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LOSS OF SESTRIN 2 POTENTIATES THE EARLY ONSET OF AGE-RELATED SENSORY CELL DEGENERATION IN THE COCHLEA

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Abstract—Sestrin 2 (SESN2) is a stress-inducible protein that protects tissues from oxidative stress and delays the aging process. However, its role in maintaining the functional and structural integrity of the cochlea is largely unknown. Here, we report the expression of SESN2 protein in the sensory epithelium, particularly in hair cells. Using C57BL/6J mice, a mouse model of age-related cochlear degeneration, we observed a significant age-related reduction in SESN2 expression in cochlear tissues that was associated with early onset hearing loss and accelerated age-related sensory cell degeneration that progressed from the base toward the apex of the cochlea. Hair cell death occurred by caspase-8 mediated apoptosis. Compared to C57BL/6J control mice, *Sesn2* KO mice displayed enhanced expression of proinflammatory genes and activation of basilar membrane macrophages, suggesting that loss of SESN2 function provokes the immune response. Together, these results suggest that *Sesn2* plays an important role in cochlear homeostasis and immune responses to stress.

Key words: sestrin 2 (SESN2), age-related hearing loss, cochlea, outer hair cells, macrophages, inflammation.

INTRODUCTION

Sestrins are a family of highly conserved stress-responsive proteins that are inducible in cells exposed to stress (Lee et al., 2010). Mammalian tissues express three types of sestrins (sestrin 1, sestrin 2 and sestrin 3) (Lee et al., 2010) and these proteins are transcriptionally regulated by p53 and forkhead transcription factors (Budanov et al., 2010). The discovery of sestrins as p53 targets suggests that these proteins are stress-inducible and can modulate tissue response to stress (Budanov et al., 2002; Velasco-Miguel et al., 1999). Sestrins protect cells from various stresses, such as DNA damage, oxidative stress and hypoxia (Lee et al., 2013). They act as antioxidants that control the activity of peroxiredoxins by scavenging reactive oxygen species (ROS). At the cellular level, disruption of sestrin expression compromises metabolic processes, which causes oxidative stress, fat accumulation, and mitochondrial dysfunction, resembling an accelerated aging process in tissues (Lee et al., 2013). Because sestrin-targeted molecules are associated with age-related tissue pathogeneses (Finkel and Holbrook, 2000; Kapahi and Zid, 2004; Stanfel et al., 2009) and because sestrins protect tissues from a range of age-related diseases (Budanov and Karin, 2008; Budanov et al., 2004; Lee et al., 2010), we hypothesize that sestrins play a role as anti-aging molecules in the auditory system.

Age-related hearing loss (ARHL), one of the most common disorders in the elderly, is predominately associated with sensory cell degeneration. Approximately 25% of individuals between the ages of 50 and 65 years have hearing thresholds greater than 30 dB and self-reported hearing loss can be identified in 50% of those over the age of 85 (Liu and Yan, 2007). ARHL affects both the peripheral and central auditory systems, leading to elevation of hearing thresholds and deterioration of auditory processing functions (Frisina et al., 2001; Khullar and Babbar, 2011; Ohlemiller and Frisina, 2008). The magnitude of hearing dysfunction is affected by an individual's genetic background and lifetime insults to their auditory organ (Bielefeld et al., 2010; Ohlemiller, 2009). As age increases, the ability of the cochlea to tolerate stress decreases increasing susceptibility to ARHL.

Among the known biological changes that occur with aging, oxidative stress has been known as one of the key protagonists in the pathophysiology of ARHL. Aged cochleae display increased production of free radicals, a byproduct of aerobic metabolism (Sohal and Weindruch, 1996) and weakened antioxidant capacity

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Abbreviations: ABR, auditory brainstem response; ARHL, age-related hearing loss; DAPI, 4′,6-diamidino-2′-phenylindole, dihydrochloride; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; KO, knockout; OHC, outer hair cell; PBS, phosphate-buffered saline; ROS, reactive oxygen species; RT-qPCR, quantitative reverse transcriptase-polymerase chain reaction; Tlr, Toll-like receptor.

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(Jiang et al., 2007; Staecker et al., 2001; Tadros et al., 2014; Tanaka et al., 2012). While ROS can function as biological molecules for many physiological processes (Sena and Chandel, 2012), excessive ROS is toxic to cells and causes apoptosis (Kamogashira et al., 2015; Linehan and Fitzgerald, 2015; Orr and Sohal, 1994; Someya and Prolla, 2010). Moreover, ROS activities can interact with other pathological activities, such as inflammatory responses to potentiate the age-related sensory cell degeneration.

Sestrin 2 (SESN2), a key member of the sestrin family, has been investigated in the heart of aging mice (Morrison et al., 2015; Quan et al., 2017)and in colorectal cancer in human and murine populations (Ro et al., 2016; Wei et al., 2015). SESN2 is crucial during myocardial ischemia to promote AMPK activation and regulate cellular energy homeostasis (Morrison et al., 2015), suggesting a role for SESN2 in the AMPK signaling pathway. Decreased SESN2 expression in human colorectal cancer tissues is associated with poor prognosis (Wei et al., 2015). This has been attributed to overproduction of ROS, which are genotoxic, tumor progression and metastasis (Lee and Kang, 2013; Waris and Ahsan, 2006; Wei et al., 2015). SESN2 acts as a tumor suppressor in the colon; the colon of *Sesn2* knockout (KO) mice was more prone to inflammation (Ro et al., 2016). Additionally, patients with chronic colon inflammation have elevated levels of SESN2, whereas patients with colon cancer have very low levels of SESN2 (Wei et al., 2015). Despite the importance of SESN2 in other fields, little is known about its functional roles in cochlear homeostasis and pathogenesis.

To investigate the function of SESN2 in cochlear sensory cell homeostasis and age-related degeneration, we assessed the expression of SESN2 in the sensory epithelium of mouse cochleae. SESN2 was downregulated with age. Importantly, loss of SESN2 function accelerated age-related sensory cell degeneration and auditory dysfunction. Cochlear pathogenesis was accompanied by enhanced inflammatory activity. Our study implicates SESN2 in sensory cell integrity and pathogenesis.

EXPERIMENTAL PROCEDURES

Animals and genotyping

Sesn2 KO mice (male and female) backcrossed for at least 9 generations with C57BL/6J mice were compared to C57BL/6J mice to determine how the deletion of the SESN2 protein affects the ARHL and hair cell degeneration. *Sesn2* KO mice, developed on the C57BL/6J background were generated in the Laboratory of Gene Regulation and Signal Transduction of the Department of Pharmacology at University of California, San Diego, La Jolla, CA, USA (Budanov and Karin, 2008). The *Sesn2* KO breeder mice provided by Dr. Ji Li (University of Mississippi Medical Center, Department of Physiology and Biophysics) were backcrossed to C57BL/6J mice for at least 9 generations (personal communication, Dr. Ji Li and Dr. Michael Karin, University of California, San Diego). C57BL/6J mice (The Jackson Laboratory, Bar Harbor, ME, USA) were used as controls. Because the C57BL/6J strain is homozygous for a recessive AHL-susceptibility allele $Cdh23^{753A}$, we confirmed that C57BL/6J controls and *Sesn2 KO* mice have the same genotype for *Cdh23*. Briefly, DNA from the tails of these mice was amplified using PCR and the region of DNA containing the 753rd nucleotide in the *Cdh23* gene was sequenced ($n = 3$). The following primers were used for PCR: Cdh23-F 5′-GATCAAGACAAG ACCAGACCTCTGTC-3′; Cdh23-R 5′ GAGCTACCAG GAACAGCTTGGGCCTG-3′. The size of amplified PCR product was 360 bps. We confirmed that all the C57BL/6J control and *Sesn2* KO mice had the same $Cdh23^{753A/753A}$ genotype (Fig. 1).

74 animals were used in this study (30 *Sesn2* KO mice and 44 C57BL/6J control mice). The KO and C57BL/6J control animals were divided into three age groups: 4–6 weeks, 3 months and 5 months. We limited the age range of the mice to 5 months because the C57BL/6J control mice develop significant high-frequency hearing loss after the age of 5 months (Someya et al., 2009) that could complicate the interpretation of the results. Both cochleae of each mouse were collected and processed for different experimental tests. The numbers of animals used in each experimental condition are presented in the Results section. All procedures involving the use and care of the animals were approved by the University at Buffalo Institutional Animal Care and Use Committee.

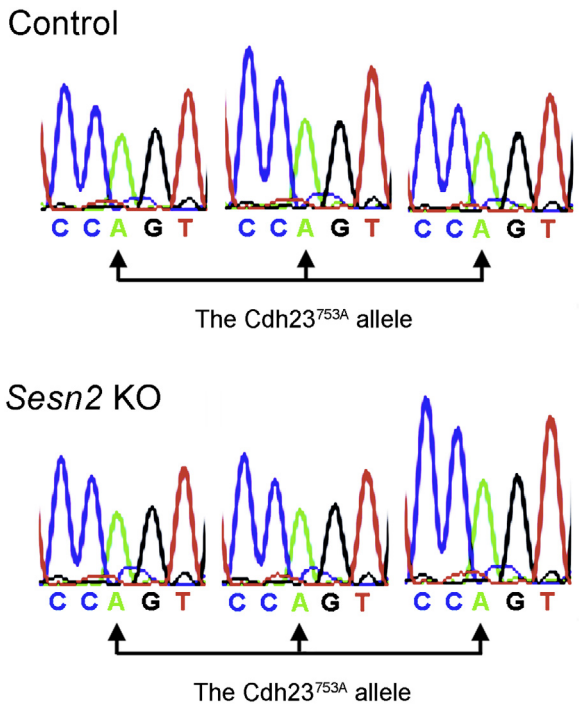

Fig. 1. Genotyping of Cdh23 gene showing the same genotype between the control and *Sesn2* KO mice. The *Cdh23* gene was sequenced in three control (C57BL/6J) and three *Sesn2* KO mice that had been backcrossed to C57BL/6J for at least 9 generations. Both the control and *Sesn2* KO animals have the $Cdh23^{753A/753A}$ genotype.