A therapeutic role for sirtuins in diseases of aging?

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The sirtuins are a group of proteins linked to aging, metabolism and stress tolerance in several organisms. Among the many genes that have been shown to affect aging in model organisms, sirtuin genes are unique in that their activity level is positively correlated with lifespan (i.e. they are anti-aging genes). Sirtuins are a druggable class of enzymes (i.e. amenable to intervention by small molecules) that could have beneficial effects on a variety of human diseases. In view of the many functions of Sir2 (SIRT1) in cells, this review focuses on its role in regulating important aspects of mitochondrial biology. Mitochondria have been linked to aging, and also to diseases of aging. Thus, sirtuins might provide a key link between mitochondrial dysfunction, aging and metabolic disease.

Therapeutic applications of sirtuins for metabolic diseases

Sirtuins are a family of related proteins that were first linked to longevity and stress tolerance in budding yeast and other lower eukaryotic organisms [1,2]. Genes encoding sirtuins are distinguished from many other genes that affect aging because they are anti-aging genes (i.e. increasing their activity extends life span). As such, in Saccharomyces cerevisiae (yeast), Caenorhabditis elegans (worms) and Drosophila melanogaster (flies), Sir2 (SIRT2) orthologs retard aging as a function of their gene dosage [3–5].

The biochemical activity of SIR2 orthologs is unique. They are NAD-dependent protein deacetylases [Class 3 histone deacetylases (HDACs)] [6,7]. NAD and NADH are involved in hundreds of metabolic (usually catabolic) reactions in cells. Thus, the biochemical activity of SIR2 led to the idea that sirtuins connected diet and metabolism to aging. It has long been known that low calorie diets, known as ‘calorie restriction’ (CR) extend the lifespan of a wide variety of organisms, including yeast, C. elegans, Drosophila and rodents [8] and it has been proposed that sirtuins might, in part, mediate this effect [9].

Sirtuins apparently mediate their life-extending effects in different organisms by targeting different pathways. For example, in yeast Sir2p deacetylates histones at several loci, including the rDNA (ribosomal DNA) locus, thereby stabilizing the rDNA from recombination and reducing the rate of formation of toxic rDNA circles [10]. These circles limit the replicative lifespan of mother cells in most yeast strains. However, in C. elegans, SIR2.1 functions as a coactivator of the forkhead transcription factor DAF-16 in a complex induced by heat or oxidative stress and mediated by 14–3–3 proteins [11]. DAF-16 can be activated to turn on target genes (such as that for super-oxide dismutase 3) by low insulin signaling, as well as by stress. SIR2.1 is required only for the stress-responsive pathway of DAF-16 activation.

In mammals, SIRT1 deacetylates many key transcription factors and co-factors, such as p53 [12], FOXO (forkhead) proteins [13,14], peroxisome proliferation activating receptor (PPAR)-gamma co-activator-1alpha (PGC-1alpha) [15] and nuclear factor-kB (NF-kB) [16] thereby affecting crucial cellular pathways involved in stress resistance and metabolism. There are several factors supporting the action of sirtuins in mediating salutary physiological effects of CR in mammals:

(i) The known effects of SIRT1 on the transcription factors and co-factors mentioned above trigger stress tolerance (p53, forkhead, Ku70/BAX [17] and metabolic changes (PGC-1α, FOXO 1, FOXO3, FOXO4) reminiscent of CR.

(ii) CR upregulates the levels of SIRT1 in several metabolic tissues, including liver, muscle and brain [18,19].

(iii) SIRT1 knockout mice fail to display a prominent phenotype of CR (i.e. increased physical activity) [20].

In addition to SIRT1, there are six other mammalian sirtuins, SIRT2–7 [21]. Broad mechanisms of action and potential therapeutic applications are summarized in Table 1. SIRT1, 2, 3, 6 and possibly 5 are NAD-dependent deacetylases, SIRT4 and 6 are ADP-ribosyltransferases, and an activity for SIRT7 has not yet been described. Sirtuin-mediated deacetylation and ADP-ribosylation are related in that both cleave NAD as the initial chemical step in the reaction cycle, as shown in Figure 1. In deacetylation, the ADP-ribosyl transfer directly participates in the removal of the acetyl group from the protein substrate to generate 2,3-O-acetyl-ADP-ribose, whereas in ribosylation, the ADP ribosyl moiety is transferred to the protein substrate [12,22–24]. Deacetylation of sirtuin substrates can inhibit or induce their activities, whereas ADP-ribosylation has only been shown to be inhibitory to date (see following section).

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we focus on the role of SIRT1 in regulating important aspects of mitochondrial biology. Mitochondria have been linked with aging and also diseases of aging, so sirtuins might provide a key link between mitochondrial dysfunction, aging and metabolic disease. An elegant study showed that elderly subjects, matched appropriately for lean and fat mass against their young counterparts, were more likely to be insulin resistant and also had a 40% reduction in oxidative phosphorylation [25]. Mitochondrial energy coupling did not change (i.e. reactive oxygen species (ROS) levels were not affected) so the drop in oxidative phosphorylation was probably owing to a reduction in either the number or function of mitochondria in the elderly. Furthermore, another study by the same group showed that the otherwise healthy offspring of patients with type 2 diabetes had impaired mitochondrial function potentially because of an inherited defect in oxidative phosphorylation [26]. Thus, any regulator of mitochondrial function might be particularly relevant to metabolic disease.

Current treatments for type 2 diabetes address only the symptoms of diabetes through lifestyle changes or drugs such as metformin, which do not directly address contributions to the disease from any underlying mitochondrial defects. Therapeutics that can increase mitochondrial biogenesis or alter the function of mitochondria might represent a novel mechanism of action to address the causes of type 2 diabetes, and other diseases of aging. In this review, we focus on connections between sirtuins and mitochondria, which might present opportunities for new strategies for treating indications that are dramatically influenced by the function of this key cellular organelle.

SIRT1

SIRT1 seems to have a significant role in mammalian metabolism. It has been shown to deacetylate and thereby

### Table 1. Mechanism of action and therapeutic potential of sirtuins

<table>
<thead>
<tr>
<th>Sirtuin</th>
<th>Mechanism of action</th>
<th>Therapeutic area</th>
</tr>
</thead>
<tbody>
<tr>
<td>SIRT1</td>
<td>NAD-dependent deacetylase, deacetylates proteins (e.g. PGC-1α)</td>
<td>Metabolic, mitochondrial, cardiovascular</td>
</tr>
<tr>
<td>SIRT2</td>
<td>NAD-dependent deacetylase</td>
<td>Cancer, neurological</td>
</tr>
<tr>
<td>SIRT3</td>
<td>NAD-dependent deacetylase, deacteylates and activates mitochondrial acteylCoA synthase</td>
<td>Metabolic, mitochondrial, cardiovascular</td>
</tr>
<tr>
<td>SIRT4</td>
<td>ADP-ribosyltransferase</td>
<td>Metabolic, mitochondrial, cardiovascular</td>
</tr>
<tr>
<td></td>
<td>ADP-ribosylates and inhibits mitochondrial glutamate dehydrogenase</td>
<td></td>
</tr>
<tr>
<td>SIRT5</td>
<td>NAD-dependent deacetylase</td>
<td>To be determined</td>
</tr>
<tr>
<td>SIRT6</td>
<td>NAD-dependent deacetylase</td>
<td>Cancer</td>
</tr>
<tr>
<td>SIRT7</td>
<td>ADP-ribosyltransferase</td>
<td>To be determined</td>
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![Figure 1. Sirtuin biochemistry. Sirtuins catalyze either ADP-dependent deacetylation or ADP-ribosylation reactions. Both mechanisms cleave NAD to release nicotinamide (NAM).](www.sciencedirect.com)
activate a crucial cofactor in mitochondrial biogenesis: PGC-1α [15]. Why is mitochondrial biogenesis beneficial? Mitochondrial activity in metabolically active tissues, such as muscle, will increase metabolic rate, drive glucose metabolism, and thereby improve insulin sensitivity. Thus SIRT1 activation is a promising strategy for treating type 2 diabetes, obesity, and metabolic syndrome (Figure 2). In addition, the number of functional mitochondria is known to decrease with aging, so an increase in mitochondrial biogenesis could exert an anti-aging effect by buffering this decline.

A second, more subtle, aspect of mitochondrial biogenesis might also have a role in anti-aging. A leading hypothesis of a cause of aging is the oxidation of macromolecules in cells owing to the generation of ROS by mitochondria [27]. One mechanism for generating ROS is the stalling of electrons along the electron transport chain. Electron transport can become stalled when protons are pumped to the cytoplasm across a hyper-polarized mitochondrial membrane, that is, when the demand for ATP synthesis is high and the charge gradient (charge difference per surface area) is steep (Figure 3). An increase in mitochondrial biogenesis will increase mitochondrial surface area, thereby reducing hyper-polarization of the membrane and leading to the production of ROS. By this reckoning, mitochondrial biogenesis could be beneficial even if it did not result in a measurable increase in metabolic rate (because of some other limiting component), and it has been found that humans on CR induce mitochondrial biogenesis without an observed increase in metabolic rate [19,28,29].

More tangible evidence that SIRT1 activation might have benefit via mitochondrial function comes from studies of the polyphenol, resveratrol, in mice. Resveratrol and other polyphenolic compounds are made by plants in response to stress. Resveratrol was recently shown to affect the activity of SIRT1 in vitro [30] although its effects seem to depend on the nature of the substrate for deacetylation [22,31]. However, in vivo, resveratrol has been shown to exert effects dependent on sirtuin orthologs – extension of lifespan in yeast, C. elegans and Drosophila, and metabolic effects on mammalian cells [30,5,24]. Two recent studies show that deleterious effects of high fat, high caloric diets in mice were mitigated by resveratrol feeding [32,33]. In one study, the shortening of lifespan by the high fat diet was reversed. In a second study, resveratrol increased SIRT1 activation, PGC-1α deacetylation, and mitochondrial biogenesis in muscle. These studies provide a powerful indication that SIRT1 activation offers a promising approach for treating metabolic disorders. However, one must be aware of possible targets other than SIRT1 for resveratrol, for example, AMP-dependent protein kinase [34].

There is also evidence to suggest neuroprotective effects of SIRT1 activation. The Wallerian mouse, which is protected against neuronal degeneration, harbors a mutation increasing levels of an NAD biosynthetic enzyme, Nmnat1. In addition, NAD itself protects dorsal root ganglions (DRGs) against chemical or physical assaults in culture, and this effect was shown to require SIRT1 [35]. These findings have been confounded by a report showing that NAD also protects DRGs in SIRT1+/− mice [36]. We surmise that NAD might have multiple protective effects on neurons, at least some of which are mediated by SIRT1. Several other studies have shown neuroprotective effects of SIRT1 or resveratrol or NAD in cultured neurons [35,37,38].

Neurons are the most metabolically active cells in the body and as such might depend most crucially on functional mitochondria. Many neurodegenerative diseases, such as Huntington’s disease and Parkinson’s disease might be marked by mitochondrial dysfunction, and therefore activation of mitochondrial biogenesis via SIRT1 and PGC-1α might hold promise in treating these diseases [37,39].
SIRT3 and SIRT4
SIRT3 is produced in brown adipose tissue in response to cold and its expression levels seem to be directly related to metabolism. Specifically, overexpression of SIRT3 leads to increased respiration and also to a reduction in ROS [40]. In addition, SIRT3 was recently shown to deacetylate and activate mitochondrial acetyl-CoA synthetase (ACS) [41,42] to enable metabolism of acetate sourced from the diet or produced endogenously. SIRT4 ADP ribosylates and inhibits the mitochondrial enzyme glutamate dehydrogenase (GDH) [43] thereby inhibiting the conversion of glutamate (and glutamine) to the TCA cycle intermediate, α ketoglutarate. SIRT4 provides perhaps the clearest example of a physiological response to CR that is mediated by a sirtuin. Under CR, SIRT4-mediated inhibition of GDH is alleviated thereby facilitating the use of glutamate and glutamine as a source of carbon and energy for gluconeogenesis in the liver. In addition, the down-regulation of SIRT4 by CR enables these amino acids to function as insulin secretagogues in the β cells of the pancreas. Because this regulation enables the animal to use amino acids as energy sources during the energy limitation imposed by CR, it makes sense that it also sensitizes their β-cells to respond to amino acids by secreting insulin.

SIRT6 and 7 might also have metabolic functions. SIRT6 has been linked to base excision repair and glucose homeostasis [44]. SIRT6 knockout mice show increased DNA damage, have low levels of IGF1, and have a catastrophic decline in blood glucose beginning two weeks after birth. SIRT7 is the only mammalian sirtuin localized to nucleoli and has been shown to be a positive regulator of ribosomal RNA (rRNA) synthesis, and therefore of ribosome biogenesis [45].

Both SIRT3 and SIRT4 are attractive candidates as potential therapeutics for metabolic disease, given their location in the mitochondrion and their links to metabolism described above [46–49,24]. SIRT4 is particularly interesting because it presents a case in which a sirtuin inhibitor might have efficacy for individuals in a pre-diabetic state, in this case by stimulating insulin secretion in individuals with glucose intolerance. It remains to be seen whether the mitochondrial sirtuins SIRT3, SIRT4 and SIRT5 will also regulate electron transport and ATP synthesis directly. This function could enable these sirtuins to exert direct effects on mitochondrial activities related to aging – ROS production by the electron transport chain, and apoptosis (which can be driven by release of mitochondrial components to the cytoplasm). It would be interesting, for example, if these mitochondrial sirtuins held the potential to limit ROS production, (as was suggested for SIRT3 [46]) and to reduce apoptosis during CR.

Sirtuin drug discovery
At present, several inhibitors and activators of SIRT1 have been described [50–52]. Inhibitors such as nicotinamide (NAM), sirtinol and splitomycin have been useful in helping to dissect the function of SIRT1 in the laboratory. Sirtinol and splitomycin function as competitive inhibitors by blocking the active site, whereas high concentrations of NAM drive reversal of the first step in deacetylation – the cleavage of NAD to an ADP-ribosyl intermediate and NAM. However, the therapeutic use of these inhibitors is limited, first because they have low potency, and second because they are not specific. In addition, use of NAM is complicated by the fact that it can be converted to NAD in vivo and NAM can inhibit other classes of enzymes (e.g. poly-ADP ribosyl polymerases). Inhibitors of other sirtuins might prove useful in diabetes, for example (for reasons described above) a SIRT4 inhibitor might increase insulin secretion by β cells [43].

There are two potential conceptual modes for activating sirtuins: direct and allosteric (Figure 4). An example of direct activation involves the NAM analog, iso-nicotinamide (INAM), which binds directly to SIRT1 and works by competing with the inhibitor NAM for binding to the catalytic site [53]. INAM is thought to prevent the reversal of the first step of deacetylation by inhibiting binding of endogenous NAM to the enzyme intermediate.

In the allosteric model, a protein region flanking the catalytic domain of SIRT1 might bind to it and inhibit catalysis (Figure 4a). It is also possible that inhibition of the catalytic domain by the flanking domain would occur by intermolecular interactions during oligomerization, [54]. This inhibition could be alleviated by the binding of a small molecule to SIRT1 to interfere with the inhibitory protein–protein interaction. An example of allosteric

![Figure 4. Mechanisms of activation of SIRT1: direct and allosteric. Direct activation: INAM binds directly to the catalytic domain (e.g. SIRT1) to competitively block inhibition by NAM at the catalytic site. ADPR indicates the reaction intermediate with ADP-ribosyl bound to the catalytic domain. O-AcADPR is the O-acetylated ADP-ribosyl product, Ac denotes an acetyl group. Allosteric activation: (a) A protein region flanking the catalytic domain of SIRT1 might bind to it and inhibit catalysis. (b) Flanking domain binds to protein substrate. Inhibition of the catalytic domain by the flanking domain would occur by intermolecular interactions during oligomerization. This inhibition could be alleviated by the binding of resveratrol, which could interfere with the inhibitory protein–protein interaction.](www.sciencedirect.com)
activation of SIRT1 might be provided by the action of resveratrol, other polyphenolic compounds and other small molecules such as quinoxalines [55]. Evidence for an allosteric mechanism for resveratrol awaits the demonstration that this compound directly binds to SIRT1 and can alter its conformation. Moreover, the generation and analysis of resveratrol-refractory SIRT1 mutants would provide important evidence for the direct action of this compound in vivo.

In living cells, it is likely that SIRT1 binds to its protein substrates via its long N- and C-terminal flanking domains. Therefore, one can imagine a class of activators that would function by binding to SIRT1 and increasing the affinity of the sirtuin for one or more substrates in vivo (Figure 4b). Again, it is possible that resveratrol works by this mechanism, and, moreover, it should be possible to screen for novel activators in cell-based assays that record the activity of a specific SIRT1 substrate (e.g. PGC-1α). Such an approach could have the benefit of yielding small molecules or drugs that specifically target one SIRT1-mediated pathway or another.

Conclusion
Sirtuins are anti-aging proteins that have therapeutic potential for a range of diseases of aging, including metabolic disorders, neurodegenerative disorders, cancer and cardiovascular disease. The link between sirtuin activation and mitochondrial biogenesis by the SIRT1-mediated activation of PGC-1α provides a novel mechanism of action for the treatment of diseases for which therapy is currently limited. We expect that the many other physiological pathways regulated by SIRT1, as well as the other six mammalian sirtuins, will offer further opportunities for novel therapeutic interventions.

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