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Silent Mating—Type Information Regulation 2 Homolog 1 Attenuates the Neurotoxicity Associated with Alzheimer Disease via a Mechanism Which May Involve Regulation of Peroxisome Proliferator-Activated Receptor Gamma Coactivator 1- α

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To investigate the neuroprotective role of silent mating—type information regulation 2 homolog 1 (SIRT1) in Alzheimer disease (AD), brain tissues from patients with AD and *APP/PS1* mice as well as primary rat neurons exposed to oligomers of amyloid- β peptide were examined. The animals were treated with resveratrol (RSV) or suramin for 2 months. Cell cultures were treated with RSV, suramin, and the peroxisome proliferator-activated receptor gamma coactivator 1- α (PGC-1 α) stimulator ZLN005. Cells were transiently transfected with PGC-1 α silencing RNA. The level of SIRT1 in brain tissues from patients with AD and *APP/PS1* mice, including nuclear and mitochondrial proteins, as well as in primary neurons exposed to oligomers of amyloid- β peptide, was decreased. Overexpression of *APP/PS1* impaired learning and memory of mice; produced more senile plaques, disrupted membranes, and resulted in broken or absent cristae of mitochondria in the brain; decreased levels of A disintegrin and metalloproteinase domain 10, beta-secretase 2, 8-oxoguanine DNA glycosylase-1, PGC-1 α , and NAD⁺; and increased levels of beta-secretase 1 and apoptosis. Interestingly, these changes were attenuated significantly by RSV treatment but enhanced by suramin administration. By activating PGC-1 α but inhibiting SIRT1, apoptotic cell death was significantly decreased; however, by activating SIRT1 but inhibiting PGC-1 α with small interfering PGC-1 α , these levels remained unchanged. These findings indicate that SIRT1 may protect against AD—associated neurotoxicity, which might involve PGC-1 α regulation. (*Am J Pathol* 2020, 190: 1545–1564; <https://doi.org/10.1016/j.ajpath.2020.03.015>)

Alzheimer disease (AD), with characteristic pathologic abnormalities, including senile plaques and neurofibrillary tangles, is the most prevalent neurodegenerative disease.¹ β -Amyloid peptide (A β) derived by proteolytic cleavage of the A β precursor protein (APP) is the primary component of senile plaques and plays an important role in the pathogenesis of AD.² Accumulation of aggregated A β is generally believed to be responsible for disease progression,³ which may be a toxic substance that destroys the functional integrity of the brain.⁴

The silent information regulator 2 (Sir2) family of proteins [sirtuins or silent mating—type information regulation

2 homolog 1 (SIRT)], with seven known members (SIRT1 to SIRT7), constitutes a new class of histone deacetylases dependent on NAD⁺.⁵ Sirtuins are expressed throughout the mammalian body but appear to be selectively localized at

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the subcellular level; SIRT1, SIRT6, and SIRT7 are mainly nuclear; SIRT3, SIRT4, and SIRT5 are primarily mitochondrial; whereas SIRT2 is the only sirtuin located in the cytosol.⁶ This implies that SIRT proteins have variety of cellular functions. Initial studies have identified that Sir2 protein contributes to extending lifespan in yeasts, flies, and worms, providing the first evidence that SIRT has an anti-aging effect.^{7,8} The SIRT1, SIRT3, and SIRT5 catalytic mechanism involves NAD⁺ as a cofactor,⁹ and SIRT1 has been most extensively reviewed.¹⁰ SIRT2 promotes cell death under oxidative stress in AD.¹¹

SIRT1 is the most studied member of the sirtuin family and is expressed at high levels in the brain, spinal cord, and dorsal root ganglia.¹² In adult rodent brains, SIRT1 mRNA is abundantly expressed in metabolically relevant areas, including the hypothalamic arcuate, ventromedial, dorso-medial, and paraventricular nuclei, the area postrema, and the nucleus of the solitary tract in the hindbrain.¹³ In addition to these sites, SIRT1 mRNA was also abundant in the piriform cortex, hippocampus, and cerebellum, with low levels in white matter.¹³ SIRT1 is predominantly expressed in neurons.^{12,13} At the subcellular level, SIRT1 is located mostly in the nucleus.¹⁴ For example, transfection with SIRT1 cDNA shows nuclear localization in monkey kidney COS-7 cells,¹² but it also can be moved between cell nucleus and cytoplasm,¹⁵ and the cytoplasmic localization of SIRT1 has also been reported recently in murine pancreatic β -cells and neonatal rat cardiomyocytes.^{16,17} The subcellular localization of SIRT1 varied in different tissues of the adult mouse (eg, some subsets of neurons predominantly expressed SIRT1 in the cytoplasm); moreover, ependymal cells expressed it in both the nucleus and cytoplasm; on the other hand, spermatocytes expressed SIRT1 only in the nucleus.¹⁵ Cardiomyocytes in the day 12.5 mouse embryo expressed SIRT1 exclusively in the nucleus, but in the adult heart, they expressed it in both the cytoplasm and nucleus. C2C12 myoblast cells expressed SIRT1 in the nucleus, but it localized to the cytoplasm after differentiation. In a heterokaryon assay, SIRT1 shuttled between the nucleus and cytoplasm.¹⁵

SIRT1 is an NAD⁺-dependent protein deacetylase that catalyzes the removal of acetyl groups from lysine residues in substrate proteins. Histones are the primary substrate of SIRT1.¹⁸ The deacetylating activity of SIRT1, in turn, regulates diverse biological processes related to axonal integrity,¹⁹ cell differentiation,²⁰ apoptosis,²¹ autophagy,²² development²³ and metabolism.²⁴ In the adult brain, SIRT1 is essential for synaptic plasticity, cognitive functions,²⁵ and the modulation of learning and memory function.²⁶ Some research found that SIRT1 as a potential novel target in AD. Previous studies found that SIRT1 may be neuroprotective in connection with the pathophysiology of AD by attenuating oxidative stress.²⁷ Recent evidence indicates that SIRT1 may protect against AD by interfering with the generation of A β .²⁸ Treatment of neuronal cultures with NAD⁺ and resveratrol (RSV, an activator of SIRT1)

reduces the binding of A β to amyloid fibrils and enhances cellular α -secretase activity, thereby activating the protective nonamyloidogenic pathway,²⁸ and SIRT1 activation, either pharmacologically or by caloric restriction, may down-regulate the generation of A β .²⁸

In addition, SIRT1 regulates gluconeogenesis and glycolysis through the peroxisome proliferator-activated receptor gamma coactivator 1- α (PGC-1 α) transcription factor, which also results in the increased number and function of mitochondria, both *in vivo* and *in vitro*.²⁹ Mitochondrial biogenesis is regulated in part by a transcriptional coactivator of PGC-1 α ,³⁰ the activity of which is dependent on deacetylation by SIRT1.³¹ The level of PGC-1 α in the brains of patients with AD is reduced.²⁹ The SIRT1–PGC-1 α transcriptional complex may constitute a core component of brain neural circuitry involved in modulating energy homeostasis.³² It is now clear that SIRT1 stimulates mitochondrial biogenesis,³³ and aging-induced inactivation of SIRT1 has a direct and deleterious effect on mitochondria, as first suggested by the important associations between SIRT1 and PGC-1 α ; a change in the expression of mammalian homolog 8-oxoguanine DNA glycosylase-1 (OGG1), which stands for mitochondrial DNA (mtDNA) damage protein; and a reduction in SIRT1 activity that down-regulates mitochondrial biogenesis, oxidative metabolism, and associated antioxidant defense pathways, leading to damage to complex I of the electron transport chain and a decline in mitochondrial function.³⁴

According to the physiologic function and involvement of SIRT1 modification, evidence suggests that the SIRT1/PGC-1 α pathway is closely associated with APP metabolism and A β accumulation in AD. In this study, we investigated the functional role of the SIRT1/PGC-1 α pathway on regulation of apoptosis by mitochondrial damage with the brain tissues from patients with AD, mice carrying the double transgenic mutation in APP and presenilin 1 (PS1), and primary cultured neurons exposed to A β 0 to understand the neuroprotective effects of SIRT1 associated with AD.

Materials and Methods

Materials

RSV, suramin, and A β ₁₋₄₂ (Sigma-Aldrich, St. Louis, MO); ZLN005 (AbMole Bioscience Inc., Houston, TX); Cell Counting Kit-8 (Dojindo Molecular Technologies, Kumamoto, Japan); Neurobasal-A Medium, Hibernate-E Medium, B-27 Serum-Free supplement, GlutaMAX Supplement (Life Technologies, Carlsbad, CA); mouse monoclonal anti- β Amyloid, 1-16 (BioLegend, San Diego, CA); mouse monoclonal anti-SIRT1 (Abcam, Cambridge, UK); rabbit monoclonal anti-SIRT1 (Cell Signaling Technology, Danvers, MA), anti-A disintegrin and metalloproteinase domain 10 (ADAM10) antibodies (Abcam); rabbit polyclonal anti-PGC-1 α , anti-OGG1, and anti- β -secretase

Table 1 Characteristics of the Antibodies

Antibody	Raised in mice		Raised in rabbits	
	Catalog no.	Fragment size, kDa	Catalog no.	Fragment size, kDa
SIRT1	ab110304 (Abcam)	110	Unwanted	Unwanted
SIRT1	Unwanted	Unwanted	9475 (Cell Signaling Technology)	110
PGC-1 α	Unwanted	Unwanted	GTx37356 (GeneTex)	91
OGG1	Unwanted	Unwanted	GTx20204 (GeneTex)	39
A β 6E10	SIG-39321 (BioLegend)	Unwanted	Unwanted	Unwanted
ADAM10	Unwanted	Unwanted	ab124695 (Abcam)	84
BACE1	Unwanted	Unwanted	GTx78908 (GeneTex)	56
BACE2	Unwanted	Unwanted	GTx113951 (GeneTex)	56
β -Actin	Unwanted	Unwanted	GTx109639 (GeneTex)	42
Histone H ₃	2D10 (Abbkine Scientific Co. Inc.)	15	Unwanted	Unwanted
COX IV	14Y2 (Abbkine Scientific Co. Inc.)	15	Unwanted	Unwanted

A β 6E10, amyloid- β peptide 6E10; ADAM10, A disintegrin and metallopeptidase domain 10; BACE1, beta-secretase 1; BACE2, beta-secretase 2; COX, cytochrome c oxidase; OGG1, 8-oxoguanine DNA glycosylase-1; PGC-1 α , peroxisome proliferator-activated receptor gamma coactivator 1- α ; SIRT1, silent mating-type information regulation 2 homolog 1.

2 (BACE2) antibodies (GeneTex, Irvine, CA); mouse monoclonal anti- β -secretase 1 (BACE1) antibody (GeneTex); anti-rabbit IgG conjugated with horseradish peroxidase and mouse monoclonal anti- β -actin (A β) antibody (Sigma-Aldrich); anti-Histone H3 and anti-COX IV mouse monoclonal antibodies (Abbkine Scientific Co. Inc., Wuhan, China); anti-mouse IgG labeled with CY-3 and anti-rabbit IgG labeled with 488 (Thermo Fisher Scientific, Waltham, MA); PGC-1 α siRNA and control siRNA (Santa Cruz Biotechnology Inc., Dallas, TX); Hyper Performance Chemiluminescence film and Electrochemiluminescence Plus reagent (Amersham, Buckinghamshire, Sweden); kits for measuring NAD⁺/hydroxyacyl-coenzyme A dehydrogenase (KeyGen BioTech, Nanjing, China); Annexin V-EGFP/PI cellular apoptosis Kit (BestBio, Shanghai, China); nuclear protein and mitochondrial protein extraction kits (BestBio); Universal TaqMan 2 $\times$ PCR mastermix (Applied Biosystems, Foster City, CA); and all other general chemicals (Sigma-Aldrich) were purchased from the sources indicated. More detailed information concerning these antibodies is presented in Table 1.

Human Brain Tissue

The postmortem human brain samples provided by the Netherlands Brain Bank (Amsterdam, the Netherlands) were all well characterized with respect to specific clinical and neuropathological criteria. Material Transfer Agreement and Implementing Letter Regarding Project (No. 1060) were granted by the Netherlands Brain Bank and ethical permission for the study by the Ethical Committee of Guizhou Medical University (2018-067). Written informed consent was obtained from the donor or an immediate next-of-kin. The donors were diagnosed as having probable AD by excluding other possible causes of dementia on the basis of their medical history, clinical presentation, and laboratory tests. This clinical diagnosis was

performed in accordance with the National Institute of Neurological and Communicative Disorders and Stroke of the United States and the Alzheimer's Disease and Related

Table 2 Clinical and Autopsy Data on Patients with AD and Controls from Whom Brain Tissue Was Obtained

Case	Sex	Age, years	PMD, hour:minute	NFT Braak stage*	A β Braak stage†
Controls					
S00/067	M	65	14:25	0	0
S00/059	F	78	06:30	1	A
S00/097	M	78	06:55	1	A
S00/055	M	82	13:35	2	A
S00/222	F	88	05:40	2	B
S97/221	F	78	04:15	1	0
S00/040	F	83	07:45	2	0
S00/047	M	98	08:40	1	B
S15/027	F	72	04:45	2	0
S15/055	F	72	06:50	1	A
Means $\pm$ SD		79.4 $\pm$ 9.2	07:03 $\pm$ 3.4		
Patients with AD					
S00/278	M	85	07:10	5	C
S00/194	F	76	05:05	5	C
S00/206	M	73	03:35	6	C
S00/085	F	80	05:20	6	C
S01/100	M	88	05:00	4	C
S00/080	F	95	04:45	5	C
S00/318	F	72	03:45	6	C
S00/041	F	86	04:45	4	B
S00/264	F	82	06:10	5	C
S14/049	F	78	04:45	6	C
Means $\pm$ SD		81.5 $\pm$ 7.2	04:06 $\pm$ 1.3		

*NFT 0 to 6 are the Braak classification of the stages of AD based on the distribution and amount of neurofibrillary tangles.

†A β A, B, and C are the Braak classification of the stages of AD based on amyloid plaques.

F, female; M, male; AD, Alzheimer disease; PMD, postmortem delay.

Table 3 Sequences of the Primers Used to Quantify Sirt1 and Aβ mRNA by Real-Time PCR

Primary cultured rat neurons			Brain tissue		
mRNA	Sequence	Length, bp GenBank accession no.*	Sequence	Length, bp GenBank accession no.*	
SIRT1	F: 5'-TGTGTGTGGGTCTATGCCTT-3' R: 5'-CCTGCAATCCCAGGTACTTT-3'	99 NM_001372090.1	F: 5'-CAGCATATTGCCTGATTGT-3' R: 5'-GCACCGAGGAACACCTGAT-3'	83 NM_001159589.2	
Aβ	F: 5'-CACCCGCGAGTACAACCTTC-3' R: 5'-CCCATACCCACCATCACACC-3'	207 NM_031144.3	F: 5'-GTGCTATGTTGCTCTAGACTTCG-3' R: 5'-ATGCCACAGGATTCATACC-3'	174 NM_007393.5	

*Accession numbers can be searched at: <https://www.ncbi.nlm.nih.gov>.
Aβ, amyloid-β peptide; F, forward; R, reverse; SIRT1, silent mating-type information regulation 2 homolog 1.

Disorders Association (NINCDS-ADRDA) criteria³⁵ and the severity of dementia estimated on the Global Deterioration Scale. The control donors had no known history or symptoms of neurologic or psychiatric disorder. The hippocampus and temporal cortex of 10 patients with AD and 10 controls were subjected to immunohistochemical staining. The means ± SD ages of these groups at the time of death were 81.5 ± 7.1 and 79.4 ± 9.2 years, and the means ± SD times that elapsed between death and autopsy were 5.1 ± 1.0 and 8.0 ± 3.4 hours, respectively. Pathologic staging of AD performed according to the Braak system and other characteristics of the patients and control individuals are documented in Table 2.

Experimental Animals

Five 4-month-old B6. Cg-Tg (APPswe, PSEN1dE9) mice with 85Dbo/Mmjax background and 15 wild-type (WT) mice of the same strain (all with a body weight of 20 to 30 g) were purchased from Shanghai Nanfang Biological Technology Development Co., Ltd. (Shanghai, China). All animals were acclimatized at a humidity of 30% to 55% and temperature of 22°C to 25°C for 1 week, after which each female *APP/PS1* mouse underwent natural mating with three WT males. At a postnatal age of 12 to 20 days, the tip of the tail of each pup was excised and DNA extracted for genotyping by the PCR, with detection of the products by 1.5% agarose gel electrophoresis. The PCR primers for target transcripts were designed on the basis of the complete cDNA sequences in GenBank (<http://www.ncbi.nlm.nih.gov>). Using genomic DNA as a template for amplification, bands approximately 400 and 600 bp in size, consistent with the *APP* and *PS1* genes, respectively, were seen in the transgenic but not WT mice.²⁷ Starting at the ages of 4 and 8 months, respectively, the *APP/PS1* and WT mice received RSV (20 mg/kg), suramin (20 mg/kg), or physiologic saline by gavage once daily for 2 months.^{36,37} All these animal experiments were preapproved by the Ethical Committee of Guizhou Medical University, China (No. 1702110).

Cell Cultures

Primary hippocampal neurons were prepared from the brains of neonatal Sprague-Dawley rats by a previous

procedure.²⁷ In brief, the hippocampus was digested with 0.25% trypsin for 10 minutes at 37°C, after removing the meninges of the hippocampus. The digested tissue was disrupted by repeated suction through fire-polished glass pipettes in 2 mL of Neurobasal/B27 complete medium. The upper suspension of single cells was transferred into a new tube and the cells counted by trypan blue exclusion and thereafter placed onto 96- or 6-well poly-L-lysine-coated plates at a density of approximately 1.0 × 10⁴ or 1.0 × 10⁵/cm². The neurons were maintained under a humidified atmosphere containing 5% CO₂ at 37°C, with replacement of half of the medium once every 3 days. The purity of these primary neurons was evaluated by immunofluorescent double-staining with some corresponding antibodies.²⁷ After 10 days of incubation, the medium was replaced with neurobasal medium that lacked B27 and the neurons then prepared for various treatments.

SH-SY5Y cells, a human neuroblastoma cell line (the German Collection of Microorganisms and Cell Cultures), were cultured at 37°C in Dulbecco's modified Eagle's medium supplemented with 10% heat-inactivated fetal bovine serum and 25 U/mL of penicillin-streptomycin under a humidified atmosphere containing 5% CO₂.

Treatment of the Cultured Neurons with AβO, RSV, Suramin, or ZLN-005

AβO was prepared according to a previously published procedure.³⁸ The primary neurons were seeded onto 96-, 12-, or 6-well poly-L-lysine-coated plates and B27 withdrawn from the culture medium 2 hours before treatment. To determine the optimal conditions for cell survival in starvation media, cells were first exposed to different concentrations of AβO (0 to 2 μmol/L), RSV (0 to 150 μmol/L), or suramin (0 to 600 mg/mL) for 48 or 24 hours and 10 μL of solution from Cell Counting Kit-8 then added to each well. Incubation was then continued for 1 to 4 more hours and absorption at 450 nm determined. On the basis of this test for viability, suitable concentrations of and incubation times with AβO, RSV, and suramin were chosen. In addition, cells were treated by ZLN-005 (20 μmol/L) for 24 hours (as recommended by the manufacturer).

Figure 1 The viability of primary neurons exposed to oligomers of amyloid- β peptide (A β O), resveratrol (RSV), and/or suramin, as determined by CCK-8 reduction, and expression of peroxisome proliferator-activated receptor gamma coactivator 1- α (PGC-1 α) protein in the primary neurons or SH-SY5Y cells subjected to siRNA transfection [small interfering control (siControl)]. **A:** A β O (0 to 2 μ mol/L); **B:** RSV (0 to 150 μ mol/L); **C:** suramin (0 to 600 mg/mL); **D:** primary neurons subjected to siRNA transfection (5 to 24 hours); and **E:** SH-SY5Y cells subjected to siRNA transfection (5 to 7 hours). Data are expressed as means $\pm$ SD. $n = 6$ independent experiments. * $P < 0.05$, ** $P < 0.01$ versus untreated cells (controls) (analysis of variance, Student-Newman-Keul test).

PGC-1 α siRNA Transfection *in Vitro*

The cultured cells (neurons and SH-SY5Y cells) were washed twice with 2 mL of with PGC-1 α siRNA transfection medium, after which 0.8 mL of this medium containing the siRNA transfection reagent was added to each

tube. After that, the neurons were incubated for 5, 6, 7, 12, or 24 hours and SH-SY5Y cells for 5, 6, or 7 hours at 37°C in a CO₂ incubator. Thereafter, 2 mL of Neurobasal/B27 complete medium or Dulbecco's modified Eagle's medium was added and the cells incubated for an additional 18 to 24 hours.

Figure 2 Expression of silent mating-type information regulation 2 homolog 1 (SIRT1) protein in nucleus, cytoplasm, and mitochondria of human brain, mouse brain, and primary rat neurons. **A–E:** Immunofluorescence localization of SIRT1 in the neurons (**A**) and the hippocampus (**B** and **E**) and temporal cortex (**C** and **D**) of human brain (**B** and **C**) and mouse brains (**D** and **E**). The cell nuclei all stained blue (with DAPI), and SIRT1-positive neurons stained green (arrows). **F** and **G:** Nucleoprotein (**F**) and mitochondrial protein (**G**) expressions of Sirt1 are found in the brains of wild-type mice and in control primary rat neurons as determined by Western blotting. Scale bars: 25 μ m (**A**, **D**, and **E**, main images); 50 μ m (**B** and **C**; **A**, **D**, and **E**, insets). Original magnification: $\times 400$ (**A**, **D**, and **E**, main images); $\times 200$ (**B** and **C**, main images; **A**, **D**, and **E**, insets). COX, cytochrome c oxidase.

Figure 3 Expression of silent mating-type information regulation 2 homolog 1 (SIRT1) in human brains, mouse brains, and primary rat neurons. **A–D:** Immunohistochemical staining was performed for SIRT1 (brown) and senile plaques (gray-blue) in the hippocampus (**A** and **B**) and temporal cortex (**C** and **D**) of patients with Alzheimer disease (AD) (**B** and **D**) and control subjects (**A** and **C**). **Thin arrows** indicate SIRT1; **thick arrows**, senile plaques. **E:** Semiquantitative analysis of SIRT1 levels in the hippocampus and temporal cortex. **F–L:** The numbers of senile plaques detected in the hippocampus and temporal cortex (**F**) and the total protein (**K** and **L**), nucleoprotein (**G** and **I**), and mitochondrial protein (**H** and **J**) expressions of Sirt1 are found in the brains of *APP/PS1* mice at 6 or 10 months of age (**G**, **H**, and **K**) and in primary rat neurons with exposures to oligomers of amyloid- β peptide (A β O) (0.5 μ mol/L), resveratrol (RSV) (20 μ mol/L), or suramin (300 mg/mL) separately or combined treatments (**I**, **J**, and **L**) as determined by Western blotting. **M** and **N:** Sirt1 mRNA levels in the brains of *APP/PS1* mice at 6 or 10 months of age (**M**) and in primary rat neurons (**N**) with exposures to A β O (0.5 μ mol/L), RSV (20 μ mol/L), or suramin (300 mg/mL), separately or as combined treatments as determined by real-time PCR. Data are expressed as means $\pm$ SD. * P < 0.05, ** P < 0.01 versus the corresponding wild type (WT) or control animal; † P < 0.05, †† P < 0.01 versus *APP/PS1* or A β O animals (analysis of variance, paired-samples *t*-test or Student-Newman-Keul test). Scale bars = 50 μ m (**A–D**). Original magnification, $\times$ 200 (**A–D**). COX, cytochrome c oxidase.

Figure 4 Immunofluorescence of silent mating-type information regulation 2 homolog 1 (Sirt1) and the senile plaques in the hippocampus of mouse brains. **A–H:** Morphologic associations between senile plaques and Sirt1 are found in the hippocampus of the brains of *APP/PS1* mice at 6 (**A–D**) and 10 (**E–H**) months of age, as detected by confocal immunofluorescent staining. **A** and **E:** Wild-type (WT) mice, **B** and **F:** *APP/PS1* mice, **C** and **G:** *APP/PS1* plus resveratrol (RSV) group; **D** and **H:** *APP/PS1* plus suramin group. **Thin arrows** indicate cell nuclei (blue); **thick arrows**, SIRT1 (green); **arrowheads**, senile plaques (red). Data are shown in [Table 4](#). Scale bars = 50 μm. Original magnification, ×200.

Figure 5 Immunofluorescence of silent mating-type information regulation 2 homolog 1 (Sirt1) and the senile plaques in the temporal cortex of mouse brains. **A–H:** Morphologic associations between senile plaques and Sirt1 are found in the temporal cortex of the brains of *APP/PS1* mice at 6 (**A–D**) and 10 (**E–H**) months of age, as detected by confocal immunofluorescent staining. **A** and **E:** Wild-type (WT) mice; **B** and **F:** *APP/PS1* mice; **C** and **G:** *APP/PS1* plus resveratrol (RSV) group; **D** and **H:** *APP/PS1* plus suramin group. **Thin arrows** indicate cell nuclei (blue); **thick arrows**, SIRT1 (green); **arrowheads**, senile plaques (red). Data are shown in [Table 5](#). Scale bars = 50 μ m. Original magnification, $\times 200$.

Figure 6 Immunofluorescence of silent mating–type information regulation 2 homolog 1 (Sirt1) and amyloid- β peptide 1 to 42 ($A\beta_{1-42}$) in neurons. Expression of $A\beta_{1-42}$ and SIRT1 by primary rat neurons exposed to control (**A**), oligomers of amyloid- β peptide ($A\beta 0$) (**B**), $A\beta 0$ plus resveratrol (RSV) (**C**), and $A\beta 0$ plus suramin (**D**), as detected by confocal immunofluorescent staining. **Thin arrows** indicate cell nuclei (blue); **thick arrows**, Sirt1 (green); **arrowheads**, $A\beta 0$ (red). Data are expressed as means $\pm$ SD. $n = 6$ samples or 6 independent experiments (Table 5). Scale bar = 50 μ m. Original magnification, $\times 200$ (smaller images).

Assessing Spatial Learning and Memory in Mice

Spatial learning and memory in mice were evaluated using the Morris Water Maze test.³⁹ This maze consists of a circular pool (130 cm in diameter) with dark walls filled with tap water colored white by milk. An escape platform (9 cm in diameter) made of stainless steel with white walls is

submerged 0.5 cm below the surface of the water. During the navigation test, the time required for the mouse to locate the escape platform (escape latency) was determined with VideoTrack Software (ViewPoint Life Technologies, Carlsbad, CA), and after locating this platform, the animal was allowed to remain seated on it for 2 seconds. Mice who failed to find the platform within 60 seconds were guided

Table 4 Expression of Sirt1 in the Dentated Gyrus of Hippocampus and the Temporal Cortex of Mouse Brains at 6 and 10 Months of Age

Protein mice	<i>n</i>	Hippocampus (6 months)	Cortex (6 months)	Hippocampus (10 months)	Cortex (10 months)
WT	6	93.4 $\pm$ 6.65	74.8 $\pm$ 5.87	115 $\pm$ 10.2	76.5 $\pm$ 3.50
<i>APP/PS1</i>	6	43.1 $\pm$ 4.68**	51.7 $\pm$ 3.76**	74.7 $\pm$ 4.66**	52.4 $\pm$ 1.22**
<i>APP/PS1</i> plus RSV	6	59.5 $\pm$ 7.70**††	63.3 $\pm$ 1.53*†	106.5 $\pm$ 7.72††	73.8 $\pm$ 3.70††
<i>APP/PS1</i> plus suramin	6	27.3 $\pm$ 4.40**††	31.4 $\pm$ 5.19**††	38.3 $\pm$ 9.08**††	29.0 $\pm$ 4.53**††

Data are expressed as means $\pm$ SD of 6 independent experiments.

* $P < 0.05$, ** $P < 0.01$ versus WT animals; † $P < 0.05$, †† $P < 0.01$ versus *APP/PS1*.

RSV, resveratrol; SIRT1, silent mating–type information regulation 2 homolog 1; WT, wild type.

Table 5 Expression of Sirt1 by Primary Rat Neurons

Treatment	n	SIRT1
None (control)	6	245 ± 11.3
Aβ0	6	138 ± 6.03*
Aβ0 plus RSV	6	162 ± 7.76*†
Aβ0 plus suramin	6	122 ± 6.28*†

SIRT1 data are expressed as means ± SD of 6 independent experiments.
**P* < 0.01 versus control; †*P* < 0.05 versus Aβ0.
Aβ0, oligomers of amyloid-β peptide; RSV, resveratrol; SIRT1, silent mating–type information regulation 2 homolog 1.

there and then allowed to remain for 2 seconds as well, with their escape latency being recorded as 60 seconds. Each mouse was subjected to 4 such trials daily for 4 days with a 5- to 7-minute interval of rest between trials, and the results of the 4 four trials on each day were averaged for statistical analysis. Furthermore, on day 5, when the platform had been removed, the time required to first cross its original site was recorded. All these behavioral tests were conducted in a quiet environment with subdued lighting.

Levels of SIRT1 and Senile Plaques in Human Postmortem Brains Determined by Immunohistochemical Staining

Immunohistochemical staining for senile plaques in brain tissue from patients with AD was performed as described previously.⁴⁰ In brief, sections were first heated at 58°C for 1 hour and thereafter deparaffinized and hydrated. After three washes in phosphate-buffered saline (PBS) and microwaving in 0.01 mol/L citric acid buffer (pH 6.0) for 20 minutes to achieve antigen repair, the sections were placed in blocking buffer (Agilent-Dako, Santa Clara, CA) for 30 minutes at

room temperature. Subsequently, they were incubated at 4°C overnight with anti-Aβ (mouse, diluted 1:100) or anti-SIRT1 (rabbit, diluted 1:100). The next day, these sections were incubated for 1 hour with secondary antibody [ie, biotinylated goat anti-mouse IgG or biotinylated goat anti-rabbit IgG (diluted 1:200 in PBS with Tween 20) for 60 minutes at room temperature]. Subsequently, the sections were incubated with the avidin-biotinylated enzyme complex and then placed in peroxidase reaction solution that contained diaminobenzidine. As a negative control, sections were processed in the same manner but without incubation with primary antibody. The senile plaques in these sections were also quantified as described previously.⁴⁰ The numbers of cells in three random fields (×100) of the hippocampus and temporal cortex staining positively for SIRT1 were determined with the Image Pro Plus software version 6.0 (Media Cybernetics Inc., Rockville, MD).

Electron Microscopic Examination of the Subcellular Morphology of the Cortex of Murine Brains

Blocks of cortical tissue (1 mm³) from the murine brain were prefixed for 2 hours at 44°C in 4% paraformaldehyde solution that also contained 0.1 mol/L phosphate buffer (pH 7.3) and 2.5% glutaraldehyde. The tissue was then rinsed in this same buffer and postfixed in 1% osmium tetroxide in 0.1 mol/L phosphate buffer (pH 7.3). Postfixation was followed by dehydration in ethanol, embedding in Epon 812 and polymerization. Thereafter, the samples were sectioned with an LKB 2088 ultramicrotome, stained with 1% uranyl acetate and lead citrate, and examined under a transmission electron microscope (JEM-2000 EXII, 80 KV, Jeol Ltd., Tokyo, Japan).

Figure 7 Electron microscopic examination of mitochondria. **A:** 6-month-old wild-type (WT) mice. **B:** 6-month-old *APP/PS1* mice; **C:** 6-month-old *APP/PS1* mice treated with resveratrol (RSV). **D:** 6-month-old *APP/PS1* mice treated with suramin. **E:** 10-month-old WT mice. **F:** 10-month-old *APP/PS1* mice. **G:** 10-month-old *APP/PS1* mice treated with RSV. **H:** 10-month-old *APP/PS1* mice treated with suramin. Original magnification, ×15,000. M, mitochondria; N, nucleus.

Figure 8 Expression of α - and β -secretase proteins in mouse brains and primary rat neurons. A disintegrin and metallopeptidase domain 10 (Adam10) (A and B), beta-secretase 1 (Bace1) (C and D), and beta-secretase 2 (Bace2) (E and F) protein expressions are shown in the brains of *APP/PS1* mice at 6 or 10 months of age (A, C, and E) and by primary rat neurons (B, D, and F) with exposures to oligomers of amyloid- β peptide ($A\beta$) (0.5 μ mol/L), resveratrol (RSV) (20 μ mol/L), or suramin (300 mg/mL) separately or as combined treatments as determined by Western blotting. Data are expressed means $\pm$ SD. $n = 6$ samples or 6 independent experiments. * $P < 0.05$, ** $P < 0.01$ versus the corresponding wild-type or control animal; $^{\dagger}P < 0.05$, $^{\dagger\dagger}P < 0.01$ versus *APP/PS1* or $A\beta$ animals (analysis of variance, Student-Newman-Keul test).

Protein Levels of Sirt1, Ogg1, Pgc-1 α , Adam10, Bace1, and Bace2 in Mouse Brains or Primary Cultured Neurons and Sirt1 in Isolated Nucleus and Mitochondria Determined by Western Blotting

Primary cultured neurons or brain tissue from mice (0.05 or 0.1 g) was homogenized in PBS that contained a complete mixture of protease inhibitors using a glass homogenizer. This homogenate was then centrifuged at $12,000 \times g$ for 40 minutes at 4°C ; in addition, the nucleoprotein and mitochondrial protein in primary cultured neurons or brain tissue were extracted from mice (0.05 or 0.1 g; BestBio), and the protein concentration of the resulting supernatant was determined with the BCA protein assay kit (Thermo Fisher Scientific). Next, these proteins were subjected to 10% SDS-PAGE and then blotted onto polyvinylidene difluoride membranes with a transfer unit (Bio-Rad Laboratories, Hercules, CA). For quantification of SIRT1, these

polyvinylidene difluoride membranes were incubated thereafter with anti-SIRT1, anti-OGG1, anti-PGC-1 α , anti-ADAM10, anti-BACE1, and anti-BACE2 antibodies diluted 1:1000, 1:500, 1:600, 1:1000, 1:500, 1:800, respectively, at 4°C overnight. After washing, the membranes were incubated with horseradish peroxidase-conjugated anti-rabbit IgG (1:10,000) or anti-mouse IgG (1:5000) for 60 minutes. Finally, these membranes were incubated in Electrochemiluminescence Plus reagent for 30 seconds to 5 minutes and the signals thus obtained visualized by exposure to Hyper Performance Chemiluminescence film.

Levels of Sirt1 mRNA Determined by Quantitative Real-Time PCR

Total RNA was isolated from primary cultured neurons or brain tissue of mice with the Trizol reagents (Invitrogen, Carlsbad, CA), using DNase I to remove residual

Figure 9 Expressions of 8-oxoguanine DNA glycosylase-1 (Ogg1) and peroxisome proliferator-activated receptor gamma coactivator 1- α (Pgc1- α) in mouse brains and primary rat neurons. Ogg1 (**A** and **B**) and Pgc1 α (**C** and **D**) protein expressions in the brains of *APP/PS1* mice at 6 and 10 months of age (**A** and **C**) and in primary neurons (**B** and **D**) exposed to oligomers of amyloid- β peptide (A β O) (0.5 μ mol/L), resveratrol (RSV) (20 μ mol/L), or suramin (300 mg/mL) as determined by Western blotting. Data are expressed as means $\pm$ SD. $n = 6$ mice or independent experiments. * $P < 0.05$, ** $P < 0.01$ versus the corresponding wild-type animals or untreated cells (controls); † $P < 0.05$, †† $P < 0.01$ versus *APP/PS1* or A β O animals (analysis of variance, Student-Newman-Keul test).

genomic DNA. Thereafter, 3 μ g of total RNA was converted with the first-strand cDNA synthesis kit (Promega Corp., Madison, WI) and oligo-d (T)18 primers following the protocol recommended by the manufacturer. The real-time PCR primers for target transcripts were designed on the basis of the complete cDNA sequences in GenBank (<https://www.ncbi.nlm.nih.gov>; GenBank accession numbers NM_001372090.1, NM_001159589.2, NM_031144.3, and NM_007393.5) (Table 3). Quantitative real-time PCR was performing using the ABI PRISM 7300 Sequence Detection System (Applied Biosystems) and the results analyzed with the GeneAmp 7300 Sequence Detection Software version 1.4 (Applied Biosystems). In brief, the 10- μ L reaction mixture contained 1 μ L of first-strand cDNA, 5 μ L of 2 $\times$ SYBR Green Master (Rox) Mix, 0.5 μ L each of the forward and reverse primers (10 mol/L), and 3 μ L of DNase and RNase-free H₂O. The thermal cycling conditions were 2 minutes at 50°C and 10 minutes at 95°C, followed by 40 cycles at 95°C for 15 seconds and then 1 minute at 60°C. The levels of the *SIRT1* and A β transcripts were calculated as $2^{-\Delta\Delta CT}$, where

ΔCT represents the difference between the threshold cycle values for the target gene and A β .

Levels of Sirt1 and Senile Plaques in Murine Brain Tissues and SIRT1 in Primary Neurons Determined by Immunofluorescence

Double immunofluorescent labeling of A β and SIRT1 involved pretreatment of the sections with 0.3% Triton X100 for 10 minutes, followed by incubation in 2% fetal bovine serum in 1 $\times$ PBS with Tween 20. Subsequently, the sections were incubated with a cocktail of primary antibodies (anti-6E10/anti-SIRT1) overnight at 4°C, followed by a secondary antibody cocktail that consisted of Cy3-conjugated goat anti-mouse and 488-conjugated goat anti-rabbit antibodies (each diluted 1:300). As a control, the primary serum was omitted, resulting in no detectable staining. The morphologic association between senile plaques and SIRT1 was monitored with a laser scanning confocal microscope (Olympus, Tokyo, Japan).

Figure 10 Learning ability and spatial memory of mice in different groups. **A:** Escape latency for each group. **B:** Number of times the target quadrant was crossed by each group. **C:** Time spent in the target quadrant by each group. Data are expressed as means $\pm$ SD. $n = 6$ trials in each case. * $P < 0.01$ versus wild-type animals; † $P < 0.05$, †† $P < 0.01$ versus APP/PS1 animals (analysis of variance, Student-Newman-Keul test).

Content of NAD⁺ in Primary Cultured Neuron and Brain Tissue

The primary cultured neurons or brain tissue (0.05 or 0.1 g) was homogenized in 1× PBS that contained

complete protease inhibitors in Epp and the content of NAD⁺/hydroxyacyl-coenzyme A dehydrogenase then measured with the appropriate kits (KeyGen BioTech).

Characterization of Apoptosis Detected by Flow Cytometry

The cultured cells were washed twice with PBS before centrifugalization and suspension in 1× binding buffer. Annexin V–fluorescein isothiocyanate (5 μ L) was mixed with 400 μ L of suspension that contained 1×10^6 cells, followed by incubation at 4°C for 15 minutes. Then, 10 μ L of propidium iodide was added for 5 minutes. Finally, the scatter parameters of the cells (10,000 cells per experiment) were analyzed using the FACS Calibur system (Becton Dickinson, Franklin Lakes, NJ).

Statistical Analysis

The values for the different groups are expressed as means $\pm$ SD. Statistical analysis was performed with analysis of variance, followed by the Student-Newman-Keuls test or the two-paired t -test using the SPSS software version 22.0 (SPSS Inc., Chicago, IL).

Results

Viability of Primary Rat Neurons Exposed to A β 0, RSV, or/and Suramin

As assessed by the CCK-8 test, the viability of the primary hippocampal neurons was significantly reduced on treatment with ≥ 0.5 μ mol/L of A β 0 (Figure 1A) for 48 hours or with ≥ 20 μ mol/L of RSV (Figure 1B) or ≥ 300 mg/mL of suramin (Figure 1C) for 24 hours. In this study, suramin was used as an opposite reagent for the effect of RSV because it can target the nicotinamide-binding pocket to inhibit the activity of sirtuins.

Protein Expression of PGC-1 α in the Primary Neurons or SH-SY5Y Cells Subjected to siRNA Transfection

As determined by Western blotting, the level of PGC-1 α protein was significantly reduced on siRNA transfection for 24 hours in the primary neurons (Figure 1D) and for 7 hours in SH-SY5Y cells (Figure 1E), which was selected as an appropriate treatment time for the cell cultures.

Distribution of SIRT1 Expression in Neurons of Human or Mouse Brains and Primary Cultured Neurons

SIRT1 was widely distributed in the nucleus and cytoplasm of the primary cultured neurons (Figure 2A) and neurons of human brains (Figure 2, B and C) determined by immunofluorescence or immunohistochemical staining, respectively.

Figure 11 The amount of nicotinamide adenine dinucleotide (NAD⁺) in the brains of *APP/PS1* mice and primary rat neurons. **A:** *APP/PS1* mice at 6 and 10 months of age. **B:** Primary rat neurons exposed to oligomers of amyloid- β peptide (A β 0) (0.5 μ mol/L), resveratrol (RSV) (20 μ mol/L), or suramin (300 mg/mL). Data are expressed as means $\pm$ SD. $n = 6$ mice or independent experiments. * $P < 0.05$, ** $P < 0.01$ versus the corresponding wild-type animals or untreated cells (controls); † $P < 0.05$, †† $P < 0.01$ versus *APP/PS1* animals or treatment with A β 0 (analysis of variance, Student-Newman-Keul test).

In the hippocampus (Figure 2D) and cortex (Figure 2E) of mouse brains, SIRT1 was located in the nucleus of neurons of these regions as measured by immunofluorescence. Furthermore, SIRT1 was expressed in the nucleus (Figure 2F) and mitochondria (Figure 2G) of neurons extracted from mouse brains and the primary cultured neurons as determined by Western blotting.

Decreased Expression of SIRT1 in Brains of AD Patients and *APP/PS1* Mutation Mice and Primary Neurons Exposed to A β 0 Compared with Controls

Compared with age-matched controls (Figure 3, A and C), the expressions of SIRT1 in AD brains (Figure 3, B and D) were significantly decreased (Figure 3E). Meanwhile, large numbers of senile plaques were seen in AD brains (Figure 3, B, D, and F). In the brain tissues of *APP/PS1* mutation mice compared with WT mice, SIRT1 levels were significantly decreased in the nucleus (Figure 3G) and mitochondria (Figure 3H) at 6 and 10 months of age. The similar changes of SIRT1 in the nucleus (Figure 3I) and mitochondria (Figure 3J) extracted from primary neurons exposed to A β 0 were observed compared with untreated cells.

Attenuation of RSV but Enhancement of Suramin on the Decreased Expression of Sirt1 in Mouse Brains with *APP/SP1* Mutation and Primary Neurons Exposed to A β 0

Interestingly, the decreased levels of Sirt1 in *APP/SP1* mouse brains at 6 or 10 months of age (Figure 3K) or primary neuron exposure to A β 0 (Figure 3L) were significantly attenuated by the treatment of RSV but enhanced by suramin. The corresponding changes of Sirt1 mRNA were obviously found in *APP/SP1* mouse brains at 6 or 10 months of age (Figure 3M) or primary neuron exposure to A β 0 (Figure 3N). Furthermore, the attenuation of RSV but enhancement of suramin on the decreased level of Sirt1 in the hippocampus (Figure 4) or cortex (Figure 5) of *APP/SP1* mouse brains at 6 or 10 months of age as well as primary neuron exposure to A β 0 (Figure 6) were also obviously

observed by the detection of immunofluorescence (Tables 4 and 5).

Ultrastructural Changes in the Mitochondria of Mouse Neurons as Revealed by Electron Microscopy

The nuclei of neurons in WT mice were round, with distinct membranes and well-distributed chromatin (Figure 7, A and E). In the temporal cortex or hippocampus of *APP/PS1* mice at 6 and 10 months of age, the membrane of mitochondria was ruptured, their cristae disrupted or absent, and the electron density of their matrix lowered (Figure 7, B and F). In *APP/SP1* mice 10 months of age, there were some U-shaped or megamitochondria, and the distribution of nuclear chromatin was abnormal, occasionally being gathered at the perimeter. Furthermore, RSV attenuated (Figure 7, C and G) but suramin (Figure 7, D and H) augmented these nuclear and mitochondrial alterations.

Expression of Adam10, Bace1, and Bace2 in Mouse Brains and Primary Neurons

As determined by Western blotting, the levels of the Adam10 (Figure 8A) and Bace2 (Figure 8E) proteins in the brains of the *APP/PS1* mice at 6 and 10 months of age were significantly lower than in WT mice, whereas the level of Bace1 (Figure 8C) was higher. Similar changes occurred in primary neurons exposed to A β 0 (Figure 8, B, D, and F). Furthermore, RSV attenuated and suramin enhanced these changes as well (Figure 8).

Expression of Ogg1 and Pgc-1 α in Mouse Brains and Primary Rat Neurons

As determined by Western blotting, the levels of the Ogg1 (Figure 9A) and Pgc-1 α (Figure 9C) proteins in the brains of the *APP/PS1* mice at 6 and 10 months of age were significantly lower than in WT mice. Similar changes occurred in primary neurons exposed to A β 0 (Figure 9, B and D). Furthermore, RSV attenuated and suramin enhanced these changes as well (Figure 9).

Figure 12 Apoptotic death among primary rat neurons and SH-SY5Y cells exposed to oligomers of amyloid- β peptide (A β O), resveratrol (RSV), suramin, or siRNA [small interfering control (siControl)] transfection. Apoptotic death among primary rat neurons exposed to A β O, RSV, or suramin (**A**: control; **B**: A β O; **C**: A β O plus RSV; **D**: A β O plus suramin; and **E**: Statistics of the apoptotic death among primary rat neurons); SH-SY5Y cells (**F** and **G**) and primary rat neurons (**H** and **I**) exposed to ZLN005 (0.5 μ M/L), suramin (300 mg/mL), RSV (20 μ M/L) with peroxisome proliferator-activated receptor gamma coactivator 1- α (PGC-1 α) siRNA (siPGC-1 α) transfection. Data are expressed as means $\pm$ SD. $n = 3$ independent experiments. * $P < 0.05$, ** $P < 0.01$ versus untreated cells (control); † $P < 0.05$, †† $P < 0.01$ versus primary rat neurons treated with A β O (analysis of variance, Student Newman-Keul test).

Spatial Learning and Memory

The escape latency time (Figure 10A) of *APP/PS1* mice at 6 and 10 months of age was clearly longer than that of WT animals, indicating impaired capacity for spatial learning. During the probe trial (when the platform in the target quadrant had been removed), the *APP/PS1* mice spent less time in the target quadrant and more in the peripheral regions (Figure 10, B and C), demonstrating cognitive damage. In addition, the number of times the platform was crossed and the time spent at the position of the platform

were lower in 6- and 10-month-old *APP/PS1* mice, suggesting poorer memory. Interestingly, treatment of the transgenic mice with RSV attenuated this impaired learning and memory, whereas suramin augmented it.

The Amount of NAD⁺ in the Brains of *APP/PS1* Mice and Primary Rat Neurons

The amount of NAD⁺ in brains isolated from *APP/PS1* mice (Figure 11A) and in primary rat neurons treated with

Figure 13 Proposed neuroprotective role of silent mating-type information regulation 2 homolog 1 (SIRT1)—peroxisome proliferator-activated receptor gamma coactivator 1- α (PGC-1 α) in neurons affected by Alzheimer disease (AD). SIRT1 may be located in the nucleus, cytoplasm, or mitochondria. In the pathogenesis of AD, the increased level of amyloid- β peptide (A β) can inhibit the production of extracellular NAD⁺, which can decrease the expression of SIRT1 and result in the damage of neurons. The stimulation of SIRT1 by resveratrol (RSV) may increase the expression of PGC-1 α by migrating SIRT1 from the nucleus to the cytoplasm. On the one hand, this stimulation promotes the nonamyloid metabolic process of A β precursor protein (APP); on the other, it could simultaneously act on mitochondrial DNA damage-related proteins [8-oxoguanine DNA glycosylase-1 (OGG1)] and mitochondrial SIRT1, reducing mitochondrial damage and apoptosis. Furthermore, exposure to SIRT1—PGC-1 α may play a neuroprotective role by decreasing the formation of A β and against the production of apoptosis in the pathogenesis of AD.

A β O (Figure 11B) was significantly lower than that in corresponding WT animals and untreated cells, respectively. Interestingly, treatment of the transgenic mice with RSV attenuated, whereas suramin augmented these differences (Figure 11).

Apoptosis in Primary Rat Neurons Exposed to A β O and RSV or Suramin and among Cultured Cells Subjected to siRNA Transfection

The extent of apoptotic death among primary rat neurons incubated with 0.5 μ mol/L of A β O increased in a time-dependent manner (Figure 12, A–E), and this increase was attenuated or augmented by pretreatment with 20 μ mol/L of RSV or 300 mg/mL of suramin, respectively, for 24 hours (Figure 12, A–E). The extent of apoptotic death among cultured cells was significantly decreased on activation of Pgc-1 α by inhibiting Sirt1 (Figure 12, F–I). However, this effect was not attenuated by reactivation of Sirt1 (Figure 12, F–I).

Discussion

AD is a progressive and neurodegenerative disorder of the cortex and hippocampus, which eventually leads to cognitive impairment. Although the cause of AD remains unclear, the presence of A β peptides in these learning and memory regions is a hallmark of AD.⁴¹ In the current investigation, we used postmortem brain tissue from patients with AD, APP/PS1 double-transgenic mice, and primary neurons exposed to A β O as models of AD to determine the neurotoxicity and examine the potential protective role of SIRT1, which plays an important role in the regulation of senescence⁴² and in connection with neurodegenerative diseases.⁵

The protocols used successfully produced mice with elevated expression of APP and PS1 and isolated neurons from the hippocampal region of rat brains with a purity of approximately 90%.

Although these mechanisms remain partly obscure, the activation of sirtuins, notably SIRT1, may be a critical step in the pathogenesis of this disease; therefore, its modulation may aid in the prevention of AD.⁴¹ In this context, there is evidence of a connection between sirtuins and AD.⁴³ Initially, studies in caloric restriction verified that the overexpression of SIRT1 reduced AD pathologic findings,⁴⁴ wherein decreased formation of A β protein and senile plaques in transgenic mice was observed.⁴⁵ These findings of lowered expression of SIRT1 proteins in these models of AD are consistent with previous reports.⁴⁶ Previous reports indicated that the loss of SIRT1 might be linked to the number of senile plaques.²⁷ Later, it was demonstrated that mice overexpressing SIRT1 significantly reduced their A β production, whereas cognitive deficits in SIRT1 knockout mice were aggravated.⁴⁷

Numerous studies have revealed an elevated content of A β ₁₋₄₂ and activity of β -secretase in the brains of patients with AD, as well as decreases in α -secretase (particularly ADAM10).⁴⁸ Our present results indicate that SIRT1 is involved in the amyloid cascade hypothesis linked to the pathogenesis of AD. The reductions in SIRT1 mRNA and protein levels observed in the brains of patients with AD are correlated with the stage and duration of the disease.⁴⁹ SIRT1 shifts the balance between amyloidogenic and non-amyloidogenic processing of APP *in vitro* and in transgenic mouse models.⁵⁰

Many studies have found that RSV has antioxidant, anti-inflammatory, and neuroprotective properties and can decrease the toxicity and aggregation of A β in the hippocampus of patients with AD, promote neurogenesis, and

prevent hippocampal damage; in addition, the antioxidant activity of RSV plays an important role in neuronal differentiation through the activation of SIRT1.⁴¹ It was also verified that RSV, acting as a sirtuin-activating compound, attenuated A β -induced cell death,⁵¹ strengthened A β clearance,⁵² and barred memory loss by decreasing cognitive impairment.^{51,53} These results are consistent with our findings. In addition, in our primary results, senile plaques were not formed in the brains of *APP/PS1* mice before 4 months of age but gradually appeared at 5 to 6 months (data not shown); therefore, the mice were treated at 4 and 8 months with RSV and suramin for 2 months, respectively, to observe the effect of RSV on the prevention and treatment of senile plaques. In the present study, the *APP/PS1* mice that reached the age of 6 months had only a few senile plaques after 2 months of treatment with RSV, whereas animals 10 months of age had significantly fewer senile plaques than the untreated *APP/PS1* mice, suggesting that RSV reduces the formation of senile plaques in brains by activating SIRT1.

Aggregated A β plays a key role in the pathogenesis of AD and is associated with mitochondrial damage and memory deficits.⁴¹ In the navigation test of this study, *APP/PS1* mice performed more poorly than WT animals, indicating impaired spatial learning. On day 5, when the platform was removed, the time spent at its original location and the number of times this site was crossed were both less in the *APP/PS1* mice, implying impairment of long-term memory. The learning and memory abilities of these mice decreased with increasing age and the number of senile plaques. These results are similar to those reported by others.⁵⁴

Inhibition of aggregation of A β has been considered the primary therapeutic strategy for treatment of AD. In accordance with previous findings on the association between sirtuins and A β in connection with the pathogenesis of AD, enhanced Sirt1 expression improved the learning and memory of *APP/PS1* mice by reducing accumulation of A β . SIRT1 modulates learning and memory by regulating the expression of the cAMP response binding protein or attenuate mitochondrial dysfunction and protect neurons against A β -induced disruption of spatial learning and memory.⁵⁵ Some researchers have found that pharmacologic activation of SIRT1 with NAD⁺ promoted α -secretase activity and attenuated generation of A β peptides *in vitro*.⁵⁶ Our studies found that RSV activated SIRT1 by changing the level of NAD⁺ as a substrate to catalyze the deacetylation of various substrates and then promoted the α -secretase non-amyloidogenic pathway of APP processing, attenuating A β generation.

In recent years, numerous studies have reported on the wide spectrum of sirtuins involved in key processes that can play a role in the development of AD, such as mitochondrial damage, neuronal apoptosis, and oxidative stress.⁵⁷ Furthermore, Sirt1 also plays an important role in exerting its beneficial influence via PGC-1 α .⁵⁸ The up-regulation of

Sirt1 and Pgc-1 α expression improved learning and memory abilities in rats with AD.⁵⁹ PGC-1 α is a key regulator of mitochondrial biogenesis⁶⁰ and is also involved in apoptosis.⁶¹ SIRT1 is primarily found in the nucleus of most cell types; however, it appears to possess both nuclear localization signals and nuclear export signals and could shuttle between the cytoplasm and the nucleus.¹⁵ This study confirmed that Sirt1 is mainly located in the nucleus of neurons from mouse brains but can be found in both the nucleus and cytoplasm in neurons of human brains and primary cultured neurons. Also, SIRT1 was located in nucleus and mitochondria extracted from mouse brains or primary neurons, which indicates that the subcellular level of SIRT1 may be related to different cell types.⁵⁶ On the other hand, SIRT1 has cytosolic substrates whose deacetylation by SIRT1 results in distinct physiologic or functional consequences.⁶²

SIRT1, a NAD-dependent protein deacetylase, is controlled by NAD⁺/NADH levels and plays an important role in deacetylation of PGC-1 α , a key transcription coactivator regulating energy metabolism.⁶³ In general, PGC-1 α normally remains in the acetylated form (unactivated) in cells; however, during exercise or muscle contraction, SIRT1 deacetylates PGC-1 α to an activated form, which then activates energy metabolism-related gene transcription.^{63,64} Elevated intracellular NAD⁺/NADH levels appear; his increase of SIRT1 expression and PGC-1 α deacetylation also play regulatory roles in energy metabolism and mitochondrial biogenesis.⁶⁵ SIRT1 and its substrate PGC-1 α regulate aspects of energy metabolism through the mitochondria, but this was initially thought to occur through their influence on nuclear localized transcription. An interesting aspect of SIRT1's activity and function that is less well defined is its extranuclear localization, particularly at the mitochondria.⁶⁶ These conclusions are similar to those of our studies, in which the expression of SIRT1 was not only in the nucleus but also in the mitochondria detected by Western blotting. Impaired PGC-1 α -mediated functions in combination with low levels of SIRT1 may contribute to the pathogenesis of AD,⁶⁷ and A β ₂₅₋₃₅-induced impairment of mitochondrial biogenesis may be associated with the inhibition of the SIRT1–PGC-1 α pathway.⁶⁸ In the hippocampus, PGC-1 α is induced by SIRT1⁶⁹ and maintained mitochondrial function. These findings are similar to those documented here. Importantly, NAD⁺ intermediate supplementation appears to restore NAD⁺ levels in both nuclear and mitochondrial compartments of cells. In one study, aging was found to trigger SIRT1 inactivation, which was reversed by supplementation of an NAD⁺ deficiency in the nuclear/cytosolic pool.⁷⁰ SIRT1 expression can alter the transcriptional activity of the mitochondrial biogenesis coactivator PGC-1 α . In addition, SIRT1 and PGC-1 α directly interact and can be co-immunoprecipitated as a molecular complex.²⁹ However, whether PGC-1 α has a direct effect on SIRT1 and produces corresponding biological effects has not been confirmed by this study.

Mitochondrial biogenesis involves the transcription of both nuclear and mtDNA-encoded genes and is orchestrated by the PGC-1 family of transcriptional coactivators, and PGC-1 α , in particular, is often cited as a master regulator of this process.⁷¹ The human OGG1 is the major DNA glycosylase responsible for the repairment of 7,8-dihydro-8-oxoguanine and ring-opened fapyguanine, critical mutagenic DNA lesions that are induced by reactive oxygen species, and acetylation of OGG1 plays an important role in response to DNA damage.⁷² OGG1 is enzyme-specific for incising 7,8-dihydro-8-oxoguanine to avoid the transversion of GC $\rightarrow$ TA or mtDNA damage-induced apoptosis.⁷³ OGG1 can rescue neurons subjected to ischemic conditions, and acetylation of OGG1 can increase the activity of the enzyme nearly 10-fold in a cell culture.⁷⁴ In the present study, a lowered expression of OGG1 protein was observed in AD models, and inhibition of SIRT1 induced by A β may result in reduced OGG1, leading to apoptosis and the low abilities of learning and memory. In addition, changing SIRT1 expression by the exposure of RSV or suramin affected the expression of OGG1. It has been reported that SIRT1 is the major acetyltransferase for OGG1 acetylation⁷² and can also activate PGC-1 α .⁷⁵ Other research indicates that p300/SIRT1 regulates OGG1 through acetylation/deacetylation and reduces oxidative damage by rescuing OGG1 activity and plaque development.⁷⁶ On the other hand, based on these results, we suspect that PGC-1 α may affect OGG1 expression through p300 protein. However, the interaction between OGG1 and the SIRT1–PGC-1 α pathway still needs to be further confirmed.

On the basis of the above observations, RSV and suramin, which influence the pressure of downstream proteins by activating or inhibiting SIRT1, were used in this study. The increased expression of PGC-1 α due to the elevated SIRT1 improves mitochondrial morphologic features and decreases neuronal apoptosis. Meanwhile, the changed levels of α - and β -secretase in the study agree with previous reports.^{27,28}

To further prove whether PGC-1 α has a key link to mitochondrial damage with lower SIRT1 expression and the subsequent apoptosis, cells were subjected to transfection of silent PGC-1 α mRNA or appropriate agonists of PGC-1 α . Interestingly, the extent of apoptotic death was lowered after activation of PGC-1 α or SIRT1, whereas these changes were not reversed by SIRT1 reactivation in the cultured cells transfected with siPGC-1 α , which indicates that the decreased apoptosis may be initially regulated by the activation of PGC-1 α , thereby maintaining neuronal mitochondrial biogenesis and memory.

In conclusion, the level of SIRT1 in brain tissues from patients with AD and *APP/PS1* mice, as well as in primary neurons exposed to A β O, was decreased, including the levels of SIRT1 proteins in nuclear or mitochondria. Overexpression of *APP/PS1* exhibited impaired learning and memory of mice; more senile plaques, disrupted membranes, and broken or absent cristae of mitochondria in the

brain; decreased levels of ADAM10, BACE2, OGG1, PGC-1 α , and NAD⁺; and increased levels of BACE1 and apoptosis. Interestingly, these changes were attenuated significantly by treatment with RSV but enhanced by suramin. By activating PGC-1 α but inhibiting SIRT1, the extent of apoptotic death in cells was significantly decreased; however, by activating SIRT1 but inhibiting PGC-1 α with small interfering PGC-1 α , these levels remained unchanged. These findings indicate that SIRT1 may protect against the neurotoxicity associated with AD, which might involve the regulation of PGC-1 α (Figure 13).

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