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SIRT1 Suppresses β-Amyloid Production by Activating the α-Secretase Gene ADAM10

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SUMMARY

A hallmark of Alzheimer's disease (AD) is the accumulation of plaques of Aβ 1–40 and 1–42 peptides, which result from the sequential cleavage of APP by the β and γ-secretases. The production of Aβ peptides is avoided by alternate cleavage of APP by the α and γ-secretases. Here we show that production of β-amyloid and plaques in a mouse model of AD are reduced by overexpressing the NAD-dependent deacetylase SIRT1 in brain, and are increased by knocking out SIRT1 in the brain. SIRT1 directly activates the transcription of the gene encoding the α-secretase, ADAM10. SIRT1 deacetylates and coactivates the retinoic acid receptor β, a known regulator of ADAM10 transcription. ADAM10 activation by SIRT1 also reduces the Notch pathway, which is known to repair neurons in the brain. Our findings indicate SIRT1 activation is a viable strategy to combat AD and perhaps other neurodegenerative diseases.

INTRODUCTION

Alzheimer's disease (AD) is a neurodegenerative disorder affecting one-third of individuals reaching the age of 80 (Tanzi and Bertram, 2005). AD displays a stereotypical brain pathology; deposition of plaques of amyloid beta (Aβ) peptides and phosphorylated tangles of the neurofibrillar protein τ (Hardy and Selkoe, 2002). Affected individuals suffer neurological damage resulting in memory loss, cognitive, and functional decline and death.

Insight into the etiology of AD has come from studies of the rare familial form of early onset AD (Selkoe, 1997). Dominant mutations have been found in the gene encoding the neuronal membrane protein amyloid precursor protein (APP), which can be cleaved in two sequential steps by the β- and γ-secretases to generate Aβ 1–40 and 1–42 amyloid peptides (Tanzi and Bertram, 2005). A second class of dominant mutations giving rise to familial AD fall in genes presenilin 1 and 2 (PSEN1 and 2), which encode components of the γ-secretase (De Strooper, 2007). These findings suggest a pathway of AD in which sequential cleavage of APP by the β and γ-secretases leads to accumulating β-amyloid and the downstream pathology of AD, such as Aβ plaques, τ tangles, and neurodegeneration.

Interestingly, the production of Aβ peptides is avoided by an alternate APP cleavage pathway mediated by the α-secretase followed by γ-secretase (Postina et al., 2004). Indeed, α-secretase cleavage of APP has been shown to be protective in AD models (Mattson, 1997; Kojro and Fahrenholz, 2005). The α- and γ-secretases also sequentially cleave the notch receptor to generate a notch intracellular domain (NICD) (Kojro and Fahrenholz, 2005; van Tetering et al., 2009). The NICD activates nuclear genes, such as HES1 and HES5 to facilitate neurogenesis during development and damage repair in adults (Kopan and Ilagan, 2009; Yoon and Gaiano, 2005; Costa et al., 2005). ADAM10 encodes the α-secretase (Saftig and Hartmann, 2005; Fahrenholz et al., 2008), and transcription of this gene is activated by the retinoic acid receptor (RAR) (Fahrenholz et al., 2008; Prinzen et al., 2005; Tippmann et al., 2009). Indeed, several studies suggest a link between retinoic acid (RA) signaling in the brain and AD (Goodman and Pardee, 2003; Corcoran et al., 2004; Goodman, 2006).

Sirtuins are NAD-dependent deacetylases that counter aging and have a wide spectrum of metabolic and stress-tolerance functions (Sinclair, 2005). Of the seven mammalian sirtuins, the SIR2 ortholog SIRT1 deacetylates numerous regulatory proteins, such as PGC-1α, p53, FOXO, HSF, and HIF-2α to trigger resistance to metabolic, oxidative, heat, and hypoxic stress (Guarente, 2009). SIRT1 has been directly implicated in neuronal protection against stress in cultured cells (Qin et al., 2006). In mice, SIRT1 has been shown to protect against neurodegeneration in the p25 overexpression model (Kim et al., 2007), as well as in Wallerian degeneration slow mice (Araki et al., 2004). More generally, SIRT1 mediates at least some of the effects of calorie restriction (Guarente, 2008), a diet reported to protect against models of neurodegenerative diseases, including AD (Patel et al., 2005).

Here, we investigate whether SIRT1 levels in brain affect the Aβ plaque formation, pathology, and cognitive decline in AD mouse model (APPswe/PSEN1dE9 double transgenic). We show that SIRT1 can suppress AD in a mouse model for this disease. The induction of brain pathology and behavioral deficits was mitigated in AD mice overexpressing SIRT1 in brain, and exacerbated with SIRT1 knocked out in the brain. SIRT1 directly activates transcription of ADAM10, which encodes the α-secretase by deacetylating RARβ. SIRT1 appears to direct APP processing toward the α-secretase and away from the β-secretase, which results in a reduction in the production of toxic β-amyloid

peptides. Furthermore, by activating ADAM10, which is also known to cleave the membrane-bound notch receptor thus liberating an intracellular domain that activates nuclear genes for neurogenesis, SIRT1 also activates notch pathway. As a result of this, SIRT1 helps to mitigate AD by increasing neurogenesis and neuroprotection that would be a second way besides suppressing β-amyloid production.

RESULTS

Effects of SIRT1 Levels in a Mouse Model of AD

We wished to test whether the levels of SIRT1 in the brain could influence Aβ plaque formation and the accruing pathological and cognitive decline in a mouse model of AD. We thus employed a model in which two linked transgenes, encoding the human APPswe and PSEN1dE9 alleles drive Aβ plaque formation and learning and memory deficits (Jankowsky et al., 2004). Plaques are evident in this model at 3–5 months of age, and they progress in number and size as mice grow older. In order to test the effects of increasing SIRT1 in these AD mice, they were crossed to strains bearing a SIRT1 transgenic allele (Tg in figures) that results in overexpression of the protein in the brain by about 2-fold (Bordone et al., 2007). Neither the SIRT1 transgene nor the knock-out allele described below affected expression of the APPswe or PSEN1dE9 disease genes (not shown). In all experiments, we compare congenic C57BL/6 littermates by quantifying cortical plaque number in serial coronal cross sections.

We observed a marked reduction of plaques in 5-month-old AD transgenic mice overexpressing SIRT1 (Tg), compared to AD control mice (Figure 1A). Wild-type (WT) mice without the AD disease genes were free of plaques. In order to delete SIRT1 in the brain, we used brain-specific knock out mice (BSKO) (Cohen et al., 2009), which lack full length SIRT1 in all neurons and glia (Figure 1B) and show normal adult brain morphology (Figure S1A available online). To test the effects of SIRT1 inactivation, BSKO mice were crossed to AD mice. Remarkably, AD-BSKO mice all died between 3 and 5 months of age, whereas AD mice or BSKO mice themselves lived well past 1.5 years of age (Figure 1C and data not shown). Although we have not pinpointed the cause of death in AD-BSKO mice, this synthetic lethality is typically indicative of a strong genetic interaction between the genes involved, further linking SIRT1 to AD pathogenesis. Consistent with this, AD-BSKO mice displayed a marked increase in plaques in 3-month-old mice compared to AD control mice (Figure 1D).

In order to monitor other features of brain pathology, we assayed for gliosis by immunological quantitation of the glial marker of inflammation, glial fibrillary acidic protein (GFAP) (Panter et al., 1985). A progressive increase in cortical GFAP staining was observed in AD control mice, and this was markedly reduced in AD-Tg mice and exacerbated in AD-BSKO mice (Figure 1E). In addition, phosphorylation of τ at Ser199 and Ser399 (Johansson et al., 2006) was evident in AD mice and suppressed in AD-Tg mice (Figures S1B and S1C).

AD mice show age-dependent decline in learning and memory (Reiserer et al., 2007), which can be measured with behavioral tests. In the fear-conditioning test (Bryan et al., 2009), mice were trained on day 1 by repeated exposure to a tone followed by a foot shock (Figure 2A). Memory of this training is evinced on day 2 by a freezing behavior when exposed to the tone without the shock. AD control mice showed a progressive decline in performance in this test at 8 and 11 months of age compared to 4 and 6 months of age (Figure 2B). This decline was clearly suppressed in older, age-matched AD-Tg mice. Wild-type and SIRT1 transgenic mice without the AD genes showed roughly normal learning at all ages (Figure 2B). Conversely, AD-BSKO mice were precociously deficient in this test at 4 months of age, whereas BSKO mice without the AD disease genes performed similarly to wild-type mice (Figure 2C).

In the Morris water maze test, mice are trained to use visual cues to find a submerged platform in an opaque pool (Bryan et al., 2009). By determining the time required to find the platform (escape latency) as a function of days of training, we observed a marked decline in performance in 8- and 10-month-old AD mice compared to 4-month olds (Figure 2D). This decline was significantly mitigated in AD-Tg mice. In mice without the AD genes, the SIRT1 transgene had no effect at any ages (Figure 2D), indicating that the suppression of decline in AD-Tg mice is not due to an inherent increase in learning and memory in SIRT1 overexpressing mice. As in the fear conditioning test, AD-BSKO mice displayed a precocious defect at 4 months of age (Figure 2F). We observed no differences in swim speed in any mice tested (Figure S1D).

SIRT1 Activates α-Secretase and Reduces Production of β-Amyloid

Because Aβ plaques were reduced in SIRT1 transgenic mice, we quantitated Aβ levels in brains of AD mice overexpressing or lacking SIRT1 by ELISA. Strikingly, Aβ 1–42 levels were substantially reduced in AD-Tg mice and enhanced in AD-BSKO mice (Figure 3A). SIRT1 affected Aβ 1–40 in a like manner (Figure S2A). The reduction in Aβ levels in SIRT1 overexpressing mice was largest in 4-month-old mice and grew smaller, but was still evident, in older mice. A second assay using immunoblotting also showed similar effects on Aβ levels by SIRT1 (Figure S2B).

A reduction of Aβ levels by SIRT1 could reflect a reduced production of the amyloid peptides. To address the potential of mice to produce Aβ, we prepared brain extracts and assayed the α and β-secretases (Kojro and Fahrenholz, 2005; Fahrenholz et al., 2008; Rockenstein et al., 2005). Remarkably, AD-Tg mice showed a doubling of α-secretase activity in 2-, 4-, and 6-month-old mice compared to AD-controls (Figure 3B). Conversely, there was a small but significant reduction in BSKO mice with or without the AD genes.

In addition, we observed a small decrease in β-secretase activity (Figure 3C) and BACE1 (β-secretase) RNA (Figure S2C) in SIRT1 overexpressing mice at all ages. However, because β-secretase was induced by the progression of disease in AD mice (Fukumoto et al., 2004), it is likely that the small decrease in this activity in AD-Tg mice is a secondary consequence of a slower disease onset and progression. Consistent with this idea, β-secretase activity (Figure 3C) and BACE RNA levels (Figure S2C) were higher in AD-BSKO mice compared to AD mice. Examination of individual γ-secretase subunits pen2 (Figure S2D), Aph1a, Aph1b (Figure S2E), and presenilin 1 (Figure S2F) showed no differences in mice with altered levels of SIRT1.