial localization sequence (MLS) and creates a 28 kDa short form of SIRT3 that processes deacetylase activity. (Jin et al, 2009a; Bao et al, 2010) The human full length SIRT3 is proposed to be exclusively located in the mitochondria, whereas the short isoform can be detected in mitochondria, the nucleus, and the cytoplasm. (Iwahara et al, 2012; Onyango et al, 2002; Scher et al, 2007) Sundaresan and others have reported that SIRT3 found in the nucleus under basal conditions can translocate to the mitochondria during cellular stress and protect cell from stress-mediated cell death by deacetylation of Ku70, a protein required for non-homologous end joining pathway of DNA repair. (Scher et al, 2007; Sundaresan et al, 2008) These studies bring controversy to the subcellular location of SIRT3 which is critical for understanding the role of SIRT3 in the regulation of variety of targets and function.

Mitochondrial targets of SIRT3

SIRT3 regulates the expression and function of many mitochondrial proteins via deacetylation. SIRT3 activates several key enzymes associated with fatty-acid metabolism, electron transport chain function, antioxidant defenses, apoptosis, acetyl-CoA metabolism, and amino acid catabolism. (Osborne et al, 2016) These targets, as shown in Figure 1, include acetyl-CoA synthase 2 (AceCS2), hydroxyl methylglutaryl CoA synthase 2 (HMGCS2), succinate dehydrogenase (SDH), glutamate dehydrogenase (GDH), isocitrate dehydrogenase 2 (ICDH2), long-chain acyl-CoA dehydrogenase (LCAD), and ornithine transcarbamoylase (OTC) in the urea cycle. (Hallows et al, 2006; Hallows et al, 2011; Hirschey et al, 2010; Shimazu et al, 2010)

AceCS2, which catalyzes the conversion of acetate to acetyl-CoA, is the first discovered SIRT3 mitochondrial target. (Fujino et al, 2001) The enzymatic activity of AceCS2 has been shown to be regulated by reversible acetylation at Lys-642 residue in the active site of the enzyme. (Schwer et al, 2006) Deacetylation of AceCS2 at Lys-642 residue by SIRT3 activates AceCS2, whereas acetylation of AcsCS2 inhibits its activity. (Hallows et al, 2006;Schwer et al, 2006) Since acetyl-CoA is an important intermediate of biosynthesis of cholesterol and fatty acid, it is possible that SIRT3 modulates those metabolic pathways.

Succinate dehydrogenase flavoprotein (SdhA), a subunit of SDH, and ICDH2 which is involved in the process of oxidative phosphorylation in the TCA cycle, along with GDH that provides substrates for the TCA cycle by converting glutamate to α -ketoglutarate, have been shown to be colocalized, deacetylated and activated by SIRT3 in the mitochondria. (Schlicker et al, 2008;Lombard et al, 2007;Cimen et al, 2010) SdhA is hyperacetylated at lysine residues K179, K485, K498, and K538 and these acetylation sites are conserved among species. (Cimen et al, 2010) In ICDH2 two acetylation sites are found at K75 and K241 and are regulated by SIRT3. (Schlicker et al, 2008) Moreover, deacetylation and activation of ICDH2 has been shown to increase NADPH levels and the ratio of reduced to oxidized glutathione, thus enhancing the mitochondrial glutathione antioxidant system in mice. (Someya et al, 2010) In addition to the role of SIRT3 in modulating TCA cycle, Ahn and colleagues demonstrated that SIRT3 can directly interact with and activate one or more subunits of Complex I including NDUFA9 via deacetylation. (Ahn et al, 2008) These results suggest that SIRT3 plays an important role in regulating mitochondrial energy metabolism.

SIRT3 deacetylates and activates OTC, a rate-limiting enzyme in the urea cycle at K88 residue, and the fatty acid metabolism related enzyme LCAD at lysine residue 42. (Hallows et al, 2011;Hirschey et al, 2010) Moreover, Shimazu and colleagues have shown that HMGCS, an enzyme mediating the rate-limiting step in β -hydroxybutyrate synthesis, is hyperacetylated at lysine residues 310, 447, and 473 in the absence of SIRT3, but the level of β -hydroxybutyrate is decreased in SIRT3 KO mice. (Shimazu et al, 2010;Hegardt, 1999) These studies further link SIRT3 to the regulation of cellular metabolism and age-related oxidative damage.

Initial studies have mostly focused on metabolic targets of SIRT3 in liver, skeletal muscle, and non-cardiac cells, their relevance to cardiac metabolism has not been fully validated. There are accumulating studies looking at SIRT3 activity in cardiac tissue and cells. SIRT3 can modulate proteins that contribute to mitochondrial dynamics and permeability, including Cyclophilin D (CypD) that regulates mitochondrial permeability transition pore (mPTP) opening. (Hafner et al, 2010) Opening of the mitochondrial mPTP is associated with a decline in mitochondrial function. (Hafner et al, 2010) mPTPs are composed of voltage dependent anion channel (VDAC) in the outer membrane, the adenine nucleotide translocator (ANT) in the inner membrane and CypD in the matrix space, as well as other proposed components including hexokinase, Bax and creatine kinase. (Beutner et al, 1996;Crompton et al, 1998;Halestrap and Davidson, 1990;Marzo et al, 1998) Triggering of the mPTP can result in mitochondrial swelling, membrane de-polarization, the removal of defective mitochondria by autophagy, and even apoptosis under acute conditions. (Kim et al, 2007) CypD modulates the opening of mPTP by binding to ANT and creating a connection

between the mitochondrial matrix and the cytosol. (Lemasters et al, 2009) SIRT3 deacetylates CypD on lysine residue 166, which promotes the dissociation of CypD and closure of the mPTP. (Hafner et al, 2010) Consistent with these findings, SIRT3 deficient cardiac myocytes exhibit an increase in mitochondrial swelling due to increased mPTP opening and SIRT3 KO mice are hypersensitive to cardiac stress and show increased cardiac hypertrophy and fibrosis. (Hafner et al, 2010)

Consistent with these findings, SIRT3 can activate forkhead box O3a-dependent (Foxo3a-dependent) antioxidant system in cardiomyocytes and block the cardiac hypertrophic response. (Sundaresan et al, 2009) Foxo3a is a transcription factor, and when activated, translocates into the nucleus and promotes the transcription of antioxidant genes including MnSOD and catalase (Foxo3a-dependent) in cardiomyocytes. (Sundaresan et al, 2009) Moreover, Tao and colleagues demonstrated that SIRT3 can also mediate deacetylation of MnSOD at lysine residue 122 and induce MnSOD activity in response to stress. (Tao et al, 2010)

In addition to the role of SIRT3 in the induction of the antioxidant system, SIRT3 is a stress-responsive deacetylase which protects cardiomyocytes from stress-mediated cell death via deacetylation of Ku70. (Sundaresan et al, 2008) Deacetylation of Ku70 by SIRT3 promotes its interaction with the pro-apoptotic protein Bax, thus preventing stress-induced apoptosis of cardiomyocytes. (Sundaresan et al, 2008) Optic atrophy 1 (OPA1) protein is also regulated by SIRT3 deacetylation at lysine residues 926 and 931. (Samant et al, 2014) OPA1 mediates mitochondrial inner fusion. (Chan, 2006) OPA1 is hyperacetylated and its GTPase activity is reduced in hearts under pathological stress, whereas SIRT3 can deacetylate and increase its GTPase activity. (Samant et al, 2014)

SIRT3 in cardiac function and cardiovascular disease

SIRT3 and cardiac function

Cardiac function decreases with aging, and understanding the molecular mechanisms underlying this dysfunction is crucial for the development of therapies. SIRT3 activity declines with age, suggesting a link between SIRT3 and the development of heart failure in aging. Cardiac fibroblasts isolated from patients with heart failure exhibit reduced expression of SIRT3 when compared to cells from control patients. (Sundaresan et al, 2015) Indeed, several studies have focused on the functional role of SIRT3 in the regulation of cardiac function using global SIRT3 KO mice. (Hafner et al, 2010; Koentges et al, 2015; Koentges et al, 2016b; Porter et al, 2014; Sundaresan et al, 2009) These SIRT3 deficient mice have decreased systolic function as evidenced by a reduction in ejection fraction (EF) and fractional shortening (FS) associated with an increase in left ventricle (LV) dilation in advanced age, whereas younger animals have normal contractile function even though decreased cardiac capillary density can already be observed. (Zeng et al, 2014a; Zeng et al, 2015; Sundaresan et al, 2009; Koentges et al, 2015) Interestingly, Koentges and colleagues also report that there is already a difference in cardiac function between SIRT3 deficient and WT mice at 8 weeks of age, using the isolated working heart model in two different studies. (Koentges et al, 2015; Koentges et al, 2016b) The authors further speculate the possibility that neuro-hormonal compensatory mechanisms, such as renin-angiotensin-aldosterone-

system and autonomic nervous system, prevent the manifestation of cardiac dysfunction in younger age, but that with increasing age SIRT3-deficiency-mediated cardiac remodeling may overcome these compensatory mechanisms resulting in detectable cardiac dysfunction. (Koentges et al, 2016a) However, such compensating mechanisms remain to be proven.

Although young SIRT3 KO hearts do not exhibit any functional deficit, additional studies investigating the role of SIRT3 in cellular metabolism and mitochondrial function suggest that SIRT3 is essential for maintaining mitochondrial homeostasis and cardiac function. Koentges and colleagues reported that cardiac contractile function, palmitate oxidation and glucose oxidation are all decreased in hearts from 8-week-old SIRT3 KO mice using an *ex vivo* isolated working heart model, while functional change cannot be detected in these mice *in vivo*. (Koentges et al, 2015) Additionally, consistent with previous studies, (Ahn et al, 2008; Hirsche et al, 2010) a defect in mitochondrial energetics is found in mitochondria isolated from 8-week-old SIRT3 KO hearts, characterized by a reduction in State 3 respiration and ATP synthesis in association with hyperacetylation and decreased activity of LCAD and GDH. (Koentges et al, 2015) Furthermore, increasing cardiac energy demand and workload by transverse aortic constriction (TAC) in SIRT3 KO mice results in an accelerated development of contractile dysfunction, cardiac fibrosis, and hypertrophy. (Koentges et al, 2015) However, the same group report that SIRT3 deficiency does not impair further cardiac function following myocardial infarction or ischemia/reperfusion (I/R) despite the pre-existing cardiac dysfunction and decreased mitochondrial respiratory capacity in SIRT3 KO mice, which creates some controversy about the role of SIRT3. (Koentges et al, 2016b) The explanation of the authors for this controversy is that SIRT3 deficient mice either possibly develop an unspecific compensatory adaptation to the genetic manipulation, or that age-related changes determine the vulnerability to IR injury. (Koentges et al, 2016b) Further studies are warranted to fully understand the discrepancy between these different models of cardiac stress.

In a gain of function study, SIRT3 transgenic mice are protected from hypertrophic stimuli induced by angiotensin II (ANGII) infusion, while SIRT3 deficient mice develop severe cardiac hypertrophic response, fibrosis, and cardiac dysfunction. (Sundaresan et al, 2009) Similarly, our study demonstrates that apelin gene therapy attenuates myocardial apoptosis and improves systolic function in WT diabetic mice subjected to permanent coronary artery ligation, whereas these protective effects are lost in SIRT3 KO mice. (Hou et al, 2015) Moreover, our study shows that ablation of SIRT3 exacerbates MI-induced injury and impairs cardiac recovery post-MI in SIRT3 KO mice, whereas overexpression of SIRT3 preserves cardiac function in post-MI mice. (He et al, 2016) Taken together, these studies suggest that SIRT3 is required to maintain cardiac contractile function under pro-hypertrophic or ischemic stress.

SIRT3 and cardiovascular disease

Heart failure develops with advanced age, hypertension, and metabolic syndrome. (Brouwers et al, 2013; Lam et al, 2011; Mohammed et al, 2015) Accumulating evidence suggests that SIRT3 plays a critical role in protecting the heart from cardiac hypertrophy, cardiac dysfunction associated with heart, and in the protection of cardiac cells from stress-mediated

cell death.(Hafner et al, 2010;Koentges et al, 2015;Sundaresan et al, 2008;Sundaresan et al, 2009) SIRT3 deficiency is associated with pulmonary arterial hypertension in rodents and humans.(Paulin et al, 2014) In addition, SIRT3 deficiency accelerates body weight gain and impairs rapid metabolic adaptation to changes in nutrient supply in LDL receptor KO mice, indicating SIRT3 plays an important role in delaying the development of cardiovascular risk factors.(Winnik et al, 2014) A recent study from Sundaresan and colleagues have shown that SIRT3 inhibits transformation of fibroblasts into myofibroblasts and tissue fibrosis via deacetylation and activation of glycogen synthase kinase 3 β , which suppresses TGF- β 1 signaling.(Sundaresan et al, 2015) These findings indicate that SIRT3 is pivotal for maintaining heart function in the process of aging and in the development of cardiovascular disease.

Mitochondrial dysfunction has been observed in several cardiovascular diseases, including hypertrophic and dilated cardiomyopathy, heart failure, pulmonary hypertension, and endothelial dysfunction in early atherogenesis.(Madamanchi et al, 2005;Winnik et al, 2015) In addition to the role of SIRT3 in mitochondrial energy homeostasis, SIRT3 protects cells from overproduction of ROS by upregulation of antioxidant enzymes, such as MnSOD, catalase, and peroxidases, in cardiomyocytes. Numerous studies suggest that SIRT3 decreases the ROS levels by inducing deacetylation and nuclear translocation of Foxo3a transcription factor, thereby activating antioxidant-encoding genes such as MnSOD and catalase.(Sundaresan et al, 2009;Tao et al, 2010) In addition, SIRT3 can also activate MnSOD via direct deacetylation.(Tao et al, 2010) Honokiol, a natural biphenolic compound, blocks and reverses cardiac hypertrophy by specifically upregulating and activating SIRT3 mediated anti-oxidant mechanism.(Pillai et al, 2015) Moreover, the same group recently reports that SIRT3 protects from doxorubicin-induced mitochondrial DNA damage and cardiomyopathy through preservation of 8-oxoguanine-DNA glycosylase-1 (OGG1),(Pillai et al, 2016) which is in agreement with another study suggesting that a SIRT3/OGG1 pathway mediates mitochondrial DNA repair.(Cheng et al, 2013) These studies provide additional evidence for antioxidant and repair mechanisms mediated by SIRT3. In addition, mPTP opening is involved in cell death due to mitochondrial swelling and membrane depolarization in response to I/R.(Heusch et al, 2010) CypD, one of the components that control the opening of the mPTP, is deacetylated and inhibited by SIRT3, thus leading to closure of the mPTP.(Sack, 2012) Cardiac myocytes from SIRT3 KO mice exhibit increased Ca²⁺ sensitivity due to the opening of mPTP and an age-dependent increase in mitochondrial swelling.(Hafner et al, 2010) These findings suggest that SIRT3 regulates cellular resistance to stress in the heart via mPTP.

A new paradigm proposes that comorbidities associated with HFpEF, including obesity, hypertension, diabetes mellitus and chronic kidney disease, result in systematic inflammation that causes coronary endothelial dysfunction and decreased bioavailability of NO, which leads to impaired diastolic function.(Paulus and Tschope, 2013) Clinical studies have demonstrated that HFpEF in patients present with abnormalities in coronary microcirculation related to endothelial dysfunction and coronary microvascular rarefaction.(Mohammed et al, 2015) Consistent with these clinical observation, our study revealed that global SIRT3 KO mice develop coronary microvascular dysfunction and diastolic dysfunction, associated with decreased myocardial capillary density and pericyte coverage.

(He et al, 2016) Similarly, endothelial-specific SIRT3 knockout (ECKO) mice also exhibit decreased coronary function and diastolic function. (He et al, 2017) These studies indicate that endothelial function plays an important role in cardiac homeostasis involving SIRT3-mediated cellular function. However, a direct link between SIRT3 and endothelial function is still unclear.

SIRT3 and endothelial physiology

Much attention has been focused on the role of cardiomyocytes in the progression of cardiac dysfunction and heart failure. Although changes in endothelial function and coronary microcirculation have been shown to play an important role in the progression towards heart failure, the link among SIRT3-endothelial cell-cardiac function is still unknown.

SIRT3 and endothelial cell metabolism

SIRT3 resides primarily in the mitochondria and has been shown to regulate energy metabolism. However, the role of SIRT3 in EC metabolism has not been investigated. Interestingly, despite their immediate access to oxygen in the blood, ECs utilize glycolysis for generating ATP rather than oxidative phosphorylation to maintain their normal functions and promote EC sprouting, proliferation and migration.(De et al, 2013;Xu et al, 2014) With lower oxygen consumption in the ECs, the mitochondrial content is also relatively low when compared to oxidative cell types such as cardiomyocytes.(Eelen et al, 2015) In contrast, ECs have much higher glycolytic rates than cardiomyocytes, accounting for up to 85% of the ATP produced in these cells, which is comparable to or higher than what can be observed in cancer cells.(De et al, 2013;Eelen et al, 2015) On the other hand, neither inhibition of mitochondrial ATP production nor stimulation of mitochondrial respiration affects vessel sprouting, whereas blockade of glycolysis by limiting glucose availability or using the glucose analogue 2-deoxy-D-glucose (2-DG) causes endothelial dysfunction and cell death.(Chung et al, 2004;Ghesquiere et al, 2014) Although quiescent ECs already possess high glycolytic rates for maintaining homeostasis and normal EC function, glycolytic flux can be further increased, in part by upregulating the expression of 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase-3 (PFKFB3), when ECs are activated.(De et al, 2013;Eelen et al, 2015) Recent reports suggest that PFKFB3 is one of the key regulators in the process of glycolysis, and inhibition of PFKFB3 reduces EC glycolytic flux by about 40%.(De et al, 2013;Ghesquiere et al, 2014) These studies suggest that glycolytic function in ECs plays a critical role in regulating EC function. Our recent study reveals that the expression of PFKFB3 and glycolysis is decreased in SIRT3 deficient ECs, whereas mitochondria oxygen consumption rate is increased. (He et al, 2017) These findings suggest that SIRT3 deletion may reprogram EC metabolism and shift metabolism from oxygen-independent glycolysis to oxygen-dependent oxidative phosphorylation in ECs. More studies are warranted to elucidate the exact role of SIRT3 in the regulation of PFKFB3, endothelial glycolysis or EC metabolism.

A recent literature highlights the important role that SIRT3 plays in glucose metabolism in skeletal muscle.(Huynh et al, 2015) Lantier *et al.* explored the mechanism by which SIRT3 regulates glucose uptake in muscle via regulating the cellular localization and activity of hexokinase II (HKII).(Lantier et al, 2015) Glucose uptake in skeletal muscle is impaired in

SIRT3 KO mice fed with high-fat diet, which results in glucose intolerance and increased reliance of cells on fatty acids.(Lantier et al, 2015) Lantier *et al.* proposed that SIRT3 promotes HKII-VDAC-ANT complex formation to increase HKII activity and glucose uptake, which enhances glycolysis and reduces reliance on fatty acids as a fuel source; conversely SIRT3 deficiency inhibits the formation of HKII-VDAC-ANT complex and leads to decreased HKII activity, decreased glucose uptake, and increased metabolic substrate switch from glucose to fatty acids.(Lantier et al, 2015) However, the mechanisms by which SIRT3 influences the formation of the HKII-VDAC-ANT complex and whether these mechanisms occur in ECs are still unknown.

Certain types of cancer cells share metabolic characteristics with that of ECs, such as high glycolytic flux and low mitochondrial respiration, a process also known as “Warburg effect”. (Bayley and Devilee, 2012;Cui et al, 2015) Cui and colleagues have shown increased expression of SIRT3 in gastric cancer cells.(Cui et al, 2015) SIRT3 overexpression promotes glucose uptake, glycogen formation and lactate production along with increased ATP generation and MnSOD activity, whereas SIRT3 knockdown reverses these effects.(Cui et al, 2015) These effects were proposed to be mediated by increased activity of lactate dehydrogenase A (LDHA) via deacetylation by SIRT3.(Cui et al, 2015) However, no exact acetylation site in LDHA was identified in this study. Nonetheless, LDHA has been shown to play an important role in aerobic glycolysis and tumorigenicity in malignant cells.(Fantin et al, 2006;Le et al, 2010) In addition to LDHA, overexpression of SIRT3 also induced expression of genes associated with glucose uptake and glycolysis, such as HKII, monocarboxylate transporter (MCT4), and glucose transporter 4 (GLUT4).(Cui et al, 2015) Although ECs and cancer cells are very different cell types in nature, their similarity in metabolism may shed some light on the role of SIRT3 in endothelial metabolism, especially glycolytic function.

Endothelial glycolysis and EC/cardiomyocyte interactions

EC/cardiomyocyte interactions are essential for sustaining cardiomyocyte function. (Lim et al, 2015;Wan and Rodrigues, 2016) Despite immediate access to oxygen in the blood stream, ECs rely on glycolysis rather than oxidative phosphorylation to maintain its function. (De et al, 2013;Vallerie and Bornfeldt, 2015) By promoting glycolysis to generate ATP, ECs save oxygen for the adjacent cardiomyocytes. (Wan and Rodrigues, 2016) Moreover, ECs support cardiomyocyte function by increasing angiogenesis and releasing regulatory proteins. Up to date, several of EC-derived cardiac regulatory proteins have been identified including apelin and angiopoietins. (Lim et al, 2015;Wan and Rodrigues, 2016) Disruption of EC/ cardiomyocyte communication resulted in the development of cardiac structural and functional abnormalities. (Lim et al, 2015;Wan and Rodrigues, 2016;Wang et al, 2014;Wang et al, 2013) Our recent study demonstrated that glycolytic enzyme PFKFB3 was dramatically decreased in the Sirt3 KO-ECs, along with impaired glycolytic function. Intriguingly, loss of Sirt3 increased oxygen consumption rate in ECs and production of ROS. (He et al, 2017) These data suggest that there is a shift in metabolism and increased oxidative stress in Sirt3 KO-ECs, such that they are more dependent on oxidative phosphorylation and thus less flexible in metabolism. Most importantly, increased oxygen consumption in Sirt3 KO EC may limit oxygen accessible to cardiomyocyte, which could

cause cardiac hypoxia and dysfunction. Since angiogenesis and the secretion of cardiac regulator proteins from EC are highly depend on glycolysis, disruption of endothelial Sirt3 and reprogramming glycolytic metabolism may contribute to coronary microvascular rarefaction and cardiomyocyte stiffness in HFpEF.

SIRT3 and endothelial function

SIRT3 is involved in the regulation of endothelial insulin resistance and vascular dysfunction in obesity.(Yang et al, 2016) Yang and colleagues have demonstrated that the expression of SIRT3 is decreased in mesenteric arteries from insulin-resistant morbid obese subjects, and downregulation of SIRT3 using siRNA reduces insulin response in human umbilical vein endothelial cells (HUVECs), whereas overexpression of SIRT3 improves insulin resistance induced by palmitate, thus suggesting an association between SIRT3 and insulin sensitivity in ECs.(Yang et al, 2016) Furthermore, SIRT3 deficiency in mice exacerbates endothelial dysfunction in diet-induced obesity as evidenced by impairment of endothelium-dependent vasorelaxation to both insulin and acetylcholine but not endothelium-independent relaxation to sodium nitroprusside (SNP).(Yang et al, 2016) In addition, the authors have shown that downregulation of SIRT3 increases mitochondrial reactive oxygen species (mtROS) generation, accompanied by decreased phosphorylation of Akt and endothelial nitric oxide synthase (eNOS) and subsequent reduction in NO release.(Yang et al, 2016) Although endothelial mitochondria occupy a small volume of EC and generate a small portion of total ATP compared to glycolysis, endothelial mitochondria still play a critical role in endothelial energetics and homeostasis. In fact, endothelial mitochondria may function as signaling organelles via the production of ROS for maintaining EC homeostasis and function. (Davidson, 2010;Quintero et al, 2006) SIRT3 improves ROS detoxification through activation of MnSOD and catalase.(Nogueiras et al, 2012) Excessive production of ROS diminishes NO bioavailability to cardiomyocytes.(Liu et al, 2015) Yang and colleagues further showed that scavenging mitochondrial ROS significantly rescued the NO production and endothelium-dependent relaxation in SIRT3 deficient ECs, suggesting that prevention of mitochondrial oxidative damage could be a potential therapeutic strategy to reverse endothelial dysfunction.(Yang et al, 2016)

SIRT3 and angiogenesis

Emerging functions of sirtuin family involves genomic stability, metabolism, and angiogenesis. The most studied member of this family is SRIT1 that has been shown to associate with angiogenesis and is upregulated during blood vessel formation.(Potente et al, 2007) Downregulation of SIRT1 results in decreased sprouting angiogenesis and branching of ECs, which is consistent with the blunted ischemia-induced neovascularization in endothelial cell-specific SIRT1 KO mice.(Potente et al, 2007) Conversely, SIRT1 activator resveratrol protects rat hearts from myocardial infarction by increasing capillary density via upregulation of VEGF and nitric oxide synthase.(Fukuda et al, 2006) However, there are only a few reports demonstrating a direct or indirect role for SIRT3 in the regulation of angiogenesis. SIRT3 deficient bone marrow cell (BMC)-derived endothelial progenitor cells (EPCs) exhibit decreased angiogenic capacity and colony formation as well as increased ROS formation and apoptosis.(Zeng et al, 2014b) In addition, these abnormalities limited the SIRT3 deficient BMCs mediated cardiac repair post-myocardial ischemia (MI).(Zeng et al,

2014b) The pro-angiogenic factor apelin improves BMCs-mediated cardiac repair and systolic function in post-MI mice via a mechanism potentially involving upregulation of SIRT3.(Li et al, 2013) Further studies demonstrated that the expression of SIRT3 was decreased in the heart of db/db diabetic mice and was associated with reduced capillary density, whereas apelin gene therapy increased the myocardial expression of SIRT3 and myocardial angiogenesis.(Zeng et al, 2014a) In agreement with these reports, apelin gene therapy improves myocardial angiogenesis and alleviates MI injury in diabetic STZ-WT mice, but not in STZ-SIRT3 KO mice.(Hou et al, 2015) Our recent study also demonstrates that deletion of SIRT3 results in decreased endothelial cell tube formation, migration, and aortic sprouting. (He et al, 2017) The impaired angiogenic properties of SIRT3 deficient ECs is associated with impaired hypoxic signaling pathway involving HIF-2 α , angiopoietin-1, and VEGF. (He et al, 2017) These studies imply a direct role of SIRT3 in the process of angiogenesis and endothelial function. However, more studies are needed to establish a comprehensive understanding of the role of SIRT3 in angiogenesis.

Therapeutic strategies for HFpEF

More than half of newly diagnosed HF patients present with preserved ejection fraction. (Upadhyaya et al, 2015;Dhingra et al, 2014) Diastolic dysfunction is one of the major characteristics of HFpEF, as well in some population of asymptomatic patients and patients with HFrEF.(Dhingra et al, 2014;LeWinter and Meyer, 2013;Upadhyaya et al, 2015) Although much attention has been invested in exploring the mechanisms of HFpEF, the underlying pathophysiology and molecular mechanisms of HFpEF are poorly understood. Yet the therapeutic options for HFpEF are also limited to diuretics and management of comorbidities since the guidelines for the management of HFpEF has not been updated for over a decade.(Lim and Lam, 2016;McMurray et al, 2012;Yancy et al, 2016) Indeed, previous clinical trials which investigating the effects of Angiotensin converting enzyme inhibitors (ACEi), Angiotensin receptor blockers (ARBs), aldosterone antagonists, and beta-blockers have not shown promising results. For example, large phase III clinical trials, including Irbesartan in Heart Failure with Preserved Ejection Fraction Study (I-PRESERVE), Perindopril for Elderly People with Chronic Heart Failure (PEP-CHF), and Candesartan in Heart Failure-Assessment of Reduction in Mortality and Morbidity-Preserved Study (CHARM-Preserved), have shown no or partially improved HF symptoms with no overall difference in mortality and hospitalization.(Massie et al, 2008;Cleland et al, 2006;Persson et al, 2007) Moreover, clinical trials, OPTIMIZE-HF and SENIORS, studying the effects of beta-blockers, carvedilol, or nebivolol on HFpEF symptoms, yield limited beneficial effects in HFpEF patients.(Hernandez et al, 2009;Flather et al, 2005) Another clinical trial also involving large population of HFpEF patients, such as CORONA using rosuvastatin shows no promising results either, other than improvement on LV remodeling. (Kjekshus et al, 2007)

There is accumulating evidence indicating diastolic dysfunction is associated with oxidative stress and the NO-soluble guanylate cyclase (sGC)-cyclic guanosine monophosphate (cGMP) signaling pathway. Recently, a novel paradigm for HFpEF has been proposed by Paulus et al., which myocardial remodeling and dysfunction in HFpEF is the consequence of comorbidities and insults.(Paulus and Tschope, 2013) These comorbidities and insults

include obesity and diabetes induced systemic proinflammatory state, increased ROS generation, decreased NO bioavailability in coronary microvascular endothelial cells, decreased protein kinase G (PKG) activity in cardiomyocytes, cardiomyocyte hypertrophy, and excessive cardiac fibrosis. (Paulus and Tschope, 2013) One of the central players in the pathophysiology of HFpEF is the endothelium, whose dysfunction leads to abnormal ventricular-vascular coupling, pulmonary hypertension, renal dysfunction, and exercise intolerance. (Lim et al, 2015b; Lim and Lam, 2016) Mineralocorticoid can cause endothelial dysfunction by reducing NO production and oxidative stress. (Lim and Lam, 2016) However, in clinical trial ALDO-DHF, using mineralocorticoid receptor spironolactone to treat 209 HFpEF patients, there is confined improvement in LV relaxation and no overall change in outcome. (Edelmann et al, 2013) In addition, another clinical trial TOPCAT using spironolactone to treat a much larger number of HFpEF patients (n=3445), shows no benefits in these HFpEF patients. (Pitt et al, 2014) Furthermore, the RELAX trial targeting NO-sGC-cGMP signaling pathway using phosphodiesterase 5 (PDE-5) inhibitor sildenafil, does not improve the outcome of the participating HFpEF patients regarding to exercise capacity or clinical status as compared to the placebo group in 24-week treatment. (Redfield et al, 2013) It failed probably due to the fact that sildenafil can only prolong the NO/cGMP signaling by blocking the degradation of cGMP in which NO bioavailability is decreased in the heart in the first place. Despite the disappointing results from the RELAX trial, NO donors, such as nitroprusside, has shown beneficial effects on LV diastolic function and peak systolic pressure. (Paulus et al, 1994) Also, a recent small clinical trial among 17 HFpEF patients showed significantly improved exercise capacity, increased peak oxygen consumption, improved endothelial function, and attenuation of arterial wave reflections, following a single dose of inorganic nitrate before exercise. (Zamani et al, 2015) Besides focusing on improving endothelial function, the RALI-DHF clinical trial targeting to decrease the late Na⁺ current and to improve diastolic function using acute infusion of ranolazine in HFpEF patients improves hemodynamics, although no actual improvement in LV relaxation is observed. (Maier et al, 2013)

Many of these clinical trials show negative or neutral results in treating diastolic dysfunction and HFpEF. One of the major reasons is that the fundamentals of HFpEF pathophysiology is still unclear. Studies from the past few years redirect the focus onto endothelial dysfunction, microvascular rarefaction, and oxidative stress, which are the central players in the pathophysiology of HFpEF. (Lim et al, 2015b; Lim and Lam, 2016) Our study demonstrate that inhibition of prolyl hydroxylase (PHD) by dimethyloxalyl glycine (DMOG) and reprogrammed cell metabolism ameliorate the pre-existing coronary dysfunction and diastolic dysfunction in both global SIRT3 deficient mice and endothelial-specific SIRT3 deficient mice, which provides a potential therapeutic strategy for patients with diastolic dysfunction associated with coronary microvascular dysfunction, especially in the aging population with reduced SIRT3 (He et al, minor revision). In addition, there are other promising therapeutic targets and strategies being discovered which have been shown to be effective in treating diastolic dysfunction in animal models, such as tetrahydrobiopterin (BH₄) and mitochondria-targeted antioxidants. (Jeong and Dudley, Jr., 2015) We could expect these findings will benefit the development of novel therapies.

Conclusion

Compelling evidence has demonstrated that SIRT3 has great potential to be one of the therapeutic targets for cardiovascular, metabolic, and other aging-related diseases due to its involvement in the regulation of a variety of cellular processes, including metabolism, antioxidant defenses, and apoptosis. Our studies demonstrated the critical role of SIRT3 in endothelial metabolism, angiogenesis, and cardiac function. The gravity of the cardiac dysfunction seems to be cell type-dependent: 1) endothelial deletion of SIRT3 induced HFpEF phenotype due to endothelial dysfunction (Figure 2). SIRT3 ECKO mice developed diastolic dysfunction ahead of the development of systolic dysfunction; whereas 2) global knockout of SIRT3 resulted in HFrEF phenotype that is explained by mitochondrial dysfunction and oxidative stress-induced cardiomyocyte death followed by replacement of myocardial fibers by fibrosis (Figure 3). This phenotypic difference between SIRT3 KO mice and SIRT3 ECKO mice indicates SIRT3 is also important in EC/cardiomyocyte interaction. Moreover, induction of hypoxia tolerance and reprogrammed cell metabolism by PHD inhibition may be a potential therapeutic strategy for the treatment of cardiovascular diseases in the aging population with reduced SIRT3.

However, the effect of SIRT3 on the cardiac system is much complex than what people's original thoughts. There are still many unanswered questions regarding the regulation of SIRT3 and the function of SIRT3 in cardiovascular system. The knowledge of endogenous regulators of SIRT3 is still poor, except for NAD⁺ and peroxisome proliferator activated receptor γ coactivator-1 α (PGC-1 α) mediated activation of SIRT3 during caloric restriction and estrogen related receptors (ERRs) mediated expression of SIRT3.(Finley et al, 2012b;Kong et al, 2010) In addition, little is known about the posttranslational regulation of SIRT3. According to the study of Schlicker *et al.* structural relationship between N-terminal and C-terminal modulates SIRT3 activity, in which the N-terminal may function as an autoinhibitory region, and cleavage of N-terminal at residue 113 results in higher enzymatic activity than when cleaved at residue 101.(Schlicker et al, 2008) Recently, by using a high-resolution mass spectrometry-based phosphoproteome analysis, Olsen and colleagues identified six phosphorylated serine residues between amino acids 101 and 118 which are close to the mitochondrial cleavage site, which may play a role in regulating proteolytic cleavage by MPP and the interaction between N-terminal and C-terminal that modulates catalytic activity of SIRT3.(Olsen et al, 2010) However, their biological relevance or influence on SIRT3 function has not been analyzed yet, and further investigations are needed to address the molecular mechanisms mediating the beneficial effects in the heart.

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Figure 1. Overview of the endogenous regulators and molecular targets of SIRT3. SIRT3 is activated and regulated by calorie restriction, NAD⁺ level, PGC-1α, and ERRα. Then SIRT3 activates several metabolic and stress-related pathways including mitochondrial respiration, antioxidant, apoptosis, and fibrosis.

HFpEF phenotype-endothelial SIRT3 deficiency

Figure 2. Working model of endothelial cell specific SIRT3 ablation in HFpEF.

In HFpEF, myocardial dysfunction and remodeling are driven by endothelial dysfunction. Loss of SIRT3 induces production of ROS, which diminishes NO bioavailability in cardiomyocytes. Loss of SIRT3 also impairs expression PFKFB3 and glycolytic energy production, which shifts ECs from oxygen-independent metabolism to highly oxygen-consuming metabolism and thus increases oxygen demand and decreases oxygen delivered to cardiomyocytes in coordination with microvascular rarefaction. Moreover, inhibition of PFKFB3 leads to decreased angiogenic capacity and a pro-inflammatory state. EC: endothelial cell; CM: cardiomyocyte.

HFrEF phenotype-cardiomyocyte SIRT3 deficiency

Figure 3. Working model of global SIRT3 deletion in HFrEF.

In HFrEF, myocardial dysfunction and remodeling are driven by cardiomyocyte dysfunction and death. Loss of SIRT3 impairs mitochondrial and contractile function due to myocardial energy depletion. Increased production of ROS resulted from loss of SIRT3 and hypoxia due to microvascular rarefaction may induce cardiomyocyte apoptosis which is replaced by fibroblast and fibrosis. In addition, decline of ANP may further decreases systolic function. EC: endothelial cell; CM: cardiomyocyte.