Circulation Journal
Official Journal of the Japanese Circulation Society
http://www.j-circ.or.jp

REVIEW

Mitochondrion as a Target for Heart Failure Therapy
– Role of Protein Lysine Acetylation –

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Heart failure is a leading cause of death worldwide. Despite medical advances, the dismal prognosis of heart failure has not been improved. The heart is a high energy-demanding organ. Impairments of cardiac energy metabolism and mitochondrial function are intricately linked to cardiac dysfunction. Mitochondrial dysfunction contributes to impaired myocardial energetics and increased oxidative stress in heart failure, and the opening of mitochondrial permeability transition pore triggers cell death and myocardial remodeling. Therefore, there has been growing interest in targeting mitochondria and metabolism for heart failure therapy. Recent developments suggest that mitochondrial protein lysine acetylation modulates the sensitivity of the heart to stress and hence the propensity to heart failure. This article reviews the role of mitochondrial dysfunction in heart failure, with a special emphasis on the regulation of the nicotinamide adenine dinucleotide (NAD^+/NADH) ratio and sirtuin-dependent lysine acetylation by mitochondrial function. Strategies for targeting NAD^+-sensitive mechanisms in order to intervene in protein lysine acetylation and, thereby, improve stress tolerance, are described, and their usefulness in heart failure therapy is discussed. (*Circ J* 2015; **79:** 1863–1870)

Key Words: Heart failure; Lysine acetylation; Mitochondria

Cardiovascular disease has become a leading cause of death worldwide.[1] As life expectancy improves and the mortality associated with acute ischemic events decreases, an increasing number of patients with ischemic heart disease and/or hypertension will develop heart failure (HF)[2,3] as the heart is remodeled in response to hemodynamic overload and neurohormonal stimulation.[4] Despite medical advances in the past decades, patients with late-stage HF have a 1-year survival rate of <50%. Novel concepts and strategies in the treatment of HF are urgently needed. There has been a growing interest in targeting mitochondrial function and cell metabolism for HF therapy in recent years. It is known that mitochondria play a multifaceted role in regulating cellular function and cell fate. Mitochondrial oxidative metabolism provides >95% of the energy for the cardiac contraction, while mitochondrial respiration is the major source of reactive oxygen species (ROS) in the cell. Mitochondria also regulate cellular calcium homeostasis and cell death. The delicate balance between calcium, ROS and ATP generation has been proposed as a critical determinant of cardiac response to stress.[5] With the surge of interest in the role of mitochondria and metabolism in HF, many advances have been made in the past decade. In this article, we will review the recent findings in this area that have suggested potential therapeutic targets for HF. In particular, we will focus on the emerging role of mitochondrial protein acetylation and its link to the hallmarks of HF, and discuss therapeutic strategies involving targeting the pathways mediated by nicotinamide adenine dinucleotide (NAD^+)-sensitive lysine acetylation (LysAc).

Mitochondrial and Metabolic Derangements in HF

Disrupted calcium homeostasis, increased oxidative stress and impaired energetics are the hallmarks of the failing heart.[6–8] These abnormalities are closely linked to mitochondrial function, given the central role of mitochondria in energy supply, ROS generation and scavenging, and in calcium handling. An increasing number of studies have identified the important roles that mitochondrial biogenesis,[9,10] oxidative metabolism[11] and antioxidant capacity[12] play in the pathogenesis and progression of HF. Furthermore, mitochondria-triggered cell death, especially under calcium overload conditions, is considered to play a key role in the pathological remodeling of the heart.[13,14] These observations have advanced our understanding of the role of mitochondria and metabolism in HF, and have provided opportunities for novel therapeutic targets, and at the same time have raised new questions regarding the disease mechanisms of HF.

Substrate Metabolism and Myocardial Energetics

The heart is flexible and omnivorous in using substrates for ATP generation.[15–17] Catabolism of all substrates in the mitochondria, that is, carbohydrates, fatty acids, and ketones, generates acetyl-coenzyme A (acetyl-CoA), which enters the

Received July 2, 2015; accepted July 3, 2015; released online August 4, 2015
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ISSN-1346-9843 doi:10.1253/circj.CJ-15-0742

tricarboxylic acid (TCA) cycle and produces the reducing equivalents NADH/$FADH_2$. Reducing equivalents are oxidized via electron transport chain (ETC) in order to maintain the mitochondrial membrane potential ($\Delta\Psi$) and ATP synthesis. In the failing heart, impaired myocardial energetics is evidenced by lower phosphocreatine (PCr), the energy reserve compound, in the early stage, and by lower ATP content in the advanced stage.[18–20] Decreased PCr/ATP ratio is a predictive marker of HF mortality.[20] Multiple mechanisms have been proposed for defective energetics of the failing heart, including impaired intracellular energy transfer through the creatine kinase system,[8,21] defective mitochondrial biogenesis,[22] and decreased oxidative metabolism,[23] in particular, a switch from fatty acid oxidation to reliance on glucose metabolism. While normal adult hearts primarily consume fatty acids for ATP generation, hypertrophic and failing hearts have an increased reliance on glucose and a decreased use of fatty acids.[24,25] This metabolic remodeling involves enhanced glycolysis and uncoupling of glucose oxidation from glycolytic flux.[26–29] Prior attempts to normalize substrate metabolism have targeted both glucose and fatty acid metabolism. Promotion of glucose oxidation using a variety of strategies, such as by inhibition of carnitine-palmitoyl transferase,[30] the rate-limiting enzyme for fatty acid entering the mitochondria; by partial inhibition of fatty acid β-oxidation;[31] inhibition of pyruvate dehydrogenase kinase,[32] the key inhibitor of glucose oxidation; or overexpression of insulin-independent glucose transporter,[33] improved cardiac function in pathological hypertrophy and HF. In contrast, promotion of fatty acid oxidation, which prevents the shift of substrate utilization toward glucose, also improves cardiac energetics and function in hypertrophy models,[11,34,35] while decreased fatty acid supply to the failing heart by acipimox is detrimental.[36] The role of substrate metabolism in HF has been discussed in detail in several recent reviews.[23,37,38]

Oxidative Stress and Redox Balance

The mitochondrial ETC is the major source of ROS in the cell. When the heart is stimulated for higher contractile performance, increased respiratory activity in the mitochondria would result in greater ROS load. It has been proposed that in failing hearts superoxide generated by the electron leakage from the ETC to molecular oxygen is not adequately scavenged and hence causes damage to biomolecules.[7] A normal mitochondrion is equipped with robust antioxidant systems such as the superoxide dismutase (SOD) and the glutathione detoxification system. SOD turns superoxide into hydrogen peroxide (H_2O_2), which is further detoxified by the glutathione system. Antioxidant capacity of the glutathione system is maintained by NADPH, governed by isocitrate dehydrogenase (IDH2) and $\Delta\Psi$-dependent NAD(P) transhydrogenase (NNT).[39] IDH2-deficient mice develop cardiac hypertrophy and contractile dysfunction[40] and NNT activity is lowered in human failing hearts, indicating oxidative damage.[41] In addition to the level of IDH2 and NNT, decreases in $\Delta\Psi$ in mitochondria would reduce NNT activity and thus weaken oxidative defense capability. Other sources of ROS in the mitochondria have also been proposed, such as NADPH oxidase 4, although its role seems to be location and dose dependent.[42,43] The role of mitochondrial ROS in the development of HF was recently demonstrated by overexpressing mitochondrial-targeted catalase in the heart or treating mice with mitochondrial-targeted antioxidant peptide. These studies showed marked cardioprotective effects of scavenging mitochondrial ROS in pathological hypertrophy and aging.[12,44,45] Given that clinical trial results for antioxidants are mostly negative,[46] these observations suggest that intracellular bioavailability of the antioxidant and, in particular, its bioavailability in the mitochondrial compartment, can be critical for the efficacy of the treatment.

Calcium Homeostasis and Mitochondria-Triggered Cell Death

Calcium is a second messenger in the heart: it connects muscle contraction/relaxation in the cardiac cycle and mitochondrial energy production.[47,48] Rhythmic control of intracellular calcium is not only critical for excitation-contraction (E-C) coupling but is also important for matching energy demand with metabolic activity on a beat-to-beat basis. It has been shown that mitochondria are localized in close proximity to the sarcoplasmic reticulum (SR), so that during E-C coupling a high concentration of calcium is presented to the mitochondria in anticipation of increased energy demand.[49–51] In HF, defective calcium handling due to impaired calcium re-uptake via SR calcium ATPase or calcium leak via the ryanodine receptors interrupts the rhythmic control of intracellular calcium.[6,52] Activation of Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) in failing hearts also promotes calcium leak and causes mitochondrial damage.[53–60] In addition, recent data suggest that CaMKII regulates mitochondrial calcium level and promotes the opening of mitochondrial permeability transition pore (mPTP).[61] Calcium overload is an important trigger of mPTP opening and cell death during ischemia/reperfusion injury. In HF, mitochondrial dysfunction caused by calcium overload would further exacerbate calcium homeostasis as the cycling of intracellular calcium through its stores consumes ATP, thus creating a vicious cycle connecting calcium, mitochondrial bioenergetics and mPTP opening. Mitochondria-originated cell death has been observed in the development of HF;[13] its link to mitochondrial bioenergetics is an emerging area of research.

Protein LysAc: Emerging Role in Cell Metabolism

LysAc is an evolutionarily conserved protein modification found in the whole range of organisms from bacteria to humans, which regulates the fundamental functions in cells.[62] LysAc is found in nuclear histone proteins as well as in non-histone proteins from the nucleus, cytosol and mitochondria. LysAc in histone proteins plays an important role in regulating gene transcription.[63] LysAc in non-histone proteins is likely critical in the regulation of cell metabolism and signaling. This is based on the observation that a large number of proteins involved in metabolism have dynamic changes in LysAc, although the specific effects of LysAc on their function are still emerging.[64–68] Furthermore, LysAc is dependent on the availability of acetyl-CoA,[69] which is intimately involved in the intermediary metabolism. Thus, LysAc is considered an important sensor and regulator of cell metabolism.[70]

Biochemistry of LysAc and Deacetylation

Protein LysAc occurs when an acetyl group is added to a lysine residue by non-enzymatic chemical modification with acetyl-CoA, or by enzymatic acetylation with acetyltransferases (**Figure 1**). Lysine residues in both histone and non-histone proteins are subject to this type of modification. The process is affected by the cellular level of acetyl-CoA and/or the activity of acetyltransferases. Detailed information on the enzymes and substrates involved in protein LysAc is summarized in **Table**.[69–83] While the presence of histone acetyltransferases (HAT) in the nucleus is well established, the mode of acetyl group transfer for non-histone proteins is complex.

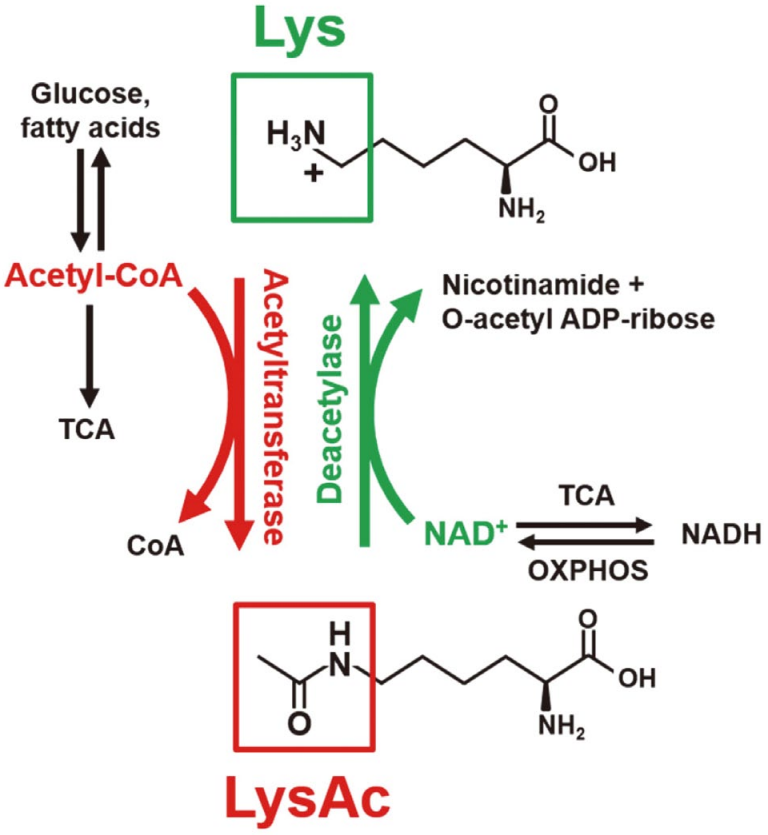

Figure 1. Regulation of protein lysine acetylation (LysAc) is closely linked to metabolism. LysAc is determined by chemical reactions involving deacetylases and acetyltransferases. The co-substrates in both reactions, acetyl-CoA and nicotinamide adenine dinucleotide (NAD^+), are key intermediates of many metabolic pathways and are important regulators of LysAc. The level of Acetyl-CoA is regulated by the balance of substrate catabolism, anabolism, and tricarboxylic acid (TCA) flux. The availability of NAD^+ for deacetylation is regulated by NAD^+ redox balance, governed by activities of the TCA cycle and oxidative phosphorylation (OXPHOS).

Table. Regulators of Protein LysAc and Their Biological Effects

Regulators	Biological effects	References
Acetylation		
Acetyl-CoA	Serves as a substrate for enzymatic catalysis of acetylation or as a direct modifier of lysine. Acetyl-CoA is regulated by glucose and fatty acid metabolism, which produce and consume acetyl-CoA, respectively.	69–71
Acetyl-transferases	HAT: nuclear localized enzymes that are linked to transcriptional activation. Non-histone targets in cytosol are reported.	63,72
	Mitochondrial protein acetyl-transferase: GCN5L1 was suggested to be responsible for enzymatic addition of the acetyl group to lysine in mitochondria.	71
Deacetylation		
Deacetylases	HDAC: class I, II, IV deacetylases, which are NAD^+ independent. Inhibition of HDAC protects the heart from ischemic and hypertrophic stresses. Sirtuins are class III NAD^+-dependent deacetylases.	73–75
NAD^+ precursors	NMN, NA, and NR: increase cellular NAD^+, activate sirtuin and reduce protein acetylation in mouse models. Supplementation of NAD^+ precursors has been shown to be beneficial in combating inflammation, hypoxia injury, oxidative stress and cancer.	76–79
NAMPT activator	P7C3: elevates NAD^+ to protect mice from brain injury.	80–82
Resveratrol	Activates Sirt1 and promotes PGC1α deacetylation and mitochondrial biogenesis.	83

CoA, coenzyme A; GCN5L1, general control of amino acid synthesis 5-like 1; HAT, histone acetyltransferase; HDAC, histone deacetylase; LysAc, lysine acetylation; NA, nicotinic acid; NAD, nicotinamide adenine dinucleotide; NAMPT, nicotinamide phosphoribosyltransferase; NMN, nicotinamide mononucleotide; NR, nicotinamide riboside; P7C3, 3,6-Dibromo-α-[(phenylamino)methyl]-9H-carbazole-9-ethanol; PGC1α, peroxisome proliferator-activated receptor γ coactivator 1-α.

Enzyme-independent acetylation by acetyl-CoA in mitochondria has been proposed.[69] The acetyltransferase GCN5L1 was found to add acetyl groups in mitochondria.[71] In cytosol, acetyl group transfer can also be facilitated by HAT.[72]

Removal of the acetyl group from lysine is mediated by deacetylases (Figure 1). There are four classes of deacetylase, including histone deacetylases (HDAC; class I, II, IV, NAD^+ independent) and sirtuin deacetylases (class III, NAD^+ dependent).[84] Like HAT, HDAC also have non-histone protein targets.[73] The sirtuin family consists of 7 members, of which Sirt1

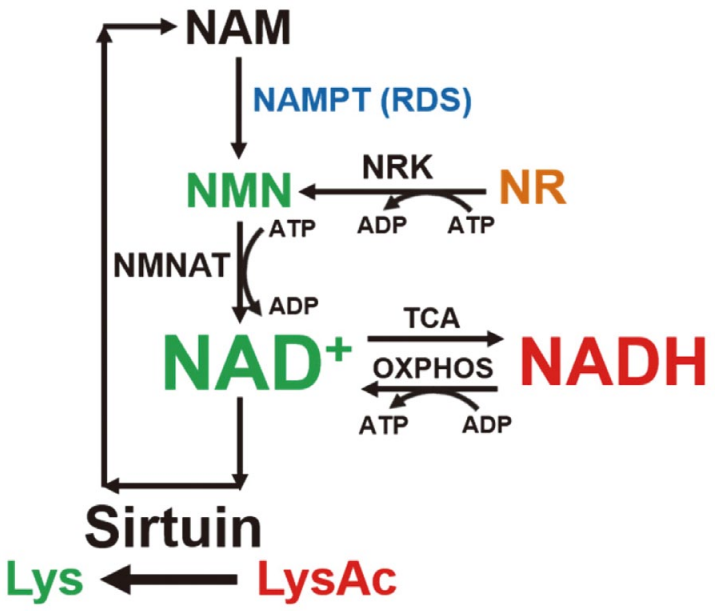

Figure 2. The level of nicotinamide adenine dinucleotide (NAD^+) is regulated by the activity of sirtuin deacetylases, NAD^+ salvage pathway and NAD^+-coupled metabolism. Sirtuins catalyze deacetylation at the expense of NAD^+ which is replenished in the NAD^+ salvage pathway. Nicotinamide (NAM), generated in the deacetylation reaction by sirtuin, is converted to nicotinamide mononucleotide (NMN) by nicotinamide phosphoribosyltransferase (NAMPT), the rate-determining step (RDS) of the pathway. Alternatively, NMN is synthesized from nicotinamide riboside (NR) and ATP by nicotinamide riboside kinase (NRK). NMN and ATP are converted to NAD^+ by nicotinamide mononucleotide adenylyltransferase (NMNAT), which has 3 isoforms localized at different subcellular compartments. The NAD^+ redox balance in the mitochondria is regulated by the tricarboxylic acid (TCA) cycle and oxidative phosphorylation (OXPHOS).

and 2 are responsible for protein deacetylation in the nucleus and cytosol,[85,86] while Sirt3 is mainly localized to the mitochondria.[87] Other sirtuins, although similar in structure, have very low deacetylase activity.[88] The deacetylation function by sirtuins requires NAD^+ as a co-substrate,[89] and it has been shown that NAD^+/NADH ratio affects sirtuin activity in vivo and in vitro.[90–92] Because of its dependence on acetyl-CoA and NAD^+/NADH ratio, LysAc is closely coupled to cellular metabolism and redox state (**Figure 1**). Consistent with this, proteomic studies have shown dynamic changes in cellular LysAc landscape in response to metabolic intervention.[93–95] Furthermore, LysAc of a significant number of mitochondrial proteins have been shown, many of which are implicated in the regulation of energy metabolism, oxidative stress and calcium homeostasis.[65,96–98]

NAD^+/NADH Ratio as a Link Between Mitochondrial Function and LysAc

As mentioned in the previous section, sirtuins are NAD^+-sensitive deacetylases; their activity is linked to NAD^+-coupled metabolism. NAD^+ is best known as an electron carrier, which receives an electron to become NADH and is readily recycled after the transfer of the electron to other factors. Thus the cellular level of NAD^+ is determined by the total pool size of NAD^++NADH, as well as the redox state, which determines NAD^+/NADH (**Figure 2**). Enzymatic removal of the acetyl group by sirtuins utilizes NAD^+ and generates nicotinamide (NAM) and O-acetyl ADP-ribose. NAD^+ is regenerated via the salvage pathway in 2 steps (**Figure 2**). Nicotinamide phosphoribosyltransferase (NAMPT), the rate-limiting enzyme of the pathway, catalyzes the conversion of NAM into nicotinamide mononucleotide (NMN). The NMN is converted to NAD^+ by nicotinamide mononucleotide adenylyltransferase. Another source of NMN is from nicotinamide riboside (NR) via reaction with nicotinamide riboside kinase.[79] NR is a vitamin B3,[99] and is found in milk.[79] NR supplementation has been found to elevate cellular NAD^+.[79]

In the mitochondria, NAD^+/NADH ratio is determined by the balance between substrate metabolism, which generates, and oxidative phosphorylation, which consumes, NADH. The ratio changes drastically during acute mitochondrial stress such as hypoxia or uncoupling.[100–102] Recent studies have shown that NAD^+/NADH ratio decreases due to the chronic stress of overnutrition, such as in obesity and diabetes, or due to impaired oxidative phosphorylation caused by mutations of mitochondrial protein(s).[79,92,103,104] Under these conditions, decreases in NAD^+/NADH ratio are coupled with increases in mitochondrial LysAc. Both can be normalized by increasing NAD^+ via supplementing its precursor.[78,79] Although mitochondrial dysfunction has been implicated in HF, whether changes in NAD^+-sensitive LysAc occur in the failing heart has not been reported.

Emerging Links Between LysAc and HF

Sensitivity to Cardiac Stresses

The Sirt3-deficiency mouse is a widely used model of increased mitochondrial LysAc. The heart of Sirt3-null mice is normal under unstressed condition but has higher sensitivity to acute and chronic stresses.[98,105,106] Similarly, increased sensitivity to stress is observed in several mouse models of decreased NAD^+/NADH and increased mitochondrial LysAc.[14,92,104,107] Increased LysAc in the heart of cyclophilin D (CypD)-null mice is associated with propensity to HF during pressure overload, metabolic inflexibility and mitochondrial swelling.[14,107] In a genetic model of mitochondrial complex I deficiency due to cardiac specific deletion of *Ndufs4*, decreased complex I function results in lower NAD^+/NADH ratio, increased mitochondrial LysAc, and higher sensitivity of mPTP opening.[92] These mice also developed accelerated HF when subjected to chronic stresses. Although the specific contribution of each LysAc site to the observed phenotype are largely unknown in these models, the observations collectively indicate that mitochondrial LysAc modulates the stress response. These studies also call for attention to the importance of NAD^+ redox imbalance and sirtuin-regulated LysAc in the development and progression of HF.

Metabolism and Oxidative Stress

A large number of proteins involved in energy metabolism undergo LysAc, and changes in LysAc are closely associated with alterations of cell metabolism. Increased LysAc in CypD-KO hearts[107] is associated with elevated glucose oxidation relative to fatty acid oxidation and increased propensity to HF.[14] Inhibition of complex I, III, and IV activity is associated with LysAc in Sirt3-KO mice.[108,109] In addition, LysAc has been observed in a number of proteins involved in antioxidant defense mechanisms, for example MnSOD, peroxiredoxin, NADPH-dependent IDH2 and thioredoxin reductase. The lack of Sirt3 results in increased ROS generation through suppression of MnSOD activity by LysAc,[97,110] and LysAc of IDH2 inhibits its activity, suppresses NADPH production and leads to redox imbalance.[111] Although an earlier study found that Sirt3-deficiency resulted in ATP depletion in the cells,[108] tissue ATP level was not affected in Sirt3 knockout mice.[112] These changes, despite a resemblance to the metabolic remodeling in HF, appear to be tolerated in the unstressed heart, consistent with the notion that LysAc modifies sensitivity to stress rather than impairs baseline function. Noticeably, metabolic intervention such as calorie restriction or fasting may increase the LysAc of some proteins while decreasing LysAc in the others in a tissue-specific manner.[94,95] Furthermore, elevation of the overall level of LysAc is not always associated with the same change in metabolism. For example, one study found that LysAc of long chain acyl-CoA dehydrogenase inhibited its activity and impaired fatty acid oxidation in Sirt3-KO mice,[66] while another study showed an association of increased fatty acid oxidation with higher LysAc in the heart.[103] Moreover, LysAc of the proteins regulating the mitochondria-cytosol exchange of NAD^+/NADH and acetyl-CoA, such as malate-aspartate shuttle and citrate synthase/citrate lyase, connects the fluctuations of mitochondrial metabolism and redox state to the non-mitochondrial compartments (Lee CF, Tian R, unpublished).[113] Thus, further insights into the mechanisms underlying the effects of LysAc on cell metabolism will likely depend on integrating the assessment of LysAc in the function of a given protein with an evaluation of the metabolic network.

Targeting NAD^+/NADH Ratio and LysAc for Therapy

Several strategies targeting the NAD^+-LysAc mechanism have been shown to improve stress response. Decreasing protein acetylation via the activation of sirtuins is an important mechanism associated with calorie restriction.[114] Stimulating the NAD^+ biosynthetic pathway in the absence of calorie restriction increases NAD^+ and promotes protein deacetylation by the sirtuins.[76,80] Treatment of the heart with complex I deficiency with the NAD^+ precursor NMN normalizes NAD^+/NADH ratio and restores the sensitivity of mPTP to calcium challenge.[92] NMN treatment also attenuates diet-/age-induced diabetes[76] and the effects of aging.[77] Elevating NAD^+ via another NAD^+ precursor, NR, protects mice from high fat diet-induced obesity.[79] Recently, a small molecule activator of the NAD^+ biosynthetic pathway was shown to be neuroprotective.[80]

Whether decreases in NAD^+/NADH ratio or increases in NAD^+-sensitive LysAc occur in the failing heart has not been directly determined. Previous studies have targeted histone acetylation for autophagy in the treatment of cardiac hypertrophy and ischemia/reperfusion injury.[74,75] Increased LysAc of non-histone proteins has been found in a rat model of HF and in type I diabetes mouse hearts.[115,116] Although the treatment of failing hearts with NAD^+ precursors has not been tested to date, overexpression of NAMPT, the rate-limiting enzyme in the NAD^+ salvage pathway, attenuates ischemia/reperfusion injury.[117] Additionally, activation of Sirt1-PGC1α signaling by resveratrol promotes mitochondrial biogenesis and protects against cardiac dysfunction in hypertensive and pressure overloaded animal models.[83,118,119] These data collectively demonstrate the benefits of increasing NAD^+ level and sirtuin activity, and suggest that NAD^+/NADH-sensitive LysAc is a potential target for HF therapy.

Future Directions

The literature to date supports the benefit of elevation of NAD^+ and activation of sirtuins for protection against disease susceptibility and progression associated with mitochondrial dysfunction. The efficacy of NAD^+ precursors in the treatment of HF certainly warrants further investigation. Although promising experimentally, tremendous work is required to develop the treatment regimen for human patients as well as to determine its efficacy in reversing cardiac remodeling and dysfunction in established HF. With regard to insights into the underlying mechanisms, proteomics data have identified a large number of proteins with LysAc.[70] Defining their roles in the regulation of cardiac sensitivity to stress is an important and yet challenging task. As quantitative measurements of the stoichiometry of LysAc become available,[120] future studies will be able to focus on characterizing LysAc sites that undergo dynamic changes under relevant (patho)physiological conditions. Determination of the biochemical function of LysAc sites will be important for understanding the mechanisms of mitochondrial dysfunction and identifying specific targets for therapeutics.

Acknowledgments

This work was supported in part by grants from the National Institute of Health (to R.T.), and from the American Heart Association (Postdoctoral fellowship 13POST16200007 to C.F.L.).

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