Recent Advances in the Study of Age-Related Hearing Loss: A Mini-Review

Ambrose R. Kidd III    Jianxin Bao
Department of Otolaryngology, Center for Aging, Washington University School of Medicine, St. Louis, Mo., USA

Key Words
Presbyacusis · Age-related hearing loss · Cochlea · Spiral ganglion neuron

Abstract
Hearing loss is a common age-associated affliction that can result from the loss of hair cells and spiral ganglion neurons (SGNs) in the cochlea. Although hair cells and SGNs are typically lost in the same cochlea, recent analysis suggests that they can occur independently, via unique mechanisms. Research has identified both environmental and genetic factors that contribute to degeneration of cochlear cells. Additionally, molecular analysis has identified multiple cell-signaling mechanisms that likely contribute to pathological changes that result in hearing deficiencies. These analyses should serve as useful primers for future work, including genomic and proteomic analysis, to elucidate the mechanisms driving cell loss in the aging cochlea. Significant progress in this field has occurred in the past decade. As our understanding of aging-induced cochlear changes continues to improve, our ability to offer medical intervention will surely benefit the growing elderly population.

Introduction
Age-related hearing loss (AHL), or presbyacusis, is a complex degenerative disease that affects tens of millions of people worldwide. It is one of the most prevalent chroni-
sen to focus on recent work that has improved our understanding of the cellular and molecular mechanisms that may cause age-related loss of sensory and neural cells in the cochlea. Our goal here is to give an overview of recent progress towards understanding these phenomena.

**Noise Exposure and Presbyacusis**

Exposure to damaging levels of noise may be the most studied environmental factor that affects hearing loss. Indeed, exposure to intense noise can cause temporary and permanent hearing loss in humans and animal models [6]. Longitudinal studies showed that presbyacusis is more severe in people thought to have suffered cochlear damage in their youth than in others [6]. It is believed that cochlear damage from noise exposure that causes temporary or no immediate hearing loss may, in fact, accelerate presbyacusis. Unfortunately, it is not possible to fully understand the long-term impact of noise exposure on presbyacusis in humans because so many factors cannot be controlled. However, in animal models, early noise exposure that only causes temporary threshold increases can cause permanent hearing loss [7–9]. Anatomically, the loss of SGNs is associated with early loss of synaptic terminals between inner hair cells and SGNs [8, 10].

**Pathology of Hair Cells and SGNs in AHL**

Patients and animals with AHL typically show degeneration and death of multiple cell types. Unfortunately, it is difficult to understand whether the pathology of hair cells and SGNs are connected [10]. The prevailing view has long been that age-related loss of SGNs occurs as a consequence of hair cell loss, synaptic loss, or both (fig. 1). SGNs do begin to die after mechanical or chemical damage of hair cells, although the rate is species specific. This led to the hypothesis that SGNs rely on hair cells for trophic support [11, 12]. However, SGN loss can also occur without damage or death of hair cells [13–15]. Thus, it is unclear, in animals with hair cell and SGN loss, whether SGN loss occurs in parallel to or as a consequence of hair cell loss. A conclusive answer to that question has proven difficult to find. For example, C57BL/6 mice, a common model strain with presbyacusis caused by multiple mutations [16–18], showed some mild aberrant pathology of SGNs prior to obvious hair cell loss [19]. However, the researchers could not conclusively say that hair cell degeneration had not begun, or that the abnormal pathology indicated that SGNs were dying. Our group addressed the question directly by analyzing mice genetically engineered to express either excess or deficient levels of neuregulin-1 (NRG1), which directly modulates synaptic transmission between hair cells and SGNs [20, 21]. We found that, at 12 months of age, transgenic mice that overexpress NRG1 in SGNs had lower hearing thresholds, while NRG1−/− mice had higher hearing thresholds than control mice. We discovered that the improved hearing in these transgenic mice occurred because elevated NRG1 expression enhanced the synaptic transmission between SGNs and hair cells. However, in aged mice, NRG1 overexpression did lead to increased inner hair cell (IHC) death compared to aged NRG1−/+ and control animals. This was in contrast to a similar loss of SGNs in mice overexpressing NRG1−/+ and NRG1 that was not significantly different from that observed in control animals. Together, these experiments mark the first evidence that enhanced synaptic transmission between hair cells and SGNs is unable to prevent SGN loss in aged animals and that age-associated IHC loss does not always cause SGN degeneration [21].

The disconnection between age-related loss of auditory neurons and synaptic changes has also been observed in another system. For example, previous studies identified an age-related functional decline in the medial olivocochlear (MOC) efferent system that occurs before age-related outer hair cell (OHC) loss [22–24]. We recently published work that asks whether age-related synaptic loss of the MOC efferent innervation was independent of age-related OHC loss [25]. We utilized a transgenic mouse model that expresses yellow fluorescent protein under the control of a neuron-specific promoter to facilitate visualization of the synaptic connections between MOC efferent fibers and OHCs. We observed striking synaptic loss between the MOC efferent fibers and the OHCs in aged mice. Importantly, the loss of efferent synapses is independent of age-related loss of OHCs. Thus, age-related loss of efferent synapses does contribute to the functional decline of the MOC efferent system. However, this synaptic loss does not cause OHC loss. This independent mechanism may be a common cellular pathway directing age-related changes in the peripheral nervous system.

**Molecular Mechanisms of Presbyacusis**

**Oxidative Stress Pathways**

Numerous studies have focused on the hypothesis that age-related mitochondrial dysfunction is an underlying pathology that can cause or hasten presbyacusis. This is...
supported by the observation that many genetic conditions linked to hearing loss impair mitochondrial function, and maternally inherited mutations of the mitochondrial genome can cause deafness [26–29]. The cell normally uses a network of proteins and antioxidants to ensure that it has sufficient, but not excessive, reactive oxygen species (ROS) [30]. This network becomes less efficient with age, leading to the increased ROS levels believed to cause a variety of age-associated maladies, including hearing loss [31]. Some groups have searched for, but not yet identified, genetic variants in ROS-signaling genes that are associated with presbyacusis [32, 33]. However, animal models susceptible to oxidative stress display a range of aging-related phenotypes, and results suggest the cochlea is, for unknown reasons, hypersensitive to ROS-induced mitochondrial damage [27]. For example, mice missing the gene encoding Cu/Zn superoxide dismutase 1 (SOD1) show premature presbyacusis [34, 35]. Similarly, mice deficient for glutathione peroxidase suffer accelerated AHL and are more sensitive to noise-induced hearing loss (NIHL) [36]. Importantly, the normal system maintains an oxidative balance that cannot be improved by adding exogenous SOD1 and is sufficient for normal function when the protein level is halved [35, 37]. NIHL may result from a similar sensitivity, as noise exposure is noted to cause elevated ROS levels [38].

Based on the responsiveness of the cochlea to ROS, several groups have sought to prevent or ameliorate presbyacusis by adding exogenous antioxidants. The results are varied, as some studies have shown clear benefit provided by antioxidant treatment [39, 40], but others show no effect [41, 42]. One recent study took advantage of SMP30/GNL rats, which cannot synthesize vitamin C. This allowed the researchers to control vitamin C levels exogenously. Consistent with previous work showing the utility of antioxidants, researchers found that lack of vitamin C accelerated hearing loss and caused loss of SGNs, while diets with additional vitamin C prevented hearing loss and preserved SGNs [43]. The techniques of the many antioxidant studies vary, and many of the different outcomes may be accounted for by differences in the dosing or delivery of antioxidants. In total, the research to date suggests that oxidative imbalance does contribute to presbyacusis, but also indicates that antioxidant therapy is not a magic elixir that will prevent or treat hearing loss associated with aging.

**Cell Death Pathways**

A great deal of the damage caused by ROS produced in the mitochondria occurs in the immediate environment. Not surprisingly, deletions of mitochondrial DNA are more common in presbyacusis patients than in those...
with normal hearing [44]. Some have hypothesized that damage to mitochondrial DNA leads to decreases in energy production that can ultimately cause cell death. Multiple strategies have been employed by researchers attempting to analyze the effect of mutating mitochondrial DNA on aging and cochlear function. For example, some have generated mice that fail to produce a specific DNA polymerase that is required for repair of mutations in mitochondrial DNA. These mice accumulate mitochondrial mutations more rapidly than wild-type mice. Interestingly, these mice also develop premature hearing loss [45, 46]. By contrast, mice subjected to caloric restriction, which slows the age-related decline of mitochondrial function, have delayed presbyacusis [47, 48]. These findings lend credence to the idea that the cell death that causes presbyacusis results from accumulated damage to mitochondria.

Many research groups have labored to determine whether active or passive mechanisms of cell death occur in the cochleae of those with presbyacusis. Distinguishing different forms of cell death is often complicated, especially in vivo and in aged subjects. Not surprisingly, researchers have found evidence of both necrosis and programmed cell death in aging cochleae. Multiple lines of evidence suggest that the damage and stress to hair cells and spiral ganglion cells results in programmed cell death. For example, TUNEL staining has been used repeatedly to show DNA fragmentation in hair cells and SGNs from aged animals [47, 49]. Several efforts to evaluate cell death mechanisms in the cochlea utilized qPCR and microarray technologies to analyze presbyacusis-associated gene expression changes in the cochlea. Together, they have found that numerous apoptosis-associated genes have altered expression in aged cochleae [46, 50]. The observations that caloric restriction results in reduced TUNEL-positive cells and mutation of mitochondrial DNA polymerase results in an increased number of TUNEL-positive cells indicate that programmed cell death has a role in cochlear decline [47, 51]. Also, mice that overexpress the human X-linked inhibitor of apoptosis (XIAP) protein have less hearing loss than wild-type siblings. Consistently, fewer hair cells died in mice with excess XIAP [52]. While microscopic analysis showed that dying hair cells in the cochleae of older mice typically appeared to be undergoing apoptotic death, there was also evidence of some cells undergoing necrotic death. As might be expected, these authors found expression of molecular markers consistent with caspase-dependent and caspase-independent cell death [53]. By contrast, our group found that neither overexpression of the proapoptotic protein BCL2 or deletion of the gene encoding that protein affected AHL [54]. However, Someya et al. [55] found that mice with a deletion of the proapoptotic gene Bak are resistant to AHL and SGN death. Overall, it appears that certain types of programmed cell death may contribute to age-related loss of cochlear function. However, it is not clear whether other forms of cell death are not relevant. Additional work is required to clarify the role of nonapoptotic cell death and to describe the apoptotic pathway functioning in hair cells and SGNs further.

### Calcium-Signaling Pathways

Aberrant calcium homeostasis has repeatedly been suggested as a contributor to age-related impairment of neuronal function [56–58]. Elderly women using calcium channel blockers were found to have lower hearing thresholds [59], suggesting that calcium regulation contributes to presbyacusis. Hair cells and SGNs have several types of calcium channels, including L- and T-type voltage-gated calcium channels [60–62]. The T-type, or low-voltage, calcium channel family is comprised of three members (Ca3.1, Ca3.2, and Ca3.3), based on their main pore-forming α-subunits, α1G, α1H, and α1I, respectively [63]. The α1G and α1I subunits are weakly expressed in OHCs and IHCs and moderately expressed in SGNs. The α1H subunit is highly expressed in SGNs and absent from OHCs and IHCs [62]. Excitingly, our group recently reported a significant delay of AHL and preservation of SGNs in mice missing the gene encoding the Ca3.2 T-type calcium channel. Additionally, we showed that wild-type mice treated with T-type calcium channel inhibitors had significant preservation of hearing thresholds and SGNs as compared to untreated controls [64]. These T-type calcium channel inhibitors can likewise prevent NIHL [65]. Together, these findings strongly suggest that additional research to study the link between calcium signaling and hearing loss is warranted. Research into the potential therapeutic value of T-type calcium channel inhibitors is ongoing.

### Other Pathways

It is clear that AHL is a complicated disorder that is not uniform in cause or etiology. Thus, many other mechanisms have been implicated as contributors to AHL but have not been studied extensively. These areas, described below, may prove to be fruitful avenues for future endeavors to characterize and prevent AHL.
Glucocorticoid-Signaling Pathways

The role of glucocorticoid signaling was first suggested when Bao et al. [66] showed that deletion of the β2-subunit of the nicotinic acetylcholine receptor caused accelerated AHL associated with SGN degeneration. Subsequent work in our laboratory showed that aged mice, but not young mice, lacking high-affinity nicotinic receptors were protected from NIHL. This protection was caused by an age-related increase in corticosterone and activation of glucocorticoid-signaling pathways, not disruption of efferent cholinergic transmission. Interestingly, chronic elevation of systemic corticosterone levels resulted in extensive SGN loss, indicating a delicate balance of glucocorticoid signaling is required for proper cochlear homeostasis [65]. Similarly, loss of nuclear factor B, which can function as a key component in the glucocorticoid-signaling pathway, caused premature SGN loss in mice [67].

Sex-Specific Hormone Pathways

Many researchers have identified sex-specific differences in presbyacusis in humans and animal models [68]. Additionally, estrogen has neuroprotective effects in multiple systems [69]. In 2006, researchers discovered that postmenopausal women using progestin for hormone replacement therapy had hearing loss more frequently than women using other or no treatments [70]. Similarly, combination hormone replacement therapy, using estrogen and progestin, was found to increase the incidence of AHL [71]. The cellular and molecular mechanisms by which progestin impacts cochlear function are unclear at this point. However, this example highlights the fact that our hearing system can be quite sensitive to assaults which do not damage other biological functions.

Stress Response-Signaling Pathways

The role of stress response proteins in maintaining cochlear function was first identified in studies of NIHL. These studies showed that mice missing heat-shock factor 1 (Hsf1) are less able to recover from noise-induced cochlear damage than control mice [72, 73]. A role for universal stress response proteins in presbyacusis was seen in recent work that demonstrated that the stress-responsive proteins HSP70 and HSP110 are upregulated in the cochlea of control mice (CBA/N) as compared to mice that are prone to AHL (DBA/2J). Interestingly, the authors also showed that addition of geranylgeranylace-tone, which induces HSP expression in the cochlea, to the diet of AHL-sensitive mice prevented hearing loss, although the protection was specific to the apical portion of the cochlea [74].

Glutamate-Signaling Pathways

The search for genetic determinants of presbyacusis led researchers to implicate aberrant glutamate signaling as a potential cause. Specifically, variants in GRM7, the gene encoding metabotropic glutamate receptor type 7, have been associated with susceptibility to AHL [75]. Although the exact mechanisms by which GRM7 variants may cause hearing loss are unknown, the authors demonstrated that GRM7 is expressed in SGNs and hair cells, and postulate that the causative alleles ultimately result in glutamate toxicity similar to that previously seen in SGN explants [76] (fig. 2).

Fig. 2. Mapping the causes of age-related loss of hair cells and SGNs. Contributions to age-related loss of hair cells and SGNs are presented as a flow chart. Causes involving mitochondrial function and ROS are outlined in the top half (dark gray boxes). Other contributions are outlined in the bottom half (light gray boxes). Verified interactions are shown with black lines. Interactions that are likely but not conclusively demonstrated are shown with gray lines.
Conclusions

A number of animal models of presbyacusis have been developed to allow detailed study of disease progression and causes. Recent studies in the animal models have effectively revealed numerous cellular and molecular mechanisms that contribute to AHL. It is clear that noise exposure is a critical environmental factor and that genetic aberrations can predispose one to age-related cochlear damage and dysfunction. Also, there is little doubt that damage to mitochondria and their subsequent dysfunction are often precursors to eventual disease phenotypes. Also, calcium signaling, glucocorticoid signaling, sex-specific hormones, and stress response pathways can contribute to presbyacusis. However, it is unclear whether these signaling pathways are universally involved in presbyacusis. Future studies that rely on more detailed analysis, including cell type-specific transgenic models, genomic, and proteomic techniques, to ensure the most detailed understanding and effective treatments for presbyacusis.

Acknowledgements

The authors wish to thank Drs. Kevin Oehlemiller and Barbara Bohne for helpful discussions while preparing the manuscript. A.R.K. is supported by a grant from the National Organization for Hearing Research Foundation.

References


