Point of VIEW

ACS chemical biology

Linking SIRT2 to Parkinson's Disease

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ABSTRACT A recent study has identified selective inhibitors of the human silent information regulator 2 NAD^+-dependent protein deacetylase, SIRT2, and has shown that these compounds protect against α-synuclein-mediated toxicity in cellular models of Parkinson's disease. The inhibitors were found to ameliorate dopaminergic cell death *in vitro* and in a *Drosophila* model of Parkinson's disease. Although the molecular mechanism of action is unclear, the compounds may function by promoting the formation of enlarged inclusion bodies, which are suggested to provide a cell-survival advantage.

The silent information regulator 2 (Sir2 or sirtuins) NAD^+-dependent histone deacetylases (HDACs) participate in a number of age-related phenomena, including life-span extension, glucose homeostasis, and neurodegeneration (*1*). Sir2-dependent life-span extension has been observed in organisms as diverse as *Saccharomyces cerevisiae* (*2*), *Caenorhabditis elegans* (*3*), and *Drosophila melanogaster* (*4*). Sirtuins may also mediate several pathways that dictate life span in mammals (*1*). However, the role of sirtuins in the molecular mechanisms underlying aging remains unclear. Targeting key players in the aging process may allow therapeutic intervention in age-associated diseases such as neurodegeneration. In a recent study, Outeiro *et al.* (*5*) suggest that inhibition of the human sirtuin homologue SIRT2 rescues cells from α-synuclein-mediated toxicity in Parkinson's disease (PD) (Figure 1).

Of the seven human sirtuins (SIRT1–SIRT7), the neuroprotective role of SIRT1 is the most established. For example, SIRT1 protects from axonal degeneration in response to increased NAD^+ biosynthesis (*6*). SIRT1 is thought to achieve axonal protection primarily through regulation of gene expression, not through deacetylation of proteins involved in axon stability. Other evidence suggests that SIRT1 protects against Alzheimer's disease (AD) at several levels. Indirectly, SIRT1 combats AD by significantly reducing nuclear factor kappa B (NF-κB) signaling in nearby glia (*7*), thereby alleviating amyloid-β (Aβ) peptide-induced neuronal death. In a recent study, SIRT1 was found to protect mouse models of AD and amyotrophic lateral sclerosis, possibly by affecting pathways involving deacetylation of p53 and PGC-1α (*8*).

The roles of SIRT2 in neurodegenerative diseases are less established. In a recent study, SIRT2 was established as an inhibitor of oligodendroglial differentiation/aging *via* deacetylation of the microtubule cytoskeleton (*9*). This may serve to protect oligodendrocytes for the purposes of remyelination or central nervous system self-repair. In some instances, the effects of SIRT2 appear to be detrimental to neuronal health. For example, SIRT2 may oppose resistance to axonal degeneration (*10*).

Outeiro *et al.* (*5*) report that small-molecule inhibition of SIRT2 may provide a unique means for therapeutic intervention in PD. PD is characterized by the development of α-synuclein-containing Lewy bodies and the loss of dopaminergic neurons in the *substantia nigra* (Figure 1) (*11*). In addition, it is becoming increasingly evident that properly balanced protein acetylation status plays a crucial role in neuronal vitality (*12*). Indeed, small-molecule HDAC inhibition provides protection in many models of neurodegeneration (*13*). Thus, SIRT2 may provide a unique target for intervention in PD.

Bodner *et al.* (*14*) identified a small-molecule (compound B2; Figure 2) that was associated with increased size of α-synuclein inclusion in cells transfected with a tagged α-synuclein construct. It is thought that the larger α-synuclein aggregates may have reduced toxicity relative to

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Published online August 17, 2007
10.1021/cb700160d CCC: $37.00

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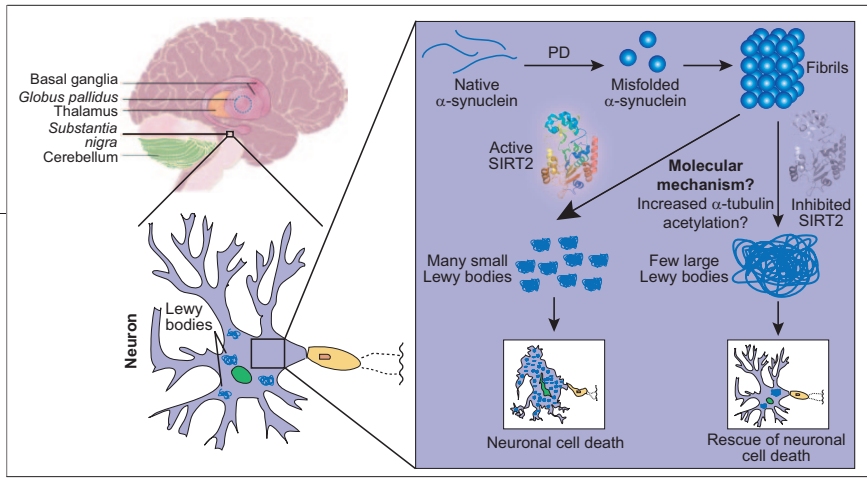

Figure 1. Putative biological mechanism of SIRT2 inhibition for treating PD. PD is characterized by buildup of α-synuclein-containing Lewy bodies within the *substantia nigra* region of the brain. Lewy bodies are composed of fibrils of misfolded α-synuclein. Small-molecule inhibition of SIRT2 results in a reduced occurrence of small Lewy bodies. Instead, a smaller number of larger Lewy bodies are formed, which are proposed to offer protection from neuronal cell death. The mechanism of increased Lewy body size is unknown, but increased α-tubulin acetylation may play a role.

their smaller-sized counterparts (*15*) (Figure 1). Outeiro *et al.* (*5*) tested the inhibition of B2 against a variety of HDACs, proteases, and chaperones and found weak but selective inhibition of SIRT2. A library screen of 200 analogues of B2 and known aggregation modifiers identified two notable compounds, AGK2 and AK-1 (Figure 2). In a fluorometric assay, AGK2 exhibited 10-fold more potent SIRT2 inhibition compared with B2. In addition, AGK2 exhibited >14-fold selective inhibition of SIRT2 compared with SIRT1 and SIRT3. The authors also demonstrated that the acetylation state of α-tubulin, a known substrate of SIRT2 (*16*), increased in a dose-dependent manner upon treatment with AGK2 in HeLa cells. Interestingly, another tubulin deacetylase, HDAC6, is believed to have a neuroprotective role (*17*) and has been shown to function in complex with SIRT2 (*16*). If α-synuclein-mediated toxicity in models of PD is due in part to deacetylation of α-tubulin by SIRT2, one might expect a similar effect from inhibition of HDAC6. However, the ability of HDAC6 to clear misfolded protein aggregates from the cytoplasm (*17*) may counter this effect.

Having identified novel SIRT2 inhibitors, Outeiro *et al.* employed molecular docking experiments of AGK2 and AK-1 to suggest a mechanism of inhibition. Using the known structure of SIRT2 (*18*), the authors used virtual ligand modeling to identify low-energy binding conformations within the nicotinamide-binding site of NAD^+. From these observations, they propose that inhibition occurs through competition at the nicotinamide binding site of cosubstrate NAD^+ (Figure 2). However, direct biochemical evidence for competitive binding between these inhibitors and NAD^+ was not presented. It is interesting that if AGK2 and AK-1 utilize the nicotinamide binding pocket, they may alleviate nicotinamide-induced product inhibition (*19*). Future biochemical experiments evaluating the mode of inhibition will be needed to provide insight into the mechanism of action for these compounds.

To examine these compounds in a cellular context, Outeiro *et al.* showed that human neuroglioma cells (H4) transfected with α-synuclein could be rescued from α-synuclein-mediated toxicity in a dose-dependent manner when treated with AGK2 or subjected to small interfering RNA (siRNA) knockdown of SIRT2. Taken together, these experiments suggest that SIRT2 may be a direct *in vivo* target for the AGK class of molecules, but they do not rule out indirect pathways. Interestingly, AGK2 and AK-1 treatment of H4 cells cotransfected with α-synuclein and synphilin-1 (to promote inclusion formation) increased the size of the inclusions, a possible mechanism for cytoprotection (*14*) (Figure 1). However, the authors did not provide genetic evidence that siRNA knockdown of SIRT2 also resulted in increased inclusion size. In a complementary experiment, Outeiro *et al.* established that AGK2 and AK-1 protect against α-synuclein-mediated dopaminergic cell death. Again, the absence of genetic evidence for the direct involvement of SIRT2 makes it unclear whether the rescue is due to specific SIRT2 inhibition.

Finally, Outeiro *et al.* evaluated the protective effects of AGK2 and AK-1 in a *Drosophila* model of PD. Notably, they found that administration of these compounds caused a dose-dependent rescue of dorsomedial neurons. However, it is not clear which sirtuin homologue, if any, was being targeted in *Drosophila* and whether the acetylation status of α-tubulin is affected. If the compounds are indeed inhibiting a *Drosophila* Sir2 homologue, then this raises some concern about the actual selectivity of the compound because of the great diversity among sirtuin orthologues from flies to humans. Further *in vivo* studies will be needed to demonstrate selectivity for SIRT2 and to establish the true target in *Drosophila*.

Many other SIRT2 inhibitor compound classes have been described recently. In the past two years, there have been several reports of SIRT2 inhibitors with IC_{50} values <25 μM. These include phloroglucinol (*20*), indole (*21*), and suramin derivatives (*22*) as well as adenosine mimetics (*23*). These compounds possess comparable potency to that displayed by AGK2 and AK-1 but exhibit significantly lower selectivity for SIRT2. It will be useful to examine these compounds in the context of PD models and establish SIRT2 as a direct target. In addition, it is unclear whether compounds that inhibit SIRT2 deacetylation through competition for the adenosine portion of NAD^+ or the acetyl-lysine binding site would provide protection in PD models.

SIRT2 has been implicated in a variety of other cellular processes. Previous studies have shown that in addition to α-tubulin (*16*), SIRT2 deacetylates p53 (*24*), FOXO3a (*25*), and histones (*26*). These observations indicate a potentially broad regulatory role for SIRT2, particularly in mitosis/cell cycle

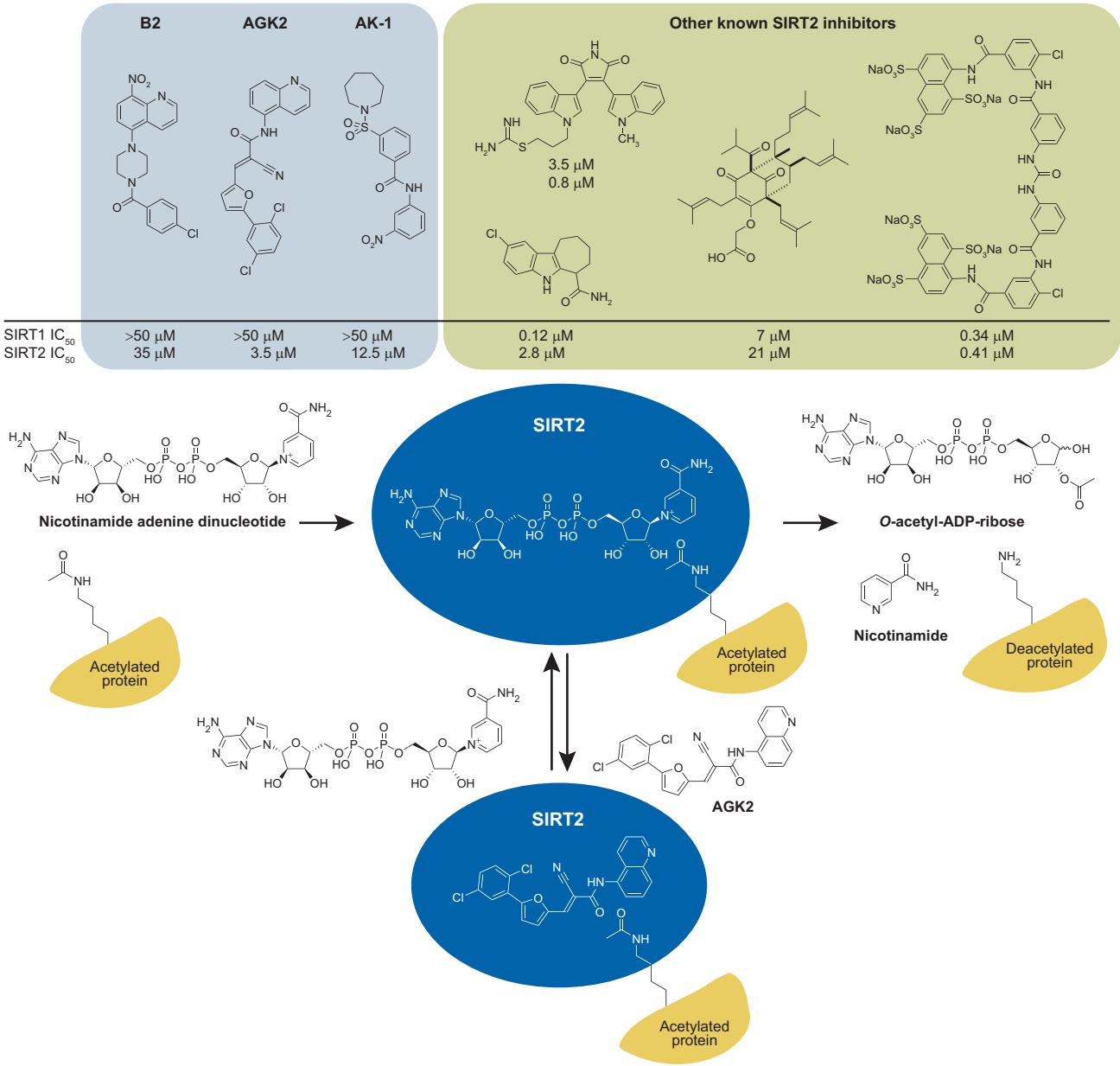

Figure 2. Structures of known SIRT2 inhibitors. The compounds from Outeiro *et al.* are hypothesized to function through competition for the nicotinamide binding site within SIRT2.

regulation (*27, 28*), apoptosis (*24*), and aging by oxidative stress (*25*). It will be crucial to establish how SIRT2 inhibition affects these pathways and their effects on neuronal health.

In summary, Outeiro *et al.* report an intriguing connection between SIRT2 and PD. Although the molecular basis of how SIRT2 is involved in α-synuclein aggregation remains unclear, the compounds identified in this screen provide an exciting starting point in combating PD. Future studies comparing the role of SIRT1 and SIRT2 in neurodegenerative diseases will be necessary to dissect the apparent paradox of the neuro-

protective properties of sirtuins. In particular, why does activation of SIRT1 but inhibition of SIRT2 deacetylase activity appear to promote neuroprotection? Furthermore, if deacetylation of α-tubulin confers neurotoxicity in models of PD, what role does HDAC6 play? Because reversible acetylation appears to play a significant role in a variety of other neurodegenerative disorders such as Alzheimer's and Huntington's diseases (*12*), similar classes of therapeutics might target multiple disorders. Future work aimed at the identification of more potent and selective inhibitors/activators of sirtuins will be critical in manipulating the pathways responsible for neurodegeneration and may provide avenues for therapeutic intervention.

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