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BRAIN RESEARCH

Research Report

Genes encoding mitochondrial respiratory chain components are profoundly down-regulated with aging in the cochlea of DBA/2J mice

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ABSTRACT

Age-related hearing loss (AHL) is the progressive loss of auditory function with aging. Mutations in the Cdh23 gene of DBA/2J mice result in AHL by 3 months of age. Hearing function was analyzed by auditory brainstem response (ABR) which confirmed that severe age-related hearing loss occurred in 8-month-old mice, whereas mild hearing loss occurred in 2-month-old mice. Cochlear gene expression of 2-month-old and 8-month-old DBA/2J mice was measured using Affymetrix microarrays. Comprehensive gene expression analysis identified significant expression changes correlated with AHL in over 4000 cochlear genes. AHL-correlated genes in the cochlea of 8-month-old DBA/2J mice were statistically associated with 15 mitochondrial process categories, including "mitochondrial electron transport chain", "oxidative phosphorylation", "respiratory chain complex I", "respiratory chain complex IV", and "respiratory chain complex V". Furthermore, 31 genes encoding components of the mitochondrial respiratory chain complexes I, II, III, IV, and V were significantly down-regulated in the cochlea. Quantitative RT-PCR (QRT-PCR) validated the microarray data in a selected set of genes. Thus, these observations provide evidence that AHL is associated with profound down-regulation of genes involved in the mitochondrial respiratory chain complexes in the cochlea of aged DBA/2J mice.

1. Introduction

Hearing function declines with advancing age in most individuals. AHL (Short et al., 2005) is a complex progressive disease characterized by an age-associated loss of hair cells and spiral ganglion cells in the cochlea of the inner ear (Fischel-Ghodsian, 2003; Someya et al., 2007). It has been postulated that AHL occurs gradually as a result of the cumulative effect of aging, exposure

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Abbreviations: ABR, auditory brainstem response; AHL, age-related hearing loss; DBA, DBA/2J; DAVID, Database for Annotation, Visualization, and Integrated Discovery; EASE, Expression Analysis Systematic Explorer; FBSN, familial bilateral striatal necrosis; FDR, false discovery rate; GO, Gene Ontology; MELAS, mitochondrial encephalomyopathy, lactic acidosis and stroke-like episodes; MERRF, myoclonic epilepsy and ragged red fibers; BOR, branchio–oto–renal; MILS, maternally inherited Leigh syndrome; mtDNA, (mitochondrial DNA); NARP, retinitis pigmentosa; QRT-PCR, quantitative RT-PCR; SAM, Significance Analysis of Microarray

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to noise, diet, and mitochondrial DNA (mtDNA) mutations. However, the molecular mechanisms of AHL remain unclear and there is no cure for this age-related disease.

DBA mice have been used as a model of AHL and exhibit progressive, severe age-related hearing loss by 3 months of age (Zheng et al., 1999). The Cdh23 gene encodes a cadherin protein that is a key component of the stereocilia hair bundle of the hair cells in the inner ear (Noben-Trauth et al., 2003). Because this strain is homozygous for the *Cdh23* mutation and possible mutation of other genes, the animals have significantly increased susceptibility to early onset of AHL (Zheng and Johnson, 2001). DBA mice also display age-associated, progressive cochlear pathology, including loss of hair cells and spiral ganglion neurons (Willott et al., 1995). However, it is unclear how cochlear degeneration develops with aging.

mtDNA mutations and the resulting mitochondrial dysfunction have been postulated to contribute to aging and age-associated diseases (Kujoth et al., 2005). We have shown previously that mtDNA mutations and mitochondrial dysfunction are associated with the development of AHL in mice carrying a mutator DNA polymerase γ (Someya et al., in press). Defects of the mitochondrial respiratory chain have also been associated with mitochondrial disorders such as mitochondrial encephalomyopathy, lactic acidosis and stroke-like episodes (MELAS), myoclonic epilepsy and ragged red fibers (MERRF), and branchio–oto–renal syndrome, the symptoms of which include hearing loss (Lin et al., 1999; Triepels et al., 2001). It has been hypothesized that damage to the mitochondrial respiratory chain accumulates with aging, leads to mitochondrial dysfunction, and results in energy depletion, accelerating the aging process.

The mitochondrial respiratory chain, or oxidative phosphorylation system, comprises five enzyme complexes. Four complexes (complexes I–IV) remove electrons from NADH or FADH2 and pass them to O_2, creating a proton gradient across the mitochondrial inner membrane. One enzyme complex, ATP synthase complex (complex V), uses the proton gradient to generate most cellular ATP (Shoubridge, 2001a,b). Previous studies have shown that damage to these respiratory chain complexes or specific mutations in the genes encoding subunits of the respiratory chain are responsible for a decline in the respiratory chain activities in aging mammals (Fischel-Ghodsian, 2003; Manczak et al., 2005; Shoubridge, 2001a).

Because DBA mice display early, severe age-related hearing loss and cochlear pathology, these mice represent a useful model for investigating mechanisms of mammalian AHL. Here, to test the hypothesis that mitochondrial respiratory chain dysfunction is associated with AHL, we performed comprehensive DNA microarray analysis using cochlear tissues from 2-month-old and 8-month-old DBA mice. We then performed data analysis to identify AHL-correlated genes and biological process categories statistically associated with AHL-correlated genes. Auditory brainstem response (ABR), histology, and QRT-PCR were performed to corroborate the microarray results.

2. Results

2.1. Assessment of hearing and histology

To confirm that AHL occurs in DBA mice, we measured ABR thresholds in 2-month-old and 8-month-old mice. A mild hearing loss was observed in 2-month-old mice (Fig. 1), whereas severe hearing loss occurred in 8-month-old mice (P value <0.05, $n=5$) (Fig. 1). To confirm that age-related cochlear degeneration occurs in DBA mice, we performed histological analysis of the cochleae from 2-month-old and 8-month-old mice. Histological analysis of the basal cochlear region confirmed that all the cochleae from 8-month-old mice showed significant loss of spiral ganglion cells and hair cells in the cochleae (Fig. 2A, lower panel), while all the cochleae from 2-month-old mice showed no loss or loss of only a few spiral ganglion cells and hair cells in the cochleae (Fig. 2A, upper panel). Decreased spiral ganglion density is one of the hallmarks of AHL in mammals (Keithley et al., 2004). The mean spiral ganglion density of 8-month-old DBA mice was significantly lower than that of 2-month-old DBA mice (Fig. 2B) ($P<0.05$, $n=3$). Together, these observations confirm that age-related hearing loss and cochlear degeneration occur by 8 months of age in DBA mice.

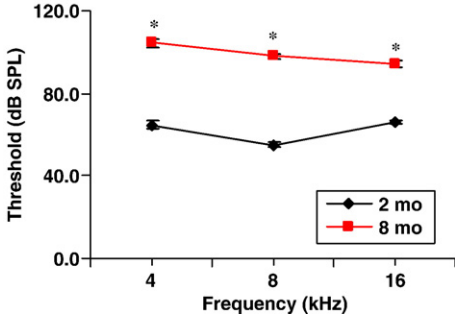

Fig. 1 – Age-related hearing loss and cochlear degeneration. The plotted data are means ± SD of ABR thresholds (dB SPL, decibels Sound Pressure Level) for 2-month-old and 8-month-old DBA mice at 4, 8, and 16 kHz. The mean ABR thresholds of 8-month-old DBA mice were significantly elevated compared to 2-month-old DBA mice (*$p<0.05$, $n=5$), showing age-related hearing loss.

2.2. Overview of microarray and statistical analysis

To identify genes associated with AHL and associated biological process categories, we conducted genome-wide gene expression analysis using RNA samples isolated from cochlear tissues of 2-month-old and 8-month-old mice ($n=3$). Using the Affymetrix GeneChip, we found that 2309 gene probe sets were significantly down-regulated, and 1996 gene probe sets were significantly up-regulated in the cochlear tissues of 8-month-old mice compared to 2-month-old mice. These significantly altered gene probe sets were further assigned to "GO: Biological Process" categories using DAVID, which assigned a classification to 2224 of the down-regulated and 1928 of the up-regulated genes. A summary of the "GO: Biological Process" categories associated with AHL-correlated genes is shown in Table 1. Table 2 shows a list of down-regulated genes encoding components of the mitochondrial respiratory chain in the cochlea. For a list of all genes identified, see Supplementary Table 3.