The sirtuin family’s role in aging and age-associated pathologies

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The 7 mammalian sirtuin proteins compose a protective cavalry of enzymes that can be invoked by cells to aid in the defense against a vast array of stressors. The pathologies associated with aging, such as metabolic syndrome, neurodegeneration, and cancer, are either caused by or exacerbated by a lifetime of chronic stress. As such, the activation of sirtuin proteins could provide a therapeutic approach to buffer against chronic stress and ameliorate age-related decline. Here we review experimental evidence both for and against this proposal, as well as the implications that isoform-specific sirtuin activation may have for healthy aging in humans.

Introduction

Human life spans have doubled since the 1800s, albeit not without complication. As average life expectancy increases, so does the propensity for aging-associated maladies. Part and parcel with this trend in life span, therefore, has been the quest to manage age-related disease and extend an individual’s healthy life. To date, the only successful intervention to significantly slow the aging process is caloric restriction (CR). CR extends life span in organisms ranging from Saccharomyces cerevisiae to rodents (1). In addition, CR can delay the onset of numerous diseases associated with aging, such as cancer and diabetes, as possibly evidenced in non-human primates (2, 3). This ability of CR to retard the aging process is dependent, at least in part, upon activation of the sirtuin family of proteins. In 1999 it was reported that a sirtuin protein in brewer’s yeast, silent information regulator 2 (Sir2), could extend life span in S. cerevisiae (4). Subsequent studies suggested that a similar effect occurred in worms and flies (5), and a large field of research into the age-related biology of sirtuins was cultivated. Although recent reports have questioned the role of sirtuins as direct determinants of longevity (6), the consensus is that they certainly can influence the physiological/pathological quality of life. Herein, we review the role of sirtuins in healthy aging, with particular emphasis on the implication for sirtuin-targeted therapy in protecting humans from age-related physiological decline.

Overview of the mammalian sirtuin family

There are 7 mammalian sirtuins, SIRT1–7, which are evolutionarily united by their highly conserved NAD+-binding, catalytic domain (7). Despite this commonality, however, a growing body of evidence suggests that their differences may far outweigh their similarities. This is highlighted by distinct expression patterns, catalytic activities, and — ultimately — biological functions. The mammalian sirtuins have discrete subcellular localizations, with a subset of sirtuins residing in predominantly nuclear (SIRT1, SIRT6, and SIRT7), cytosolic (SIRT2), or mitochondrial (SIRT3, SIRT4, and SIRT5) compartments (8). Most sirtuins display protein deacetylase activity, such that they deacetylate internal lysine residues that are acetylated upon their e-amino groups. Exceptions include SIRT4, which is known only for ADP-ribosyltransferase activity (9), and SIRT5, which has very effective demalonylase and desuccinylase activity and only weak deacetylase activity (10, 11).

In the case of acetylation, this reversible post-translational modification quickly manipulates a protein’s activity and regulates processes as diverse as metabolic flux and DNA repair (12). The specific activation of sirtuins, therefore, would favor an overall deacetylated protein state in the cell. Interestingly, dietary stress, such as that accompanying fasting or CR, can produce selective protein deacetylation (13–15), although dietary stress can induce protein acetylation as well. A significant portion of diet-induced deacetylation has been shown to occur through the enhanced activity of certain sirtuins (13), suggesting that stress resistance pathways activated by CR may depend, at least in part, on sirtuin deacetylase activity.

Among the mammalian sirtuins, SIRT1 has been the most extensively characterized for its role in aging. Although much of the attention has gone to SIRT1 and its protection from the onset of chronic diseases, its effect on longevity per se is as of yet unconvincing. Indeed, transgenic mice that overexpress SIRT1 have similar life spans as controls (16). In contrast, recent work with SIRT6 suggests that this sirtuin might hold the most potential for actual life span extension. Mice deficient in SIRT6 show severe metabolic defects and have shorter life spans (17), whereas SIRT6-overexpressing male mice (but not female mice) have increased life spans (18). Additionally, SIRT3 is the only sirtuin for which there is evidence for an association with human aging; some (but not all) studies have linked polymorphisms in the SIRT3 genomic locus to survival in elderly (19–22). Given the dearth of evidence for clear life span–extending effects for the sirtuin family as a whole, this Review will focus on the disease-ameliorating potential of the sirtuin protein family. The promise of sirtuin-directed therapies can be inferred from data acquired using sirtuin knockout and sirtuin-overexpressing transgenics, as well as data from putative small-molecule sirtuin activators and inhibitors, an area of intense interest and controversy (see ref. 23 for a review).

Disease states

Metabolic dysfunction

Declines in basal metabolic rate and physical activity contribute to an elevated incidence of insulin resistance, obesity, and metabolic syndrome with age (24). Activation of pathways that restore...
insulin sensitivity and improve the utilization of glucose and fatty acids would therefore be of benefit to stemming the pathologies associated with age-related metabolic dysfunction.

SIRT1. Many studies have indicated that SIRT1 is an important target for mitigating metabolic dysfunction. SIRT1 levels increase in response to CR, and the enzyme is able to deacetylate a variety of transcription factors and enzymes involved in central metabolism and energy expenditure (see ref. 25 for a review). Key among these factors is PPARY coactivator-1α (PGC-1α), a transcriptional coactivator that regulates expression of metabolic genes involved in processes such as hepatic gluconeogenesis, fatty acid oxidation, and mitochondrial biogenesis (26, 27). Of particular importance to metabolic syndrome, the deacetylation of PGC-1α by SIRT1 enhances PGC-1α activity, yielding increased expression of transporters and catabolic enzymes necessary for the uptake and utilization of free fatty acids (28). The process is most evident during exercise, which promotes the utilization of fatty acid in skeletal muscle (29). Consistent with these effects, SIRT1 promotes browning of adipose tissue by increasing the interaction between PPARγ and PRDM16 (30), a potent inducer of PGC1A expression (31).

Several transgenic models have shown that heightened SIRT1 activity protects against the metabolic derangement associated with obesity. More specifically, it was found that mild overexpression of SIRT1 in mice on a high-fat diet (HFD) and in diabetic db/db mice causes a significant reduction in blood glucose and plasma insulin levels without significant change in body weight or fat content (16, 32, 33). A portion of these effects may be due to the ability of SIRT1 to maintain β cell integrity and function. β Cell–specific overexpression of SIRT1 markedly improves insulin secretion and glucose tolerance (34). Similarly, pancreatic expression of SIRT1 diminishes β cell death that has been induced by the pro-diabetic drug streptozotocin (35). In addition, the protective effects of SIRT1 may occur through attenuation of inflammatory responses, as SIRT1 overexpression mitigates HFD-induced hepatic steatosis and adipose tissue–specific inflammation (33, 36). Conversely, hepatocyte-specific Sirt1 deletion induces hepatic steatosis and inflammation with chronic HFD feeding (37) and an age-dependent onset of hepatic steatosis with normal-diet feeding (38); this suggests that SIRT1 activation might be useful in treating non-alcoholic fatty liver disease, which shows a strong age-dependent onset.

Highlighting the promise for the therapeutic potential of SIRT1 activation are studies proclaiming beneficial effects of putative SIRT1 activators. One such small molecule, the pharmacologically engineered sirtuin-activating compound SIRT1720 (39, 40), was shown to improve the overall health and significantly attenuated the premature death of mice fed a HFD (41). Many of the hallmarks of metabolic syndrome such as insulin resistance, hepatic steatosis, and multi-organ inflammation — but not weight gain — were markedly improved by SIRT1720 supplementation. In obese humans, 30 days of supplementation with the putative SIRT1 activator resveratrol produced mild to moderate improvements in a number of different clinical parameters including systolic blood pressure, circulating cytokines, intrahepatic fat content, intramyocellular lipid content, and muscle mitochondrial oxidative phosphorylation capacity (42). Nevertheless conflicting results regarding the mechanism of action of these compounds has resulted in much controversy over their true mechanism (see refs. 43, 44 for reviews).

Additionally, some evidence suggests that reduced SIRT1 activity may be desirable for treating certain types of metabolic dysfunction. Transient knockdown of hepatic SIRT1 improved whole body insulin sensitivity and reduced elevated hepatic gluconeogenesis in two rodent diabetic models (45, 46). In mice with the LDL receptor deleted, whole body overexpression of SIRT1 improved glycemia but promoted hepatic lipid production and secretion and increased the development of atherosclerotic plaques (47). Sirt1–/– and Sirt1+/– mice were also found to have lower cholesterol levels and blunted responses to LXR agonists in another study (48). These data stress the importance of context and tissue specificity for the potential application of SIRT1-targeted therapy.

SIRT2. Current evidence suggests a role for SIRT2 in regulating adipose tissue development and function. Whereas SIRT2 expression is induced in adipose tissue during CR (49), its expression is significantly blunted in white adipose tissue of obese patients (50). Targets of SIRT2-mediated deacetylation include the FOXO family of transcription factors. SIRT2 deacetylation of FOXO1 leads to its retention in the nucleus and increases FOXO1-mediated repression of the master regulator of adipogenesis, PPARγ, culminating in an inhibition of adipocyte differentiation (51, 52). Also, cell-based assays have suggested a role for SIRT2 in promoting fatty acid oxidation in a PGC-1α–dependent manner (50). Thus, SIRT2 activation may prove to be protective against obesity, and its role in metabolic homeostasis deserves further exploration.

SIRT3. Of the mitochondrial sirtuins, SIRT3 is the most deacetylase and the best characterized (53). SIRT3 increases in adipose tissue, skeletal muscle, and liver during CR or fasting (13, 54–56), and conversely decreases in response to caloric excess (54, 57–59) or in diabetic models (60, 61). As such, SIRT3 functions to activate enzymes important during CR, such as 3-hydroxy-3-methylglutaryl-CoA synthase 2 for generation of ketones (62) and long-chain acyl-CoA dehydrogenase for oxidation of long-chain fatty acids (13). SIRT3 also deacetylates and activates components of the electron transport chain, thereby increasing oxidative phosphorylation (57, 58, 63–65). Accordingly, in Sirt3 germline knockout mice, a chronic HFD regimen results in lower energy expenditure, greater adiposity, and higher insulin resistance. Additionally, these mice develop severe hepatosteatosis, increased inflammatory cytokine levels, and higher plasma lipid levels (59). Another study found that SIRT3 deficiency results in glucose intolerance, decreased insulin signaling, and increased oxidative stress in skeletal muscle (60). Of note, patients with a functional SNP in SIRT3, specifically rs11246020, which results in a point mutation of its catalytic domain that decreases activity, have an increased susceptibility to developing metabolic syndrome (59). However, it is still unknown which tissue(s) are directly responsible for mediating the effects of SIRT3, and interest was recently refueled by a report of both muscle-specific and liver-specific Sirt3–/– mice lacking overt metabolic phenotypes (66).

SIRT4. In contrast to SIRT3, hepatic SIRT4 expression declines slightly during CR and increases in genetic models of diabetes (9, 55, 67). SIRT4 ADP-ribosylates and inactivates glutamate dehydrogenase, which results in decreased insulin release from β cells (9). Notably, this negative regulation of SIRT4 on insulin secretion opposes the positive regulation mentioned above with SIRT1. Likewise, SIRT4 negatively regulates fatty acid oxidation and respiration in hepatocytes and myocytes (67). Cumulatively, these data suggest reciprocal coordination, in which SIRT4 antagonizes the metabolically favorable effects of SIRT1 and SIRT3.

SIRT5. SIRT5 is only beginning to be appreciated for its role in metabolism. Carbamoyl phosphate synthetase 1, mitochondrial
ATP, and higher levels of oxidative stress (78). On the other hand, transgenic lines possessing high levels of SIRT1 geneity confers increased resistance to oxidative stress (78). Under these same conditions, low-level SIRT1 trans-

interstitial fibrosis, and senescence markers such as p15INK4b and p19ARF (78). Under these same conditions, low-level SIRT1 trans-

syntholysis of glycosylation and disruption of glucose homeostasis (71). Interestingly, mice with neural deletion of Sirt6 have normal glycemia but develop adult-onset obesity, again highlighting the importance of tissue specificity (72).

Conversely, SIRT6 overexpression protects from diet-induced obesity, with mice showing increased glucose tolerance and reduced fat accumulation (73). SIRT6 overexpression also highly reduces the IGF-1 signaling pathway, together leading to increased life span in male mice (18). Combined with other non-metabolic roles of SIRT6, including regulation of the inflammatory pathway (74), DNA damage response (17, 75), and genome integrity (76, 77), SIRT6 activation provides protection from the onslaught of metabolic dysfunction associated with aging (i.e., diabetes and obesity) and perhaps from aging itself.

Cardiovascular disease
Age is one of the major risk factors associated with cardiovascular disease. Part of this complex phenomenon is the deterioration of tissues that constitute the heart and its associated vasculature. This deterioration is aggravated by secondary stresses to the cardiovascular system from lifestyle changes (i.e., decreased physical activity), increases in inflammation and oxidative stress, and metabolic disorders (i.e., insulin resistance). Activation of restor-

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(CPS1), which limits the rate of the urea cycle in detoxification and disposal of ammonia, is a target of SIRT5-mediated deacetylation (68) and desuccinylation (10). As such, although SIRT5-deficient mice were not found to display overt phenotypes (69), elevated blood ammonia was detected during a prolonged fast (68), seem-

Also, activation of SIRT1 in endothelial tissues may be of benefit in the protection of endothelial cell function with age. Mice with endothelial-specific SIRT1 overexpression on an Apoe−/− genetic background exhibit attenuated development of atherosclerotic lesions (79). Conversely, SIRT1 insufficiency results in greater foam cell formation and atherosclerotic lesioning (80).

SIRT3. SIRT3 has also been proposed to play a role in aging-

related heart disease. SIRT3-deficient mice show mild cardiac hypertrophy and interstitial fibrosis at baseline and severe car-

diac hypertrophy in response to hypertrophic stimuli, whereas SIRT3-overexpressing mice are protected from similar stimuli (81). Although the exact mechanism for the anti-hypertrophic effects of SIRT3 remains unclear, some have suggested that the cardiac pathology of Sirt3 knockout mice stems from an increased activa-

tion of the mitochondrial permeability transition pore (mPTP), leading to apoptosis of cardiomyocytes (82). SIRT3 deacetylates and inhibits cyclophilin D, a regulatory component of mPTP, and cardiomyocytes from Sirt3 knockout mice show an age-
dependent increase in mitochondrial permeability transition (82). These data suggest that SIRT3 is necessary to prevent mitochondrial dysfunction and cardiac hypertrophy during aging.

SIRT7. Although SIRT7 lacks extensive characterization, the tip of the iceberg seems promising. SIRT7-deficient mice develop a lethal heart hypertrophy, and p53 has been shown to be a target of SIRT7 deacetylation, suggesting that in the absence of SIRT7, p53 activation leads to increased apoptosis in the myocardium (83). Further studies are required to examine whether other SIRT7 tar-

ggets are related to cardiovascular function.

Cancer
Based upon statistics from the National Cancer Institute, 54% of all cancer cases occur in people over the age of 65. This same cohort of individuals shoulders 70% of all cancer-related deaths. In this section, we review the literature for a potential role of sirtuin proteins in attenuating cancer susceptibility in the elderly.

SIRT1. SIRT1 expression is elevated in a wide array of cancers, including prostate cancers (84), acute myeloid leukemia (85), colon carcinomas (86), and non-melanoma skin carcinomas (87). This elevated expression suggests that SIRT1 may be a cancer promoter. Inhibition of SIRT1, therefore, could theoretically be a starting point for inducing cell cycle arrest and cause apoptosis in transformed cells. Indeed, some reports indicate that tempering the activity of SIRT1 by either genetic or pharmacological means results in cancer cell death (88–91). Further, the SIRT1 activator SRT1720 was recently reported to promote metastasis in a breast cancer mouse model (92). The proposed mechanisms for these effects are diverse and have been reviewed elsewhere (93).

Other research, however, points to a tumor suppressor role for SIRT1. Certain cancer types, such as mutant breast cancer 1, early onset–mutant (BRCA1) breast cancer cells, contain significantly lower levels of SIRT1 expression than BRCA1 wild-type cells; restoration of SIRT1 levels in these cells results in inhibition of tumor growth (94). Overexpression of SIRT1 has also been shown to reduce the growth rate of fibrosarcoma cells in a tissue allograft model (95). In other mouse models of cancer, SIRT1 can protect against the development of intestinal tumors in a beta-catenin-
driven colon cancer model (96), sarcomas, lymphomas, teratomas, and carcinomas arising from deletion of p53 (97), HFD-induced hepatocarcinomas (98), and age-associated spontaneous tumor development (98). It remains to be seen what cellular determi-
nants underlie SIRT1 being a tumor suppressor under some circumstances and an oncogenic promoter under others.

SIRT2. Mounting evidence implies a role for SIRT2 in cancer. SIRT2 is well appreciated for its regulation of cell cycle progression (99–101) and chromatin assembly (102, 103). These roles are best showcased in Sirt2 knockout mice, in which SIRT2 deficiency leads to tumorigenesis due to failed mitosis complex regulation and disrupted genomic integrity (104). However, others have shown that pharmacological inhibition of SIRT2 delays tumor growth through activation of p53 (91, 105). Further studies will be required to flesh out these conflicting results and determine the therapeutic potential of SIRT2-specific targeting in cancer.

SIRT3. Several studies indicate that SIRT3 is a tumor suppressor (see ref. 106 for review). Important among these are the findings that SIRT3-deficient mice develop mammary tumors, and embryonic fibroblasts from these mice exhibit a malignancy-prone phenotype with increased stress-induced superoxide levels and genomic instability (64). These cellular defects can be rescued by the expression of SOD2, a target of SIRT3 deacetylation that is involved in the detoxification of reactive oxygen species (107, 108). Coinciding with animal data, SIRT3 expression is decreased in many human cancers, with loss of at least one copy of SIRT3 occurring in 40% of human breast and ovarian cancers (64, 109). Nonetheless, there are also reports that SIRT3 overexpression promotes survival and enhances tumorigenesis, such as with oral cancer, emphasizing the importance of tumor type (reviewed in ref. 110).

SIRT6. Although the effect of SIRT6 on tumor formation and growth is a topic still in its infancy, current evidence suggests that SIRT6 is important in the preservation of genomic integrity upon DNA damage and stress stimuli (76, 77, 111). Thus, in addition to metabolic defects observed in Sirt6 knockout mice, these mice also have an impaired DNA damage response (17), including genomic instability upon irradiation. And although Sirt6 transgenic mice do not show a significant decrease in age-associated tumor formation (18), SIRT6 overexpression might still serve a protective role against tumorigenesis, and this avenue deserves further exploration.

SIRT7. An additional deacteylation target of SIRT7, H3K18, was recently identified (112). Importantly, hypoacetylation at H3K18 has been linked to aggressive cancer phenotypes (113). Indeed, SIRT7 knockdown reduces tumor growth of human cancer cell xenografts in mice (112). It will be important for future studies to analyze Sirt7 knockout mice for their resistance to tumor growth.

Neurodegeneration

Although the body’s organ systems experience a general decline in function with age, perhaps the most emotionally and physically devastating is the decline associated with the CNS. Impaired CNS function, with its effects on cognition, memory, hearing, balance, and motor control, can lead to a rapid loss of quality of life. The ability of sirtuins to ameliorate CNS-specific disorders is still a very inchoate area of investigation, yet its initial findings offer significant promise.

SIRT1. Progress in understanding the neurobiological benefits of SIRT1 has been focused on its ability to alter animal models of different human CNS diseases. In mouse models of Alzheimer’s disease, brain-specific knockout of Sirt1 caused a significant elevation in β-amyloid plaques and reactive gliosis (114). On the other hand, overexpression of SIRT1 caused a decrease in these parameters, possibly due to deacetylation by SIRT1 of retinoic acid receptor-β (114). Also, SIRT1 conferred neuroprotection in three different mouse models of Huntington’s disease (115, 116). Brain-specific deletion of SIRT1 exacerbated the neurotoxicity associated with mutant huntingtin protein, whereas overexpression of SIRT1 attenuated the toxicity. The neuprotective effect of SIRT1 in Huntington’s disease models appears to be mediated by the activation of multiple targets, including CREB, CREB regulated transcription coactivator 1, and FOXO3a. In a mouse model of Parkinson’s disease, overexpression of SIRT1 reduced α-synuclein aggregates, lessened gliosis, and attenuated lethality (117). Knockout of Sirt1, however, significantly increased aggregates, worsened gliosis, and hastened mortality (117). Deacetylation and activation of the heat shock factor HSF1 appears to be the major mechanism...
whereby SIRT1 confers protection against this mouse model of Parkinson’s disease. Lastly, in a mouse model of injury-induced axonal degeneration, NAD+ biosynthesis and its support of SIRT1 activity was shown to be essential in preventing axonal loss following axonal transection (118, 119).

SIRT2. Inhibition of SIRT2 by pharmacological and genetic means in invertebrate and cell culture models has suggested potential neuroprotective benefits. Two such studies demonstrated reduction of SIRT2 to be protective in fly and worm models of Huntington’s disease by increasing photoreceptor neuron survival (120) and decreasing sterol biosynthesis (121). Partial rescue of toxicity was also observed in a Drosophila model of Parkinson’s disease (122). Unfortunately, however, Sirt2 knockout mice lacked a remarkable phenotype related to neurodegeneration (123), calling into question the effect of SIRT2 on neuronal health in mammals. Interestingly, data from mice with Schwann cell–specific Sirt2 deletion or overexpression have revealed a role for SIRT2 in proper myelination of the peripheral nervous system, with implications for SIRT2 in demyelinating neuropathies (124).

SIRT3. Age-related hearing loss is a common disorder of the elderly that involves oxidative damage to neurons and ciliated cells of the cochlea. A recent study established that the ability of CR to slow the progression of age-related hearing loss is SIRT3 dependent (125). The authors suggested that SIRT3 mediates this effect through deacetylation of isocitrate dehydrogenase 2 and protection of oxidative stress–induced cell death. Whether SIRT3 exerts protection in other neuronal cell types remains to be seen.

Conclusion
Sirtuin proteins bolster stress resistance of mammalian cells by virtue of their ability to remodel metabolism, alter inflammatory responses, and enhance the ability to cope with oxidative species. Because many of these same pathways are pathologically altered in the aged, activation of sirtuins represents a feasible means for attenuating age-related decline in physiological function (see Figure 1). This directive is in part supported by promising preliminary studies with putative SIRT1 activators, such as SRT1720.

However, several major challenges need to be addressed before intelligent formulations of drugs can be made. The first is a more careful dissection of each sirtuin’s ability to ameliorate a particular pathophysiological state. Currently, there is little standardization of the disease models that are being employed to study sirtuin therapeutic biology, leading to contradictory reports with limited cross-validation of the findings. Future directions may also help discern whether there is cooperation or antagonism among the sirtuin paralogs to resolve age-related diseases. The second challenge is to more thoroughly reconcile whether there are any negative effects associated with chronic sirtuin activation, particularly in older cohorts. The vast majority of reports suggest that there are only benefits to be accrued from their activation, but a more skeptical approach may be warranted. The third major challenge is the development of more isoform-specific activators and/or inhibitors. The most effective class of sirtuin activators has only been reported for SIRT1. Nevertheless, as we learn more about the biology of other sirtuins, it has become apparent that activation of the paralogs of SIRT1 may be more beneficial under certain circumstances. In conclusion, although the optimism surrounding the therapeutic potential of sirtuins in treating age-related conditions should be cautious, the trend in encouraging literature suggests that good things are yet to come from the sirtuin family.

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