Neurobiology of Aging 43 (2016) 58–71

Contents lists available at ScienceDirect

Neurobiology of Aging

journal homepage: www.elsevier.com/locate/neuaging

Sirt1 deficiency protects cochlear cells and delays the early onset of age-related hearing loss in C57BL/6 mice

Chul Han [a], Paul Linser [b], Hyo-Jin Park [c], Mi-Jung Kim [a], Karessa White [a], James M. Vann [d,e], Dalian Ding [f], Tomas A. Prolla [d,e], Shinichi Someya [a,*]

[a] Department of Aging and Geriatric Research, University of Florida, Gainesville, FL, USA
[b] Whitney Laboratory, University of Florida, St Augustine, FL, USA
[c] Department of Neurology, University of Florida, Gainesville, FL, USA
[d] Department of Genetics, University of Wisconsin, Madison, WI, USA
[e] Department of Medical Genetics, University of Wisconsin, Madison, WI, USA
[f] Center for Hearing and Deafness, State University of New York at Buffalo, NY, USA

ARTICLE INFO

Article history:
Received 16 April 2015
Received in revised form 29 February 2016
Accepted 22 March 2016
Available online 30 March 2016

Keywords:
Hearing loss
Aging
Inner ear
Oxidative stress
Sirtuin

ABSTRACT

Hearing gradually declines with age in both animals and humans, and this condition is known as age-related hearing loss (AHL). Here, we investigated the effects of deficiency of *Sirt1*, a member of the mammalian sirtuin family, on age-related cochlear pathology and associated hearing loss in C57BL/6 mice, a mouse model of early-onset AHL. *Sirt1* deficiency reduced age-related oxidative damage of cochlear hair cells and spiral ganglion neurons and delayed the early onset of AHL. In cultured mouse inner ear cell lines, *Sirt1* knockdown increased cell viability under oxidative stress conditions, induced nuclear translocation of Foxo3a, and increased acetylation status of Foxo3a. This resulted in increased activity of the antioxidant enzyme catalase. In young wild-type mice, both Sirt1 and Foxo3a proteins resided in the cytoplasm of the supporting cells within the organ of Corti of the cochlea. Therefore, our findings suggest that SIRT1 promotes early-onset AHL through suppressing FOXO3a-mediated oxidative stress resistance in the cochlea of C57BL/6 mice.

1. Introduction

Sirtuins are a family of NAD^+-dependent protein deacetylases that are known to extend life span in lower organisms (Finkel et al., 2009). Although earlier studies have shown that overexpression of sirtuins increases life span in lower organisms (Rogina and Helfand, 2004; Tissenbaum and Guarente, 2001; Viswanathan et al., 2005), later studies revealed that overexpression of sirtuin genes do not increase life span when compared with a genetically standardized control strain in worms and flies (Burnett et al., 2011). Caloric restriction (CR) extends life span and delays the onset of age-related diseases, including age-related hearing loss (AHL) in mammals (Someya et al., 2010; Weindruch and Sohal, 1997). Earlier studies showed that CR increases life span by activating sirtuins in yeasts, worms, or flies (Lin et al., 2000; Rogina and Helfand, 2004; Wang and Tissenbaum, 2006). These studies have led to the development of sirtuin activators as a potential strategy to delay aging and age-related diseases in humans (Baur et al., 2006). However, subsequent studies revealed that sirtuins are not required for life span extension by CR in these organisms (Kaeberlein, 2010; Kenyon, 2010). Furthermore, overexpression of *Sirt1*, a member of the mammalian sirtuin family, increases apoptotic cell death in hearts and decreases cardiac function in mice (Alcendor et al., 2007), whereas *Sirt1* inhibition protects rat cortical neurons against oxidative stress (Li et al., 2008). Hence, the roles of sirtuins in extending health span and life span have proved controversial.

Hearing gradually declines with age in mammals, and this condition is known as AHL (Gates and Mills, 2005; Yamasoba et al., 2013). Hearing loss is the third most prevalent chronic condition in older adults and affects 40% of people older than 65 years and 80% of people older than 85 years (Gates and Mills, 2005; Yamasoba et al., 2013). Hearing loss also affects speech understanding (Frisina and Frisina, 1997), contributes to isolation and depression, and has been linked to dementia. AHL arises from age-dependent loss of sensory hair cells, spiral ganglion neurons (SGNs), and/or stria vascularis atrophy in the cochlea of the inner ear. Hair cells are the sensory receptors that transduce sound stimuli into electrical responses (Hudspeth, 1997). The inner hair cells (IHCs) are the actual sensory receptors that relay their electrical response postsynaptically to the central auditory system through the auditory

* Corresponding author at: Department of Aging and Geriatric Research, University of Florida, 2004 Mowry Road, PO Box 112610, Gainesville, FL 32611, USA. Tel.: 352-294-5167; fax: 352-294-5058.
E-mail address: someya@ufl.edu (S. Someya).

0197-4580/$ – see front matter © 2016 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.neurobiolaging.2016.03.023

nerves or SGNs, whereas outer hair cells (OHCs) receive mostly efferent input. Stria vascularis is heavily vascularized and holds numerous capillary loops and small blood vessels that are essential for transporting oxygen, nutrients, and hormones into the cochlea. Hence, these cells are essential for maintaining auditory function, and extensive loss or degeneration of the hair cells or SGNs, and/or atrophy of the stria vascularis results in hearing loss.

We have shown previously that Sirt3, a mitochondrial sirtuin, is required for the CR-mediated reduction of oxidative damage in the cochlear hair cells and SGNs and prevention of AHL in C57BL/6 (B6) mice, a mouse model of early-onset AHL and one of the most widely used mouse models for the studies of aging (Someya et al., 2010). In the present study, we examined the effects of *Sirt1* deficiency on age-related cochlear pathology and associated hearing loss in B6 mice. Our results show that *Sirt1* deficiency reduces age-related oxidative damage of cochlear hair cells and SGNs and delays the early onset of AHL by enhancing Foxo3a-mediated oxidative stress resistance in the cochlea of B6 mice.

2. Materials and methods

2.1. Animals

Male and female *Sirt1*$^{+/-}$ mice were a gift from Dr. Frederick W. Alt (Harvard University Medical School, Boston, MA, USA) and have been described previously (Cheng et al., 2003). Details on the methods used to house and feed mice have been described previously (Pugh et al., 1999). Experiments were performed in accordance with protocols approved by the University of Wisconsin-Madison Institutional Animal Care and Use Committee. Only male wild-type (WT) and *Sirt1*$^{+/-}$ littermates were used in the present study.

2.2. Genotyping and DNA sequencing

Sirt1 genotyping: *Sirt1*$^{+/-}$ males were mated with *Sirt1*$^{+/-}$ females, and the offspring from these mating were genotyped from DNA obtained by a tail clip at weaning. The following primers were used for genotyping: SIRT1SKO-F 5′-CTTGCACTTCAAGGGACCAA-3′; SIRT1SKO-R1 5′-GTATACCCACCACATCTGAG-3′; SIRT1SKO-R2 5′-CTACCACTCCTGGCTACCAA-3′. The polymerase chain reaction (PCR) cycling parameters were as follows: 94 °C for 3 minutes; 35 cycles of 94 °C for 30 seconds, 56 °C for 60 seconds, 72 °C for 60 seconds; 72 °C for 10 minutes. PCR products were separated on 1.5% agarose gel, and the expected band size for WT and knockout (KO) allele were 500 and 800 bps, respectively (Fig. 1A).

Cdh23 genotyping: male and female *Sirt1*$^{+/-}$ mice have been backcrossed for 4 generations onto the C57BL/6J mouse strain that is homozygous for the recessive AHL-susceptibility allele *Cdh23*^{753A}. To confirm that both *Sirt1*$^{+/+}$ and *Sirt1*$^{+/-}$ mice have the same genotype for *Cdh23*, we amplified the DNA region containing the 753rd nucleotide in the *Cdh23* gene by PCR reaction and sequenced the *Cdh23* gene in the DNA obtained from tails of young *Sirt1*$^{+/+}$ and *Sirt1*$^{+/-}$ mice (N = 4 each group). The following primers were used for the PCR reaction: Cdh23-F 5′- GATCAAGACAAGACCA-GACCTCTGTC-3′; Cdh23-R 5′-GAGCTACCAGGAACAGCTTGGGCCTG-3′. The size of amplified PCR product was 360 bps. We confirmed that all the *Sirt1*$^{+/+}$ mice have the *Cdh23*$^{753A/753A}$ genotype, and all the *Sirt1*$^{+/-}$ mice have the *Cdh23*$^{753A/753A}$ genotype (Fig. 1B).

2.3. ABR hearing test

At 3 and 12 months of age, auditory brainstem responses (ABRs) were measured with a tone burst stimulus at 8, 16, and 32 kHz using an ABR recording system (Intelligent Hearing System, Miami, FL, USA) as previously described (Someya et al., 2010). Mice were anesthetized with a mixture of xylazine hydrochloride (10 mg/kg, i.m.; Phoenix Urology of St. Joseph, St. Joseph, MO, USA) and ketamine hydrochloride (40 mg/kg, i.m.; Phoenix Urology of St. Joseph). We used 9–12 mice per group for ABR hearing assessment. After the ABR hearing measurements, tissues from the same mice were used to conduct histopathological and biochemical analyses.

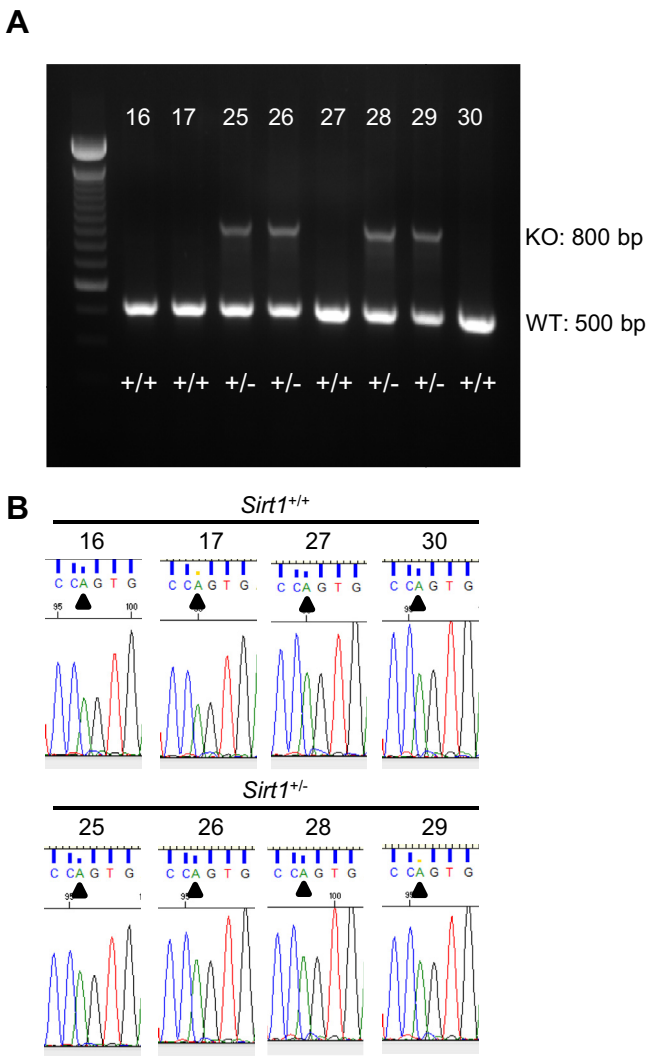

Fig. 1. Genotyping of *Sirt1*$^{+/+}$ and *Sirt1*$^{+/-}$ mice. (A) PCR products were separated on 1.5% agarose gel and the expected band sizes for WT and KO alleles were 500 and 800 bps, respectively. (B) The *Cdh23* gene in WT and *Sirt1*$^{+/-}$ mice (n = 4) was sequenced. All the *Sirt1*$^{+/+}$ mice had the *Cdh23*$^{753A/753A}$ genotype, and all the *Sirt1*$^{+/-}$ mice had the *Cdh23*$^{753A/753A}$ genotype. Arrows indicate the *Cdh23*^{753A} allele. Abbreviations: KO, knockout; PCR, polymerase chain reaction; WT, wild-type.

2.4. Cochlear histology

After the ABR hearing measurements, the animals were sacrificed by cervical dislocation, and the temporal bone was excised from the head and divided into cochlear and vestibular parts (Someya et al., 2010). The cochlea was then excised, immersed in a fixative containing 4% paraformaldehyde (Sigma-Aldrich, St. Louis, MO, USA) in phosphate-buffered saline solution for 1 day, and decalcified in 10% ethylenediaminetetraacetic acid for 1 week. The paraffin-embedded specimens were sliced into 4-μm sections, mounted on silane-coated slides, stained with hematoxylin and