Sirtuins, Aging, and Medicine

REVIEW ARTICLE

FRANKLIN H. EPSTEIN LECTURE

Franklin H. Epstein, M.D., served the New England Journal of Medicine *for more than 20 years. A keen clinician, accomplished researcher, and outstanding teacher, Dr. Epstein was Chair and Professor of Medicine at Beth Israel Deaconess Medical Center, Boston, where the Franklin H. Epstein, M.D., Memorial Lectureship in Mechanisms of Disease has been established in his memory.*

Sirtuins, Aging, and Medicine

Leonard Guarente, Ph.D.

POPULATIONS IN DEVELOPED COUNTRIES CONTINUE TO GROW OLDER, AS medical advances allow baby boomers to march inexorably onward. Many of the most important diseases that lead to disability and death occur late in life, indicating that aging itself is a key risk factor. Recent research into the science of aging has identified genes and pathways that appear to control the aging process. This review describes one such family of antiaging genes, the sirtuins, and details progress in understanding the biology that undergirds their promise as therapeutic targets.

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OVERVIEW

In the past century, medical research has led to the development of new therapeutic agents, including antibiotics and antiviral and anticancer drugs, as well as surgical interventions, such as coronary-artery bypass grafting, among others. Since many important diseases often occur later in life (e.g., diabetes, neurodegenerative diseases, cancer, cardiovascular disease, proinflammatory diseases, and osteoporosis), aging is an important risk factor for these conditions. During the past decade, research on aging, which began in simple laboratory organisms, has identified important genes and pathways that contribute to longevity. Included among these is the family of nicotinamide adenine dinucleotide (NAD)–dependent protein deacetylases termed sirtuins.[1,2] These proteins can extend the life span in model organisms and are important in mediating the salutary antiaging effects of a low-calorie diet (calorie restriction). Mammals have seven sirtuin homologues, which perform nonredundant functions in adapting human physiology to environmental stressors, such as food scarcity. Small molecules have been identified that reportedly inhibit as well as activate sirtuins in vivo and in vitro. Whether such molecules could have antiaging properties is an interesting but open question.

HISTORY OF SIRTUINS IN AGING

Sir2 is one of a complex of proteins that mediate transcriptional silencing at selected regions of the yeast genome. Mutations that extend the replicative life span of yeast mother cells have been shown to increase the silencing activity of Sir2 at the ribosomal DNA repeats.[3-5] Although the silencing of ribosomal DNA has turned out to be an idiosyncratic feature of aging in yeast, the role of Sir2-related gene products (sirtuins) in aging appears to be universal. Sir2 orthologues slow aging in the nematode *Caenorhabditis elegans*, in the fruit fly *Drosophila melanogaster*, and in mice.[6-8] The sirtuins have been shown to have NAD-dependent protein deacetylase activity, which is associated with the splitting of NAD during each deacetylation cycle (Fig. 1).[9]

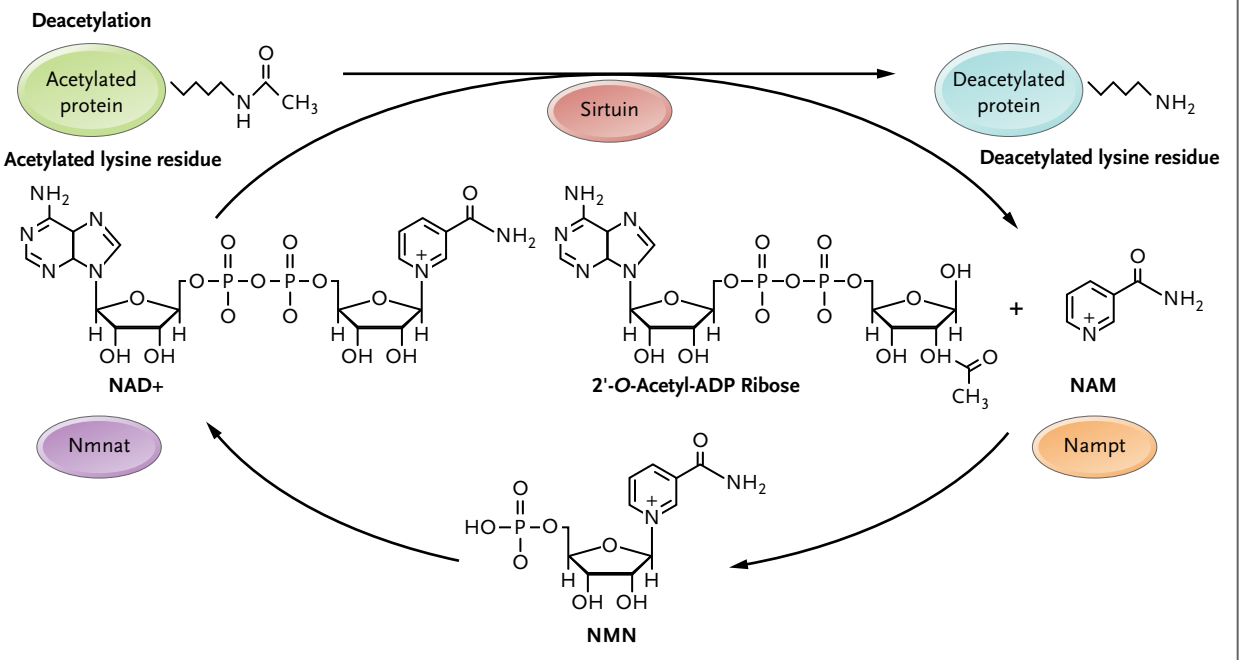

Figure 1. Enzymatic Activity of Sirtuins.

Shown is nicotinamide adenine dinucleotide (NAD)–dependent lysine deacetylation of a protein substrate by sirtuins, in which nicotinamide (NAM) and *O*-acetyl-ADP ribose are also generated. NAM can be resynthesized back into NAD in vivo by the enzymes Nampt, which generates the intermediate nicotinamide mononucleotide (NMN), and Nmnat, which generates NAD+.

Of the mammalian sirtuins, SIRT1, 2, 3, 4, 5, and 6 have been shown to have this activity.[10] Some SIRT family members (e.g., SIRT4 and SIRT6) also have ADP-ribosyltransferase activity.[1,2,10]

In mammals, the Sir2 orthologue SIRT1 is primarily a nuclear protein in most cell types and has evolved to deacetylate transcription factors and cofactors that govern many central metabolic pathways (Fig. 2). Targets of SIRT1 include transcriptional proteins that are important in energy metabolism, such as nuclear receptors, peroxisome proliferator-activated receptor-γ coactivator 1α (PGC-1α), and forkhead box subgroup O (FOXO).[11-14] SIRT1 also regulates components of the circadian clock, such as BMAL1 and PER2, which underscores the interconnectedness of protein acetylation, metabolism, circadian rhythm, and aging.[15,16] SIRT1 is also closely coupled to AMP-kinase activity in a mutually enforcing mechanism that adjusts cellular physiology for conditions of energy limitation.[17]

Other categories of SIRT1 targets relate to stress tolerance (p53, hypoxia-inducible factor 1α and 2α, and heat-shock factor protein 1),[18] DNA repair (NBS1, PARP1, Ku70, and WRN),[18] and inflammation (nuclear factor κB).[19] All told, the breadth of SIRT1 substrates indicates that its activity is poised to regulate metabolism and stress response.

SIRT1 AND METABOLIC DISEASES

One early indication that SIRT1 might be important in diseases of metabolism was the finding that the protein could influence differentiation and fat accumulation in the 3T3-L1 adipose-cell line and in primary preadipocytes in rats.[20] In a second case, calorie restriction triggered a SIRT1–PGC-1α–dependent increase in muscle mitochondrial biogenesis[21] and the activation of fatty acid oxidation by SIRT1 and peroxisome-proliferator–activated receptor α (PPAR-α),[22] which together favor insulin sensitivity and evidently a slower rate of aging-related decline. In liver, SIRT1 has been found to govern two pathways with opposing effects on gluconeogenesis. On the one hand, the

activation of PGC-1α and FOXO1 appears to favor glucose production,[12] whereas the deacetylation and destabilization of the cyclic AMP response-element–binding (CREB) coactivator CRCT2 would suppress it.[23] The relative importance of each pathway may switch as a function of the duration of fasting to fine-tune the magnitude of glucose production over time. In steady-state calorie reduction, the net effect of these pathways results in a mild elevation in glucose production.

The oral administration of the putative SIRT1-activating compounds has been shown to mitigate the prodiabetic effects of a high-fat diet without major toxic effects, which suggests that activation of SIRT1 is antidiabetic and not pharmacologically detrimental. This hypothesis was first shown with the natural product resveratrol[24,25] and was subsequently corroborated with more selective synthetic-activating compounds.[26] In the latter case, efficacy was also shown in a genetic model of murine obesity (the leptin-deficient ob/ob mouse) and in obese rats. Although these antidiabetic outcomes are consistent with known effects of SIRT1 on muscle, fat cells, and liver, recent studies have shown that this sirtuin also functions in the hypothalamus to control feeding behavior and energy expenditure.[27,28]

It has been argued that the SIRT1-activating compounds that have been described to date do not directly activate this sirtuin,[29,30] a topic that is discussed in a later section. However, it is striking that several different genetic models of SIRT1 overexpression in transgenic mice also prevent diabetes. In one model, leanness and increased insulin sensitivity were shown to occur under standard laboratory conditions.[31] In two other models, there was protection against metabolic decline induced by a high-fat diet or, more strikingly, by normal aging.[32,33] In addition, SIRT1 activation, whether chemical or genetic, has been associated with changes in genomic transcriptional patterns normally induced by calorie restriction.[34,35] All told, these genetic studies provide strong evidence that SIRT1 plays a central role in driving the phenotype of rodents toward metabolic fitness. Therefore, among the possibilities it seems most likely that the SIRT1-activating compounds work by the activation of SIRT1 in vivo (either directly or indirectly).

Studies of SIRT1 transgenic mice also suggest that activation may be protective against other diseases of aging (e.g., bone loss and an inflammation-induced model of liver cancer).[8] The anti-inflammatory effect of sirtuins may be much broader, since both SIRT1 and SIRT6 repress the activity of the major proinflammatory transcription factor, nuclear factor κB.[19,36] In other murine studies, it was found that SIRT1 may be protective against colon cancer[37] and breast cancer.[38] The fact that SIRT1 represses hypoxia-inducible factor 1α furthers its candidacy as a tumor suppressor. However, the relationship between SIRT1 and cancer may not be totally straightforward, since other studies suggest that this sirtuin may also have oncogenic properties in certain contexts.[39,40] The mitochondrial sirtuin SIRT3 (which is discussed below) has recently emerged as another interesting target for cancer therapy.

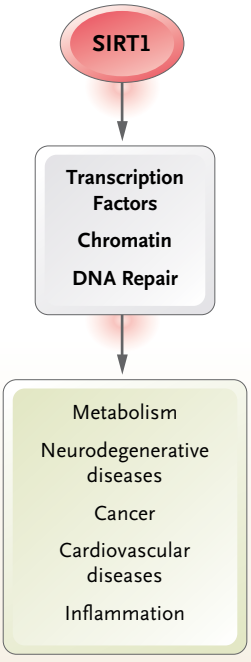

Figure 2. Categories of SIRT1 Targets for Deacetylation and Their Associated Diseases.

The protein targets of SIRT1, a sirtuin that has been shown to regulate metabolism and stress response, include transcription factors and cofactors, histones and other chromatin proteins, and components of DNA repair machinery. The depictions of the associations between SIRT1 targets and the diseases that are affected are graphical, since the exact mechanisms of most of these associations are not known.

SIRT1 AND CARDIOVASCULAR DISEASE

SIRT1 appears to possess cardiovascular protective properties beyond those deriving solely from metabolic fitness. For example, sirtuins may protect against hypertrophy in cardiac and smooth-muscle cells. Transgenic mice that overexpress SIRT1 in the heart are protected against pathological cardiac hypertrophy (Fig. 3).[41] SIRT1 also protects smooth muscle by inhibiting the expression of the angiotensin receptor AT1.[42,43] AT1$^{-/-}$ mice produce lower levels of reactive oxygen species and live longer than normal mice.[44] Further illustrating the functional interplay between SIRT1 and SIRT3, the reduction in reactive oxygen species in AT1$^{-/-}$ mice appears to be mediated by elevated levels of SIRT3.

The earliest connection between SIRT1 and endothelial cells was the finding that SIRT1 deacetylates and activates endothelial nitric oxide synthase (eNOS).[21] The activation of eNOS and repression of AT1 suggest that SIRT1 activity ought to curb high blood pressure. SIRT1 also inhibits the senescence of endothelial cells,[45] and its salutary effect on these cells may mitigate atherosclerosis. Interestingly, calorie restriction is known to protect against atherosclerosis,[46] and many of the physiological effects of calorie restriction are blunted in eNOS$^{-/-}$mice.[21] These findings all indicate that SIRT1 helps facilitate the favorable effect of calorie restriction on cardiovascular function by its effects on eNOS, AT1, and perhaps other targets.

Another function of SIRT1 that may be critical in cardiovascular disease is its regulation of fat and cholesterol homeostasis. In addition to triggering β-oxidation of fatty acids in calorie restriction,[22] SIRT1 also exerts two opposing effects on fat and cholesterol synthesis. SIRT1 deacetylates and activates the nuclear receptor liver X receptor (LXR), which up-regulates the ATP-binding cassette transporter A1 to facilitate reverse cholesterol transport from peripheral tissues.[11] Likewise, SIRT1 was also shown to deacetylate and activate the nuclear bile acid receptor farnesoid X receptor (FXR) to increase its dimerization with its partner retinoid X receptor and its activity.[47] Thus, LXR and FXR activation by SIRT1 has the potential to increase the production of high-density lipoprotein (HDL) cholesterol and protection against atherosclerosis by facilitating cholesterol removal.

But LXR also activates the gene encoding the sterol regulatory element-binding protein 1 (SREBP1) in liver to drive fat and cholesterol synthesis. Remarkably, LXR activation by SIRT1 in liver may be counteracted by an opposing SIRT1 activity, deacetylation of SREBP1 and repression of its activity.[48,49] The subtlety in control of fat synthesis is reminiscent of the control of glucose synthesis discussed above; SIRT1$^{-/-}$ mice were found to be prone to liver steatosis in some studies[22,50,51] and protected in another,[52] possibly because of differences in the experimental protocols.

Finally, the demonstrated protective effect of SIRT1 in the kidney would also be cardioprotective by aiding the control of blood pressure. In this organ, SIRT1 protects tubular epithelium[53] and medullary cells[54] and mediates kidney-protective effects of calorie restriction.[55] Although not all of the effects of sirtuin on cardiovascular health are known, this is an area with much research potential.

SIRT1 AND NEURODEGENERATIVE DISEASES

As humans are living longer, neurodegenerative diseases have become a sobering obstacle to healthy aging. It is estimated that Alzheimer's disease alone will affect up to one third of those lucky enough to win the longevity lottery.[56] Although methods to detect early stages of Alzheimer's disease have become ever more sensitive, treatment approaches remain elusive. Neuronal stress (e.g., in cultured neurons[57] or in cyclin-dependent kinase 5 transgenic mice[58]) was mitigated by the overexpression of SIRT1, prompting the question as to whether this sirtuin might restrain Alzheimer's disease.

Indeed, SIRT1 overexpression in the brain has been shown to reduce the load of β-amyloid peptide, the toxic agent that is generated by proteolytic cleavage of amyloid precursor protein in mice that overexpress two human genes that predispose to early Alzheimer's disease (Fig. 4).[59] By activating the gene encoding α-secretase, SIRT1 directed the processing of amyloid precursor protein along a pathway that avoided the production of β-amyloid peptide and thus protected against the disease. SIRT1 activated the α-secretase gene by deacetylating its transcriptional activator, the retinoic acid receptor β.

β-Amyloid peptide gives rise to a protein aggregate of plaques in the brain of patients with Alzheimer's disease. In mice overexpressing β-amyloid peptide, the level of plaques was reduced in mice that also overexpress SIRT1. Another hallmark of

Alzheimer's disease in humans is an increase in the number of aggregates or tangles of the tau protein in neurons. Indeed, β-amyloid peptide and tau have been two leading candidates for the causal agent of Alzheimer's disease.[56] In a separate study in mice,[60] SIRT1 was shown to deacetylate tau protein to destabilize it and reduce tangles (Fig. 4).

Beyond frank diseases, cognitive decline is another malady of aging. Recent studies suggested that SIRT1 also benefits learning and memory in mice[61,62] by activating the gene for brain-derived neurotrophic factor (BDNF). SIRT1 appears to potentiate activity of the BDNF transcription factor CREB and may generally promote activation of CREB target genes in the brain. Important questions remain with regard to the potential of sirtuins as therapeutic targets in the brain. Can SIRT1 affect other neurodegenerative diseases? Might other sirtuins (SIRT2, 3, 4, 5, 6, and 7) play a role in combating brain diseases of aging? Finally, will sirtuin drugs that are being developed to protect against diabetes cross the blood–brain barrier and, if not, can such drugs be developed?

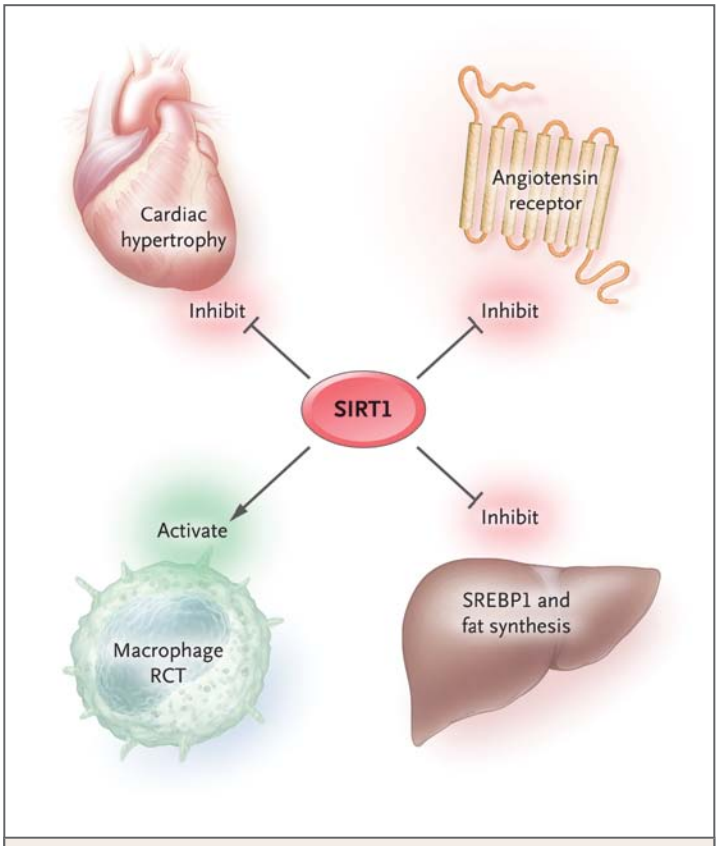

Figure 3. Four Mechanisms of SIRT1 Protection against Cardiovascular Disease.

SIRT1 appears to protect against cardiac hypertrophy in cardiac and smooth-muscle cells and also protects smooth muscle by inhibiting the expression of the angiotensin receptor AT1. Reverse cholesterol transport (RCT) is driven by SIRT1-mediated deacetylation of the nuclear receptor liver X receptor (LXR), which activates the gene encoding the sterol regulatory element-binding protein 1 (SREBP1) in liver to drive fat and cholesterol synthesis. LXR activation by SIRT1 in liver may be counteracted by an opposing SIRT1 activity through deacetylation of SREBP1 and repression of its activity.

MITOCHONDRIAL SIRTUINS — METABOLISM, CANCER, AND AGING

Studies of the mitochondrial sirtuins (SIRT3, 4, and 5) have suggested that sirtuins mediate physiologic adaptation to reduced energy consumption. All three of these sirtuins modify mitochondrial proteins governing metabolic pathways that are important in energy deprivation (Fig. 5). SIRT4 was shown to repress the enzyme glutamate dehydrogenase (by ADP-ribosylation), which determines the utilization of amino acids as energy sources.[63] In beta cells or liver, SIRT4 activity declined during calorie restriction, which allowed glutamine to feed directly into catabolic metabolism. Thus, in SIRT4$^{-/-}$ mice or in wild-type calorie-restricted mice, glutamine is a fuel source for glucose synthesis in liver and also drives insulin secretion by beta cells. Remarkably, SIRT4 depletion was also recently shown to increase fatty acid oxidation.[64] SIRT5 was shown to deacetylate and activate carbamoyl-phosphate synthase 1, the first and limiting enzyme in the urea cycle.[65,66] The demonstrated increase in SIRT5 activity during calorie restriction activates the urea cycle to facilitate the disposal of ammonia when amino acids are used as fuel sources.

SIRT3 was shown to deacetylate long-chain acyl dehydrogenase and other enzymes that are involved in β-oxidation of fatty acids, as well as the urea cycle.[67,68] A recent study in *C. elegans* also revealed the close linkage of the sirtuin SIR-2.1, fatty acid oxidation, and longevity.[69] It has been speculated that the production of ATP from catabolism of fat rather than carbohydrates may itself be protective against reactive oxygen species and aging.[70]

New findings more directly link SIRT3 and the production of reactive oxygen species. SIRT3$^{-/-}$ cells produce increased levels of reactive oxygen species and have a concomitant reduction in ATP production.[71,72] This finding suggests that SIRT3 may deacetylate components of the electron transport chain to render oxidative phosphorylation more efficient and less affected by reactive oxygen

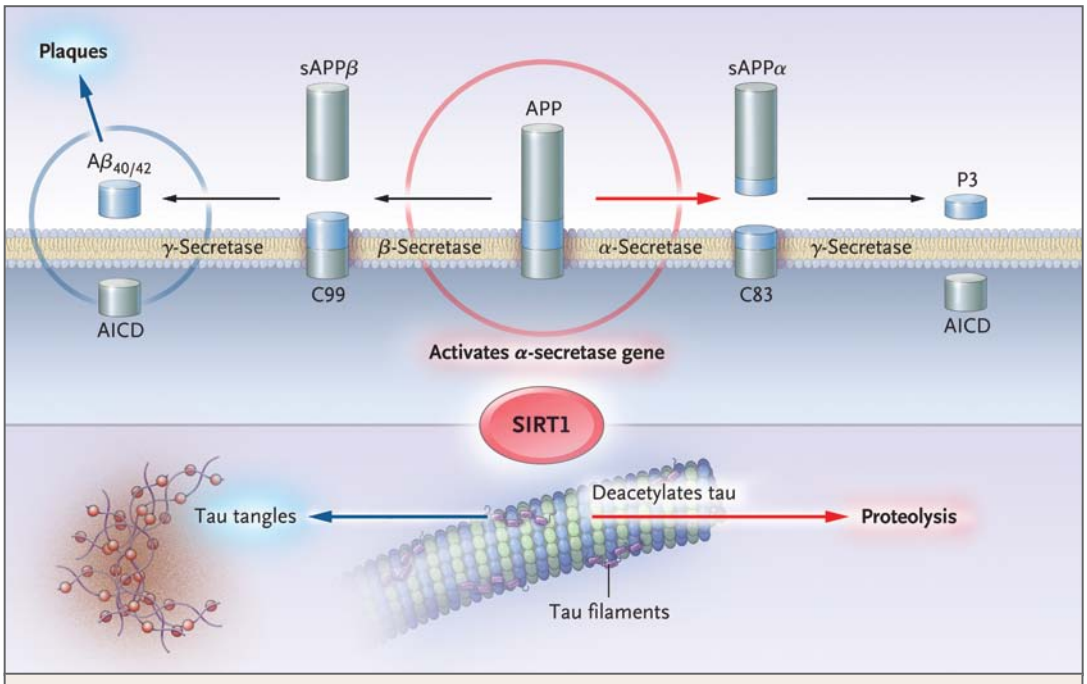

Figure 4. Two Mechanisms of SIRT1 Protection against Alzheimer's Disease.

SIRT1 directs cleavage of amyloid precursor protein (APP) away from production of the toxic β-amyloid peptide (Aβ) by activating the gene encoding α-secretase. It also deacetylates tau for ubiquitination and proteasomal destruction, thereby reducing tangles. Fragments sAPPβ and C99 are produced by β-secretase cleavage of APP, and fragments sAPPα and C83 are produced by α-secretase cleavage of APP. AICD is the APP intracellular domain produced by γ-secretase cleavage of C99 and C83.

species. Indeed, SIRT3$^{-/-}$ mitochondria show increased acetylation of electron transport chain (ETC) components and reduced activity of ETC complexes I and III in isolated mitochondria.[71,73] Moreover, SIRT3 was also recently shown to deacetylate and activate mitochondrial superoxide dismutase 2 (SOD2)[74,75] and isocitrate dehydrogenase 2 (Idh2),[76] the latter of which generates NADPH for the glutathione pathway of detoxification of reactive oxygen species in mice.

An example of the potential link between SIRT3 and aging was shown in a murine model of hearing loss.[76] By 12 months of age, wild-type mice showed complete hearing loss, which is triggered by oxidative damage in the spiral ganglia neurons and sensory hair cells in the cochlea. Hearing loss and oxidative damage were completely prevented by calorie restriction in these mice. However, SIRT3$^{-/-}$ mice were resistant to the protective effects of calorie restriction against hearing loss and oxidative damage. If this effect of SIRT3 extends to other neuronal types or more broadly to non-neuronal tissues, SIRT3 activators may be the simplest and most direct way to counteract aging itself by triggering mechanisms resembling those of calorie restriction. For example, SIRT3 was shown to protect cardiovascular function by suppressing the production of reactive oxygen species in endothelial and cardiac cells.[77]

Finally, the ability of SIRT3 to suppress reactive oxygen species suggests that it may be involved in tumor suppression. Indeed, SIRT3$^{-/-}$ mice generate more reactive oxygen species and are more susceptible to mammary tumors than normal mice.[72] Recently, SIRT3 has been linked to the metabolic reprogramming in cancer cells termed the Warburg effect, which enforces the glycolytic production of ATP. The reactive oxygen species that are produced when SIRT3 activity is suppressed activate nuclear hypoxia-inducible factor 1α,[78,79] which turns on genes for glycolysis and angiogenesis. Indeed, enforced SIRT3 expression reversed the Warburg effect in many tumor-cell lines.[79] Moreover, a large percentage of human tumors (up to 40% of breast and ovarian cancers) show inactivation of SIRT3.[72,79] It will be

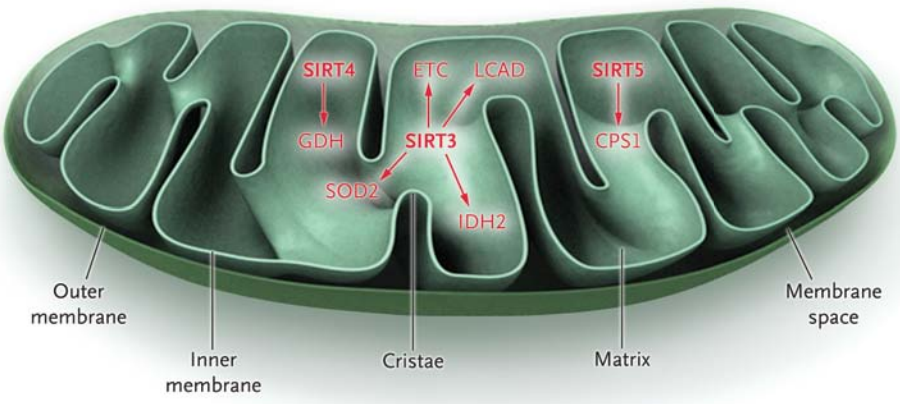

Figure 5. Three Mitochondrial Matrix Sirtuins.

SIRT4 represses the enzyme glutamate dehydrogenase (GDH) (by ADP-ribosylation) to regulate amino acid catabolism for energy. SIRT3 deacetylates long-chain acyl dehydrogenase (LCAD) to mediate β-oxidation of fatty acids. SIRT3 also deacetylates and activates mitochondrial superoxide dismutase 2 (SOD2), isocitrate dehydrogenase 2 (IDH2), and components of the electron transport chain (ETC) to suppress reactive oxygen species. SIRT5 deacetylates carbamoyl-phosphate synthase 1 (CPS1) to activate the urea cycle.

critical to further validate the possibility that the loss of SIRT3 is a major determinant of the Warburg effect and cancer.

SIRTUIN-ACTIVATING DRUGS

This section will focus on small-molecule activators of SIRT1 rather than inhibitors, which have also been described.[80] The first studies of chemical activators of sirtuins focused on SIRT1. The polyphenols, such as resveratrol, were identified by screening compounds for boosting the deacetylation of synthetic peptidyl substrates labeled with a chemical fluorophore group.[81] Subsequently, chemically distinct classes of SIRT1 activators that had higher potency than polyphenols were identified with the use of a different assay with fluorophore-containing peptides.[26] Both polyphenols and newer activating compounds were shown to increase the binding affinity of SIRT1 for the peptide substrates used.

Questions were first raised regarding the mechanism of activation by resveratrol since there was a lack of activation with the use of nonfluorophore-containing peptides.[29] It was subsequently reported that the nonpolyphenolic compounds also did not activate SIRT1 on peptide substrates lacking the fluorophore.[30] These findings led to the proposal that the activating compounds interacted with the fluorophore and that their effects in vitro and in vivo were not mediated by SIRT1. A second school of thought proposed that activators such as resveratrol might target other proteins in cells (e.g., AMP kinase[82]) to affect SIRT1 indirectly. Relevant to this idea, resveratrol has been shown to inhibit mitochondrial ATP synthase,[83,84] although such an effect may not occur at moderate yet efficacious concentrations. Finally, a more recent study responded to these challenges by showing that several classes of the nonpolyphenolic compounds can activate SIRT1 with the use of peptide substrates lacking any chemical modifications.[85] This latter study reported a mechanism that was based on allosteric modulation of SIRT1 by the drug, which was dependent on the amino acid sequence of the protein substrate.

How can these seemingly disparate findings be reconciled? The model in which the compounds do not affect SIRT1 at all seems unlikely, given the congruence of their in vivo effects and genetic activation of SIRT1. It is also inconsistent with examples in which the effects of activators in cells or mice are SIRT1-dependent.[86] To me, the most likely possibility seems to be that the compounds do bind directly to SIRT1 as described or to a component of a SIRT1 complex in cells. The

activation mechanism in vivo could involve an increase in the binding affinity for protein substrates by the allosteric mechanism mentioned above. Alternatively, the activation mechanism could relate to other proteins in cells that bind to and modulate SIRT1 activity. One interesting example is the protein called deleted in breast cancer 1 (DBC1), which is a natural inhibitor of SIRT1 activity in many cell types.[87,88] Pharmacologic activation may involve the binding of compounds to SIRT1 to release active enzyme from the SIRT1–DBC1 complex. Consistent with this idea, knocking out DBC1 increases deacetylation of SIRT1 targets in cells and elicits a phenotype resembling SIRT1 transgenic mice.[50] In any case, the intense focus on SIRT1-activating compounds should clarify mechanisms of action conclusively.

SUMMARY

Sirtuins were originally identified as antiaging proteins in model genetic organisms and have emerged as mediators of the beneficial effects of calorie restriction in mammals. The mammalian Sir2 orthologue, SIRT1, is an NAD-dependent deacetylase that is involved in many central pathways governing physiology and stress management. Genetic or pharmacologic activation of SIRT1 can benefit numerous diseases in murine models. Indeed, two different SIRT1-activating compounds are now in a diverse set of phase 1 or phase 2 human trials (ClinicalTrials.gov numbers, NCT00937326, NCT00964340, NCT01014117, NCT01018017, NCT01018628, NCT01262911, NCT01031108, and NCT01154101). Beyond SIRT1, there are six other mammalian sirtuins (SIRT2, 3, 4, 5, 6, and 7), and all may turn out to have therapeutic potential with the use of activators or inhibitors. Among these sirtuins, SIRT3 is extremely interesting, because it appears to suppress one of the contributing causes of aging itself, reactive oxygen species in mitochondria. Indeed, genetic polymorphisms in the SIRT3 promoter have been associated with extreme longevity in an Italian population,[89,90] although these studies will have to be replicated in other groups. In conclusion, sirtuins are a unique class of proteins that link protein acetylation to metabolism and exert profound effects on mammalian physiology and diseases of aging. The development of drugs that target sirtuins to treat these diseases is ongoing.

Disclosure forms provided by the author are available with the full text of this article at NEJM.org.

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