Review

Anti-apoptotic treatment in mouse models of age-related hearing loss

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Abstract

Age-related hearing loss (AHL), or presbycusis, is the most common neurodegenerative disorder and top communication deficit of the aged population. Genetic predisposition is one of the major factors in the development of AHL. Generally, AHL is associated with an age-dependent loss of sensory hair cells, spiral ganglion neurons and stria vascularis cells in the inner ear. Although the mechanisms leading to genetic hearing loss are not completely understood, caspase-family proteases function as important signals in the inner ear pathology. It is now accepted that mouse models are the best tools to study the mechanism of genetic hearing loss or AHL. Here, we provide a brief review of recent studies on hearing improvement in mouse models of AHL by anti-apoptotic treatment.

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Contents

1. Introduction

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Age-related hearing loss (AHL) is the most common sensory disorder in the elderly population, causing communication problems and adversely impacting the quality of life of affected individuals (Gates and Mills, 2005; Yamasoba et al., 2007; Op de Beeck et al., 2011). Genetic predisposition is one of the major factors in the development of AHL and

extensive candidate-gene-based association studies on AHL have been conducted recently (Newman et al., 2012; Yamasoba et al., 2013). As mice and humans share similar genetic components, anatomic structures and pathological characteristics, mouse models play a crucial role in understanding the pathogenesis associated with these genes (Angeli et al., 2012; Han et al., 2015). In fact, most inbred mouse strains display at least some degree of AHL, and the age of the onset is known to vary from 3 months in DBA/2J mice to over 20 months in CBA/CaJ mice (Zheng et al., 1999; Noben-Trauth and Johnson, 2009). Part of the early onset of hearing loss in these mice is explained through the presence of a recessive *ahl* allele in the gene of *cadherin 23* (*Cdh23*).

Cdh23, also known as gene of otocadherin, encodes for a calcium-dependent cell adhesion protein (CDH23) that is required for establishing and/or maintaining the proper organization of the stereocilia bundle of hair cells in the cochlea and the vestibule during late embryonic/early postnatal development. CDH23 and protocadherin 15 (PCDH15) interact to form the tip links in the stereocilia. They localize, respectively, to the upper and lower parts of tip links (Kazmierczak et al., 2007). Altered adhesion or reduced stability of CDH23 may confer susceptibility to AHL (Noben-Trauth et al., 2003). It is concluded that the *cadherin 23^{ahl}* (*Cdh23^{ahl}*) allele is associated with a rapid progression of AHL (Johnson et al., 2000; Ohlemiller, 2006; Op de Beeck et al., 2011). Other mapped loci, such as *ahl2* in NOD/LtJ, *ahl4* in A/J and *ahl8* in DBA/2J, et al., contribute to the earlier onset and more rapid progression of hearing loss in these strains (Noben-Trauth and Johnson, 2009).

Histopathologically and pathophysiologically, AHL may variably be accompanied by an age-dependent loss of sensory hair cells, spiral ganglion cells, and degeneration of stria vascularis cells (Op de Beeck et al., 2011; Yamasoba et al., 2013). In fact, apoptosis has been identified as the final common pathway in degradation of the organ of Corti in several types of genetic hearing loss (Bao and Ohlemiller, 2010; Cheng et al., 2011; Op de Beeck et al., 2011; Laine et al., 2007; Niu et al., 2007; Tadros et al., 2008; Schwander et al., 2009). Recent studies on mouse models of AHL, in particular, have revealed that apoptosis contributes to degeneration of cells in the cochlea and anti-apoptotic treatment improves hearing in these mouse models.

2. Current concept on the mechanism of AHL development

Although the pathology of hearing loss is very complicated, extensive genetic and molecular biological studies have provided considerable insight into understanding the mechanisms of cell death. Apoptosis in the cochlea may be triggered by oxidative stress, which produces reactive oxygen species (ROS), according to a current conceptual model (Yamasoba et al., 2007). It is accepted that mitochondria are a major source of ROS and a major site of ROS-induced oxidative damage, which has been proposed to play a causal role in AHL (Ohlemiller et al., 1999; Seidman, 2000; Liu and Yan, 2007;

Yamasoba et al., 2007; Someya et al., 2009). The aging theory predicts that in the course of time ROS concentration rises either due to depletion of antioxidant defenders or due to an elevated ROS formation. This causes mitochondrial damage and subsequent release of pro-apoptotic factors that finally induce apoptosis (Op de Beeck et al., 2011). It is hypothesized that the ROS may induce DNA damage, which results in the upregulation of P53, causing chronic activation of the mitochondrial BAK pathway, ultimately resulting in the triggering of apoptotic cell death in the cochleae (Someya and Prolla, 2010). However, other studies pointed that multiple cell death pathways, all potentially linked to oxidative stress, were involved in hair cells of the auditory organ in aging mice (Sha et al., 2009). Despite limitations in the various models of AHL, it appears that ROS formation and apoptosis are key events in the pathology of AHL (Op de Beeck et al., 2011).

3. Anti-apoptotic treatment in mouse models of AHL

3.1. C57BL/6J mice

The C57BL/6J mouse strain is a long-living strain (mean lifespan of approximately 30 months) and the mostly used mouse model for studies of aging and age-associated diseases. It is well known that hearing loss in C57BL/6J mice occurs at about 9−12 months of age. Genome-wide linkage analyses have identified an associated locus (mentioned above as *ahl*) in D10Mit5 − D10Mit31 interval on Chromosome 10 (Chr 10) and further genetic mapping delimited the interval to an 830 kilobases (kb) region on Chr10 (Zheng and Johnson, 2001; Noben-Trauth et al., 2003). Sequencing of genes in this interval identified a functional polymorphism (G753A) in the coding sequence of *Cdh23*. This single nucleotide polymorphism (SNP) occurs at the last position of exon 7 and alters the consensus splice site, leading to in-frame skipping of exon 7. One of the major genetic factors contributing to hearing loss in C57BL/6J mice is the *ahl* locus (Johnson et al., 1997). However, inbred strain variants of the *Cdh23* have been shown to influence the onset and progression of AHL in mice: the CBA/CaJ-derived *Cdh23^{Ahl+}* allele dramatically lessens hearing loss and hair cell death in an otherwise C57BL/6J genetic background, but that the C57BL/6J-derived *Cdh23^{ahl}* allele has little effect on hearing loss in an otherwise CBA/CaJ background (Kane et al., 2012). Study also indicated that loci, in addition to *ahl,* contributed to the differences in hearing loss between C57BL/6J and CAST mice. To be specific, although hearing thresholds in 24-month-old B6. CAST-*Ahl* mice were significantly elevated compared to the normal hearing wild-type CAST/Ei mice, they were still lower than in age-matched C57BL/6J mice (Keithley et al., 2004). Therefore, *Cdh23^{ahl}* homozygosity is necessary but not sufficient on its own to cause accelerated hearing loss in C57BL/6J mice.

Study has shown that AHL in C57BL/6J mice is mediated, at least partly, by Bak-dependent mitochondrial apoptosis. It is speculated that, in response to increased oxidative DNA damage in the aged cochlea, p53 translocates to mitochondria and activates *BCL2-antagonist/killer1* (*Bak*), leading to Bak-

mediated apoptosis and eventually to cochlear cell death (Someya et al., 2009). Also in this study, oral supplementation with the mitochondrial antioxidants suppresses Bak expression in the cochlea, reduces cochlear cell death, and prevents AHL (details in section of anti-oxidative stress). Thus, induction of stress-induced apoptotic cell death through activation of a Bak-dependent mitochondrial apoptotic program in response to oxidative stress may be a key mechanism of AHL in C57BL/6J mice (Someya et al., 2009).

3.2. A/J mice

A/J mice and C57BL/6J mice share the same *ahl* allele (Noben-Trauth et al., 2003). However, mice of the A/J sub-strain exhibit an early-onset progressive hearing loss with elevated auditory-evoked brainstem response (ABR) thresholds by 25 days of age, and hearing impairment progresses to near deafness by three months of age (Zheng et al., 1999, 2009). Therefore, additional genetic factors must be involved. Sequencing of the mitochondrial genome revealed a single nucleotide insertion in the tRNA-Arg gene (*mt-Tr*) that is likely responsible for the phenotypic effect (Johnson et al., 2001). However, the effect of the *ahl* locus combined with the mitochondrial effect is still not enough to account for the full extent of hearing loss exhibited by A/J mice. Linkage back-cross was used to map yet another age-related hearing loss locus (named *ahl4*) to the distal region of Chr10 (Zheng et al., 2009). As was the case with *mt-Tr,* the *ahl4* effect on hearing loss was limited to backcross mice with predisposing *ahl/ahl* genotypes. The *ahl4* locus, which could explain about 40% of the ABR threshold variation in these mice, was then identified as a mutation in gene of citrate synthase (CS, p.H55N) (Noben-Trauth and Johnson, 2009; Johnson et al., 2012).

In a recent study, we firstly showed that A/J mice displayed more severe degeneration of hair cells, spiral ganglion neurons (SGNs) and stria vascularis in the cochleae compared with C57BL/6J mice (Han et al., 2015). We then tried to figure out the reasons for the pathological impairment in A/J mice. Our results indicate that apoptosis in cochlea is related to the early onset of hearing loss in A/J mice. As A/J mice are a model of AHL, a time course caspase dependent and independent molecules were detected in this mouse. We showed that apoptosis signals of *caspase-3, caspase-9* and *Aif* (apoptosis-inducing factor) were at high levels in the inner ears of A/J mice even at postnatal day 1 (P1), and were significant higher than those of C57BL/6J mice at 2 and 8 weeks of age, indicating that apoptosis occurs at early stage of inner ear development in A/J mice.

The results of anti-apoptosis treatment improving hearing further support the idea that hearing loss in A/J mice is related to apoptosis in the inner ears. Z-VAD-FMK (z-Val-Ala-Asp (Ome)-fluoromethylketone) is a pan-caspase inhibitor that suppresses the activities of a range of caspases including caspase-3. It has been documented that Z-VAD-FMK protected against 3-NP-induced hearing loss through inhibiting progressive degeneration of the lateral-wall fibrocytes in the cochlear basal turn, as well as apoptosis of these fibrocytes

(Mizutari et al., 2008). There was also study that early direct perfusion of Z-VAD-FMK into cochlea leads to accelerated hearing recovery and reduced hair cell loss in guinea pigs suffering gunshot noise-induced trauma (Abaamrane et al., 2011). Later study showed that intra-cerebro-ventricular administration of Z-VAD-FMK to post-radiation rats resulted in reduced numbers of TUNEL-positive cells in the hypoglossal nucleus, suppressed expression and activation of caspases 3/8/9 and decreased appearance of cytochrome c in the cytosolic fraction (Li et al., 2014). In our study, Z-VAD-FMK was given intraperitoneally (1.5 μg/g mouse weight) beginning at P7 for a period of 8 weeks. The drug preserved hearing of A/J mice by reducing about 15 dB sound pressure level (SPL) of the ABR thresholds. We thus conclude that caspase-mediated apoptosis in the cochleae contributes to the early onset of hearing loss in A/J mice (Han et al., 2015).

3.3. DBA/2J mice

DBA/2J mice develop early-onset hearing loss with hearing thresholds elevated by 15−20 dB at 3 weeks of age and approaching deafness levels by 14 weeks (Zheng et al., 1999). Apart from *ahl*, a locus named *ahl8* on the distal arm of Chromosome 11 was identified as the main contributor to the early onset of hearing loss in DBA/2J mice (Johnson et al., 2008). The effects of the *ahl8* locus on hearing loss in the backcrossed mice were manifested only in mice with *ahl/ahl* genotypes (Noben-Trauth and Johnson, 2009). The *ahl8*-causative gene was then identified as *fascin-2*, which encodes an actin crosslinking protein previously thought to be retina-specific (Shin et al., 2010). Fascin-2 cooperates with β-actin to maintain stereocilia length and auditory function. Mice expressing mutant fascin-2 (p.R109H in DBA/2J mice) or lacking actin share common phenotypes including progressive, high-frequency hearing loss together with shortening of a defined subset of stereocilia in the hair cells (Perrin et al., 2013).

Studies have showed that hearing loss in DBA/2J mice is paralleled by degeneration of the organ of Corti and spiral ganglia (Willott et al., 2005). Actually, there are three major types of AHL: sensory (loss of sensory hair cells), neuronal (loss of spiral ganglion neurons) and metabolic (strial atrophy) hearing loss (Yamasoba et al., 2013). Our results demonstrated that DBA/2J mice displayed at least two features of AHL: loss of sensory hair cells and spiral ganglion neurons (Yang et al., 2015). We further found that apoptosis-related genes, especially *Bak* and *Caspase-3,* were highly expressed in inner ears of DBA/2J mice at 2 weeks of age, preceding hearing loss that occurred at around 3 weeks of age(Yang et al., 2015). We therefore suggest that apoptosis in the cochleae of DBA/2J mice may be related to the activation of Bak-dependent pathways in mitochondria, which were previously showed in C57BL/6J mice (Someya et al., 2009; Someya and Prolla, 2010). Moreover, mRNA levels of *Caspase-3* and *Caspase-9* were higher in the inner ears of DBA/2J mice than those of C57BL/6J mice at 2 and 8 weeks of age. These results indicate that apoptosis probably plays a significant role in the hearing loss of DBA/2J mice.

To determine the significance of caspase-dependent apoptosis in the hearing loss, Z-VAD-FMK was given intraperitoneally to DBA/J2 mice over an 8-week period starting at one week of age. Blockage of caspases preserved hearing in the mice by more than 10 dB SPL of the ABR thresholds and significantly reduced outer hair cell loss at the basal turns of the cochleae. These results demonstrate that the hearing loss in DBA/J2 mice can be attenuated by anti-apoptotic treatment (Yang et al., 2015).

3.4. Mouse models of DFNB12

Some mouse models of DFNB12 (a form of nonsyndromic autosomal recessive deafness with mutations in the *Cdh23* gene in humans) show AHL and apoptosis is also involved in the degeneration of cochlea (Noben-Trauth and Johnson, 2009; Schwander et al., 2009; Han et al., 2012). We previously reported a novel mouse model of DFNB12 with a mutation in *Cdh23* (*Cdh23$^{erl/erl}$*), which was induced by N-ethyl-N-nitrosourea (ENU) in the C57BL/6J mouse strain. The *Cdh23$^{erl/erl}$* mice were characterized by progressive hearing impairment beginning approximately 1 month after birth and became deaf at 3 months of age. Genetic linkage and complementation tests demonstrated that *erl* was a new *Cdh23* allele in which a point mutation in exon 3 (208T > C) leads to a substitution of amino acids (S70P) (Han et al., 2012). mRNA levels of caspase-3, caspase-8 and caspase-9 were upregulated in the inner ears of *Cdh23$^{erl/erl}$* mouse at P14 and P56, compared with the levels of C57BL/6J mice. The *Cdh23$^{erl/erl}$* mice were then injected intraperitoneally with Z-VAD-FMK beginning at P7 for a period of 3 months. The mean percentages of outer hair cell loss in the cochleae of Z-VAD-FMK-treated *Cdh23$^{erl/erl}$* mice were significantly reduced compared to those of untreated or DMSO-treated mice. Hearing was preserved (ABR thresholds were on average 20-dB lower) in Z-VAD-FMK-treated *Cdh23$^{erl/erl}$* mice compared to untreated controls (Han et al., 2012).

In a later study, we showed further that erythropoietin (EPO) had otoprotective effects on the *Cdh23$^{erl/erl}$* mice as evaluated by the measurement of ABR thresholds and amplitudes of distortion product oto-acoustic emission (DPOAE) (Han et al., 2013). EPO is a cytokine hormone with multiple functions. Similar to the way that EPO stimulates erythropoiesis by protecting the erythroid progenitor cells from apoptosis, its cytoprotective or otoprotective mechanism appears to be anti-apoptotic (Vesey et al., 2004; Johnson et al., 2006; Han et al., 2013). A more recent study showed that systemic treatment with tauroursodeoxycholic acid (TUDCA), a taurine-conjugated bile acid, significantly alleviated hearing loss and suppressed hair cell death in *Cdh23$^{erl/erl}$* mice. Additionally, TUDCA inhibited apoptotic related gene expression in the cochleae of *Cdh23$^{erl/erl}$* mice (Hu et al., 2016).

4. Antioxidants and anti-oxidative stress in mouse models of AHL

As oxidative stress causes apoptosis in the cochleae, anti-oxidative stress is important to prevent AHL. The mitochondrial theory of aging postulates that ROS generated inside of mitochondria damages key mitochondrial components, including mitochondrial NDA (mtDNA) and respiratory chain complex proteins. Such damage accumulates over time and ultimately leads to permanent age-related mitochondrial dysfunction, which in turn contributes to the aging phenotypes (Loeb et al., 2005). In line with this theory is that antioxidant defenders such as mitochondrial superoxide dismutase 2 (SOD2) decrease significantly with age in all cell types of the organ of Corti, suggesting that oxidative imbalances indeed contribute to AHL (Jiang et al., 2007).

The efficacy of the antioxidant systems, e.g., glutathione and thioredoxin, is an important factor in the pathophysiology of the aging nervous system (Kang et al., 2012). To explore the relation between expression of antioxidant-related genes in the cochlea and AHL in CBA/CaJ mice, expression levels of 56 antioxidant-related genes were analyzed using Affymetrix H GeneChip (Tadros et al., 2014). The results showed that gene of glutathione peroxidase 6 (Gpx6) was upregulated while the gene of thioredoxin reductase 1, (txnrd1) was downregulated with age/hearing loss. The heat shock protein1 (hspb1) gene was found to be downregulated in middle-aged animals as well as those with mild presbycusis, whereas it was upregulated in those with severe presbycusis. These results may facilitate development of future interventions to predict, prevent or slowdown the progression of presbycusis (Tadros et al., 2014).

Experiments in C57BL/6J mice provide evidence that mitochondrial derived ROS may play a causal role in AHL. C57BL/6J mice fed with an antioxidant supplemented diet (α lipoic acid, coenzyme Q and N-acetyl-L-cysteine) showed significantly lower ABR hearing thresholds when compared to thresholds from the control mice (Someya et al., 2009). Furthermore, hearing tests in C57BL/6J mice overexpressing mitochondrial targeted catalase (MCAT) displayed reduced ABR thresholds compared to the wildtype mice (Someya et al., 2009).

AHL is also associated with profound downregulation of genes involved in the mitochondrial respiratory chain complexes in the cochleae of aged DBA/2J mice (Someya et al., 2007). Supplementation with antioxidant alpha-lipoic acid significantly delayed the onset of AHL in DBA/2J mice (Ahn et al., 2008).

5. Trends and challenges

Genetic factors predispose people to AHL. Only when the related pathways of a mutant gene are identified, can the treatment receive better results in animal models. For example, apoptosis in the cochleae with genetic hearing impairment in C57BL/6J mice may be linked to mitochondrial related pathways (Someya et al., 2009; Someya and Prolla, 2010). Recent studies reveal that apoptotic signaling may arise from endoplasmic reticulum (ER) stress underling functional deficiency of a particular protein (Xia et al., 2010; Zhang et al., 2010; Kang et al., 2012; Blanco-Sanchez et al., 2014). Generally, products of the mutant genes are impaired in trafficking, leading to ER stress which triggers apoptosis in cells.

Apoptosis is generally involved in the process of cochlear degeneration. It is a chronic process and anti-apoptotic treatment needs a long period. Meanwhile, more anti-apoptotic drugs should be screened using the animal models.

According to the current concept, apoptosis is generally triggered by oxidative stress. Therefore, both anti-apoptotic agents and antioxidants may be employed in the treatment of AHL.

Moreover, attempts of genetic therapy for AHL should be involved in the future study.

Acknowledgments

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