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Review

Mitochondrial oxidative damage and apoptosis in age-related hearing loss

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ABSTRACT

Age-related hearing loss (AHL) is a universal feature of mammalian aging and is the most common sensory disorder in the elderly population. Experimental evidence suggests that mitochondrial dysfunction associated with reactive oxygen species (ROS) plays a central role in the aging process of cochlear cells. Although it is well established that mitochondria are the major source of ROS in the cell, specific molecular mechanisms of aging induced by ROS remain poorly characterized. Here we review the evidence that supports a central role for Bak-mediated mitochondrial apoptosis in AHL. We also propose that this mechanism may be of general relevance to age-related cell death in long-lived post-mitotic cells of multiple tissues, providing an opportunity for a targeted therapeutic intervention in human aging.

1. Introduction (age-related hearing loss and mitochondria)

Age-related hearing loss (AHL) is a universal feature of mammalian aging and is the most common sensory disorder in the elderly population. AHL affects more than 40% of people over 65 years of age in the US and is projected to afflict 28 million Americans by 2030 (Aging, 2009; Gates and Mills, 2005; Yamasoba et al., 2007). AHL is associated with an age-dependent loss of sensory hair cells, spiral ganglion (SG) neurons, and stria vascularis cells in the inner ear (Gates and Mills, 2005; Yamasoba et al., 2007). The progressive loss of these cells eventually leads to AHL in mammals because hair cells and cochlear neurons do not regenerate in these organisms.

AHL is thought to be the result of aging, oxidative damage, mitochondrial impairment, and environmental factors (Kokotas et al., 2007; Liu and Yan, 2007). Noise is the most well-documented environmental factor causing hearing loss. Outer hair cells are the primary lesion from noise exposure, and the accumulated effect of noise is thought to contribute to AHL (Liu and Yan, 2007). Ototoxic substances such as aminoglycoside antibiotics also increase susceptibility to AHL as these drugs can damage hair cells (Liu and Yan, 2007). Assuming that AHL is mechanistically similar between short-lived and long-lived mammals, it should be possible to understand basic mechanisms of aging in long-lived post-mitotic cells by understanding the molecular features that account for the different rates of progression of AHL in different species. The relatively early onset of AHL in rodents, its slow progression, and our ability to monitor its progression non-invasively, make AHL an ideal system to study basic mechanisms of aging and age-related diseases.

2. Human mitochondrial diseases associated with hearing loss

A central role for mitochondrial dysfunction in AHL is supported by the finding that a large number of genetic syndromes associated with hearing loss are due to defects in mitochondria (Kokotas et al., 2007). This observation suggests that cochlear cells are exquisitely sensitive to disturbances in energy metabolism, and that the well-reported mitochondrial decay associated with aging may selectively impact the cochlea. Diseases associated with a primary mitochondrial defect can be due to mutations in the mtDNA, or mutations in nuclear genes that encode factors that function in the mitochondria (Kujoth et al., 2007). Both types of mutations have been associated with progressive hearing loss (Table 1).

It is estimated that 20% of inherited post-lingual hearing loss is caused by mutations in the mtDNA genome (Kokotas et al., 2007) and only a few percent of cases of inherited deafness display maternal inheritance (Marazita et al., 1993). Mitochondrial genome screening in a large collection of French maternally inherited non-syndromic hearing loss suggests that approximately 30% of such cases are associated with inherited mtDNA mutations (Leveque et al., 2007). Importantly, although only a minority of

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Table 1
Human genetic disorders associated with both mitochondrial dysfunction and deafness.

Disease	Gene (reference)	Age of onset (years)
mtDNA metabolism		
ADOAD	OPA1, dynamin-related GTPase (Liguori et al., 2008; Mancuso et al., 2004)	~30
PEO	POLG1, mitochondrial DNA Polymerase (Hudson et al., 2008)	~28
Mitochondrial function		
Wolfram syndrome 2	ZCD2 (Amr et al., 2007)	~10
mtDNA mutations		
MELAS/MERRF	A3243G, $tRNA^{Leu}$ (Deschauer et al., 2001; Chinnery et al., 2000)	~3
MIDD	A3243G, tRNALeu (Laloi-Michelin et al., 2009)	~48
Non-syndromic deafness	A1555G, 12SrRNA (Malik et al., 2003; Prezant et al., 1993)	~6
Pigmentary retinopathy	G12183A, tRNAHis (Crimi et al., 2003)	~11

MIDD, maternally inherited diabetes and deafness; MELAS, myoclonic epilepsy, lactic acidosis, and stroke-like episodes, PEO, progressive external ophtalmoplegia; ADOAD, autosomal dominant optic atrophy and deafness; MERRF, myoclonic epilepsy with ragged red fibers.

cases of inherited deafness is likely to result from a primary mitochondrial defect, it is clear that mitochondrial dysfunction can lead to deafness, and presumably to AHL.

Mutations that impact mtDNA genomic stability, such as defects in the DNA polymerase γ (*POLG*) that maintains mtDNA replication fidelity (Filosto et al., 2003; Hudson et al., 2008), or the *OPA1* gene (Liguori et al., 2008; Mancuso et al., 2004), which is involved in mitochondrial fission, lead to premature hearing loss. Wolfram Syndrome, a recessive autosomal disorder associated with diabetes, optic atrophy and deafness, has been recently shown to be due to mutations in *ZCD2* (Amr et al., 2007). Mutation in *Cisd2* in the mouse leads to the onset of Wolfram Syndrome 2 clearly associated with mitochondrial dysfunction, suggesting that the encoded protein, which localizes to the mitochondria, is important for mitochondrial function in affected tissues (Chen et al., 2009). There are also a number of well-characterized multisystem syndromes due to inherited mtDNA point mutations that are associated with deafness. These include MIDD (Laloi-Michelin et al., 2009), MELAS (Deschauer et al., 2001) and MERRF (Chinnery et al., 2000; Fischel-Ghodsian, 2003), which are each associated with multiple clinical phenotypes. However, similar to most phenotypes observed in mtDNA genetic disorders, deafness is not an obligatory clinical feature. Possibly, nuclear modifying genes, as well as the level of heteroplasmy of the mtDNA mutation in various tissues determine the range of clinically relevant phenotypes in an affected individual. Importantly, these genetic observations strongly suggest that alterations in mitochondrial function with age have the potential to play a major role in AHL.

3. Animal models of mitochondrial diseases associated with hearing loss

Most inbred mouse strains display at least some degree of AHL, and the age of onset of AHL is known to vary from 3 months in DBA/2J mice to over 20 months in CBA/CaJ mice (Zheng et al., 1999). The C57BL/6J mouse strain, which is widely used for aging research, displays the classic pattern of AHL by 12–15 months of age (Hunter and Willott, 1987; Keithley et al., 2004). Strains susceptible to early onset AHL are known to carry a specific mutation in the cadherin 23 gene (*Cdh23*), which encodes a component of the hair cell tip link (Keithley et al., 2004; Noben-Trauth et al., 2003; Ohlemiller, 2006). Johnson et al. (2001) screened reciprocal backcrosses of three inbred mouse strains, A/J, NOD/LtJ and SKH2/J that display AHL, and determined that mtDNA derived from the A/J strain exerted detrimental effects on hearing as compared with mtDNA from the CAST/Ei strain. The effect was only observed in mice homozygous for the A/J allele at the *Ahl* locus, and sequencing of mtDNA revealed that the effect was due to a single nucleotide insertion in the mtDNA $tRNA^{Arg}$ gene. However, we note that inherited mtDNA mutations are only one factor in the development of AHL in A/J mice, since most of the early onset-hair cell loss in this strain appears to be due to a genetic interaction between the *Cdh23* and *Ahl4* loci (Zheng et al., 2009).

Direct evidence for mitochondrial dysfunction in AHL comes from the observation that mice engineered to carry a mutation (D257A) that disrupts the exonuclease domain of the mitochondrial DNA Polymerase γ show early onset of AHL (Kujoth et al., 2005; Someya et al., 2008). Mice carrying this mutator allele of POLG display a several hundred-fold increase in the level of point mutations in mtDNA (Vermulst et al., 2007), and this increased load of point mutations increases the levels of apoptotic cells in multiple tissues (Kujoth et al., 2005). DNA microarray analysis of cochleae from mitochondrial mutator mice was associated with transcriptional alterations consistent with impairment of energy metabolism, induction of apoptosis, cytoskeletal dysfunction, and hearing dysfunction (Someya et al., 2008). TUNEL staining and caspase-3 immunostaining analysis revealed that the levels of apoptotic markers were significantly increased in the cochleae of mitochondrial mutator mice compared to age-matched controls (Someya et al., 2008). DNA microarray analysis of the cochleae of DBA/2J mice, which show severe hearing loss by 8 months of age, is also consistent with a profound downregulation of genes involved in mitochondrial energy metabolism in AHL (Someya et al., 2007a). AHL-correlated genes that change in expression in the cochleae of 8-month-old DBA/2J mice were representative of several Gene Ontology categories linked to mitochondrial function, including the mitochondrial electron transport chain and oxidative phosphorylation. A striking observation was the downregulation of 31 genes encoding components of the mitochondrial respiratory chain complexes I, II, III, IV, and V in the cochlea (Someya et al., 2007a). Taken as a whole, our observations lead us to propose a model of how accumulation of mtDNA mutations impact cochlear function, whereby mtDNA mutations lead to mitochondrial dysfunction resulting in an associated impairment of energy metabolism, and the induction of an apoptotic program that leads to death of hair cells and neurons (Someya et al., 2009).

4. Evidence for a causal role of mitochondrial ROS in AHL

There is a growing body of evidence suggesting mitochondrial ROS contributes to AHL that is age-dependent and has no defining genetic basis. The free radical theory of aging postulates that aging is the result of accumulated oxidative damage caused by ROS (Beckman and Ames, 1998; Finkel and Holbrook, 2000; Harman, 1956; Shigenaga et al., 1994). It is now widely accepted that mitochondria are a major source of ROS and a major site of ROS-induced oxidative damage, and that ROS production increases with age (Balaban et al., 2005; Beckman and Ames, 1998; Shigenaga et al., 1994; Wallace, 2005). This theory is supported by the observations that over-expressing the mitochondrial antioxidant

gene *Sod2* (Sun et al., 2002) or the mitochondrial iron regulator protein frataxin (Runko et al., 2008) significantly increases longevity in Drosophila, while over-expressing a mitochondrially-targeted catalase gene (MCAT) results in reduced age-related pathology and moderately increases lifespan in mice (Schriner et al., 2005).

Oxidative damage caused by ROS has also been postulated to play a causal role in AHL (Darrat et al., 2007; Liu and Yan, 2007; Seidman, 2000; Seidman et al., 2000; Someya et al., 2009; Van Eyken et al., 2007; Yamasoba et al., 2007). Several studies have shown that ROS are generated in cochleae exposed to high intensity noise (Jacono et al., 1998; Ohlemiller et al., 1999). Age-related cochlear hair cell loss is enhanced in mice lacking the antioxidant enzyme *Sod1* (McFadden et al., 1999), while mice lacking the antioxidant enzymes *Gpx1* or *Sod1* show enhanced susceptibility to noise-induced hearing loss (Fortunato et al., 2004; Ohlemiller et al., 2000). Moreover, oxidative protein damage increases with age in the cochleae of CBA mice (Jiang et al., 2007; Staecker et al., 2001). We have recently shown that overexpression of mitochondrially-targeted catalase results in reduced cochlear cell damage in mice (Someya et al., 2009). Specifically, we reported that the mean ABR hearing thresholds of middle-aged MCAT transgenic mice were significantly lower than those of age-matched wild-type mice at all the frequencies tested (Someya et al., 2009). In agreement with the ABR results, cell counting demonstrated that catalase overexpression reduced outer hair cell and inner hair cell loss. Furthermore, cochlear oxidative DNA damage increased during aging, and oxidative damage to DNA was reduced by mitochondrially-targeted catalase overexpression (Someya et al., 2009). Collectively, these findings suggest that mitochondrial ROS may play a causal role in AHL in mammals.

5. Evidence for a causal role of mitochondrial apoptosis in AHL

There is a growing body of evidence suggesting that an apoptosis program contributes to aging and age-related degenerative diseases (Dirks et al., 2006; Dirks and Leeuwenburgh, 2004; Kujoth et al., 2007; Someya et al., 2008, 2009). Apoptosis can occur through two major pathways: the intrinsic pathway, also known as the mitochondrial pathway, is initiated when the outer mitochondrial membrane loses its integrity, while the extrinsic pathway is initiated through ligand binding to cell surface receptors (Lindsten et al., 2000; Youle and Strasser, 2008). In mammals, mitochondria play a major role in apoptosis that is regulated by Bcl-2 family members (Youle and Strasser, 2008). Of the Bcl-2 family members, the pro-apoptotic proteins Bak and Bax have been proposed to play a central and sometimes redundant role in promoting mitochondrial-mediated apoptosis (Lindsten et al., 2000; Takeuchi et al., 2005).

Caloric restriction (CR), the only intervention known to retard several aspects of the aging process in multiple species (Sohal and Weindruch, 1996; Walford et al., 1987; Weindruch and Sohal, 1997), may retard aging in part by preventing apoptosis. In an animal model of Parkinson's disease, CR lowers symptom severity and levels of apoptosis in neurons (Mattson, 2000). CR also reduces levels of caspase-3 and caspase-9 in the brain of aged rats, suggesting that CR is neuroprotective and that apoptosis may contribute to brain aging (Shelke and Leeuwenburgh, 2003). CR has also been proposed to promote the long-term survival of irreplaceable cells by SIRT1-mediated deacetylation of the DNA repair factor Ku70, causing it to sequester the pro-apoptotic factor Bax away from mitochondria (Cohen et al., 2004). The findings that the pro-apoptotic protein Bak is upregulated in the aging human brain (Kitamura et al., 1998), as well as in the hippocampus of Alzheimer's disease patients (Obonai et al., 1998), suggest that neurons may be an important target of an age-related mitochondrial apoptotic program.

Several studies have shown that aging is associated with increased expression of apoptosis-associated genes such as the Bcl-2 family members *Bak*, *Bax*, and *Bim* in the cochlea of several strains of mice (Someya et al., 2007a, 2008; Tadros et al., 2008). Previous studies have also demonstrated that TUNEL-positive hair cells and SG neurons were distinctly evident in the cochlea of aged gerbils (Zheng et al., 1998) and mice (Usami et al., 1997). Moreover, CR slows the onset of AHL in mice, reduces the levels of apoptosis, and reduces the expression of the mitochondrial apoptosis activator gene *Bak* in the aged cochlea (Someya et al., 2007b), suggesting that Bak-mediated mitochondrial apoptosis may contribute to AHL.

We have shown recently that deletion of the mitochondrial pro-apoptotic gene *Bak* prevents AHL in mice (Someya et al., 2009). We found that the ABR hearing thresholds of middle-aged $Bak^{-/-}$ mice were significantly lower than those of age-matched wild-type mice at all frequencies tested, but were not significantly different from those of young wild-type mice at the middle and high frequencies, indicating that Bak is required for the development of AHL (Someya et al., 2009). In agreement with the ABR results, cell counting demonstrated that Bak deficiency increased cochlear SG neuron survival (Fig. 1A–D) and outer hair cell survival. We also investigated whether age-related cochlear cell death was apoptotic and found that aging resulted in increased levels of TUNEL-positive cells in the wild-type cochlea, while levels of TUNEL-positive cells in the $Bak^{-/-}$ cochlea did not increase with age. Paraquat (PQ) is known to damage neurons and cochlear cells by generating ROS (Fei et al., 2008; Nicotera et al., 2004). We also reported that primary cochlear cells isolated from mice lacking *Bak* were resistant to PQ-induced cell death at all PQ concentrations tested (Someya et al., 2009). Furthermore, PQ-induced oxidative stress increased the expression of *Bak* mRNA in wild-type cells (Someya et al., 2009). Taken together, these findings suggest that mitochondrial apoptosis may play a causal role in AHL in mammals. We note that these findings do not exclude a role for the extrinsic apoptosis pathways or other pathways such as ER stress, because AHL is a multifactorial process.

6. Dietary antioxidant supplementation interventions prevents AHL

If ROS plays a causal role in AHL, then it is likely that enhancing antioxidant defenses through antioxidant supplementation can reduce oxidative cochlear cell damage and delay the onset of AHL. In support of this hypothesis, supplementation with the antioxidant alpha-lipoic acid (LA) significantly delays the onset of AHL in Fisher 344 rats (Seidman et al., 2000) and in DBA/2J mice (Ahn et al., 2008). Several studies have also shown that supplementation with the antioxidant acetyl-L-carnitine delays the onset of AHL in Fisher 344 rats (Seidman et al., 2000) and Wistar rats (Derin et al., 2004). Seidman investigated the effects of vitamin C and vitamin E on AHL in Fisher 344 rats and found that supplementation with both vitamin C and E slows the progression of AHL in this animal model (Seidman, 2000).

We have recently investigated the effects of 17 antioxidant compounds including LA, acetyl-L-carnitine, beta-carotene, carnosine, coenzyme Q_{10} (CQ), curcumin, *d*-alpha-tocopherol, epigallocatechin gallate, gallic acid, lutein, lycopene, melatonin, *N*-acetyl-L-cysteine (NAC), proanthocyanidin, quercetin, resveratrol, and tannic acid on AHL in C57BL/6J mice (Someya et al., 2009). All animals were fed the antioxidants orally under conditions of controlled caloric intake, and the dietary regimen was maintained for 11 months starting from 4 months of age. We found that the

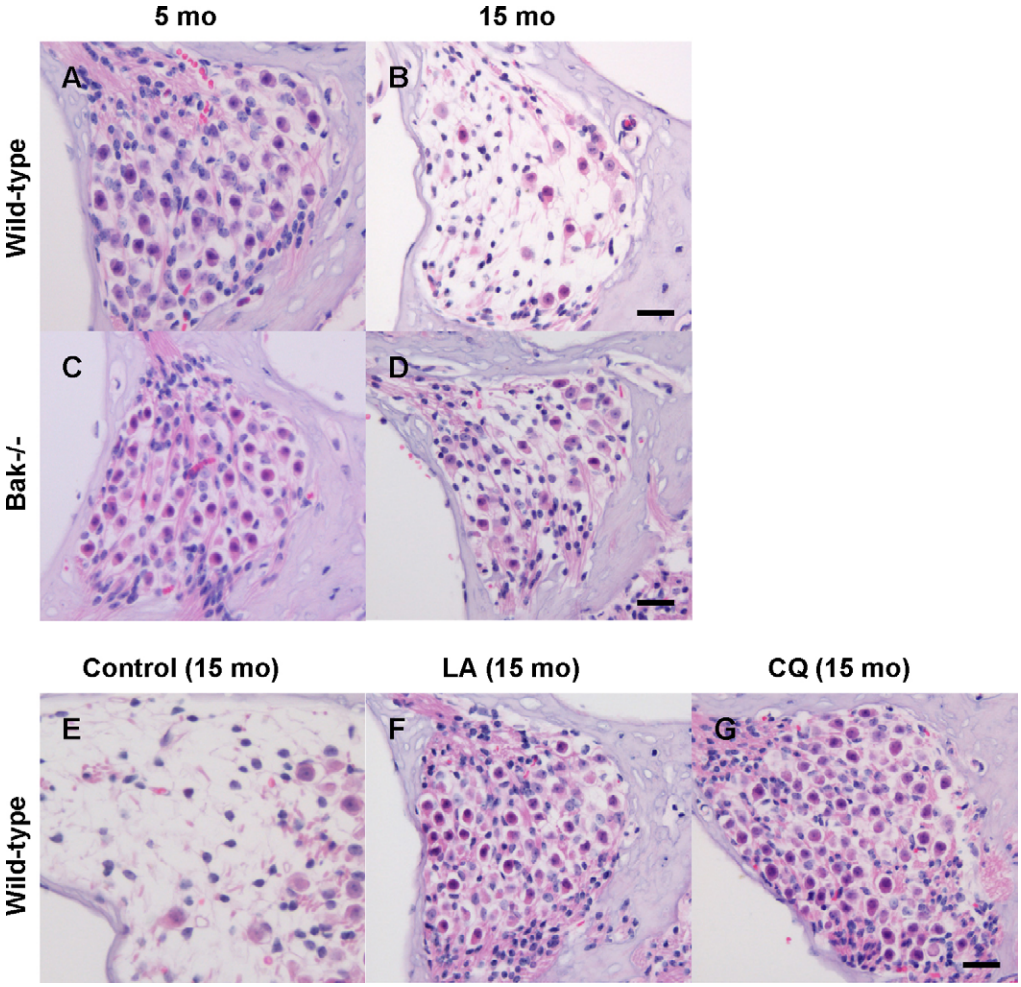

Fig. 1. Bak deficiency or antioxidant supplementation reduces cochlear neurodegeneration. (A–D) Neurons in the basal cochlear regions of wild-type and *Bak*$^{-/-}$ mice at 5 and 15 months of age. (E–G) Neurons in the basal cochlear regions of 15-month-old C57BL/6J mice fed control diet or diets supplemented with LA (alpha-lipoic acid), or CQ (coenzyme Q_{10}) (Someya et al., 2009). Scale bar = 200 μm.

mean ABR hearing thresholds from mice fed LA, CQ, or NAC were significantly lower at the high frequency than those of control diet-fed mice, and that these interventions prevented age-related cochlear SG neuron death (Fig. 1E–G). LA and NAC are thiol compounds that have been shown to reduce mitochondrial ROS production and associated mitochondrial dysfunction (Banaclocha, 2001; Hart et al., 2004; Palaniappan and Dai, 2007), while CQ is an essential component of the mitochondrial electron transfer chain and acts as a mitochondrial antioxidant (Sohal and Forster, 2007). Interestingly, we found that antioxidants that do not selectively target mitochondria did not delay the onset of AHL at all the frequencies tested (Someya et al., 2009). Together, these results suggest that a diet rich in specific antioxidants that selectively improves the mitochondrial antioxidant defense system can prevent cochlear neuron loss and retard AHL.

7. A model for the central mechanism of ROS in age-related cell death and aging

We have proposed a model of AHL that involves ROS-induced and Bak-mediated mitochondrial apoptosis, which may be of wide relevance to the aging process of multiple tissues in mammals (Someya et al., 2009) (Fig. 2). There is a growing body of evidence suggesting that oxidative damage and associated cell death contributes to the development of Parkinson's disease, Alzheimer's disease, and other age-associated neurodegenerative diseases (Darrat et al., 2007; Liu and Yan, 2007; Mattson, 2000; Sohal and Weindruch, 1996; Someya et al., 2009; Van Eyken et al., 2007; Weindruch and Sohal, 1997; Yamasoba et al., 2007). As discussed earlier, tissues such as brain and cochlea which consist of post-mitotic cells are particularly susceptible to oxidative damage since extensive cell loss in these non-regenerating tissues leads to permanent tissue dysfunction.

We also propose that a nuclear, pro-apoptotic signaling pathway is likely to play a key role in this pathway. It is well known that the nuclear transcription factor p53 is activated by DNA damage and that activation of p53 can trigger apoptosis in a wide range of cell types including neurons (Culmsee and Mattson, 2005). In response to cell stress, p53 rapidly translocates to mitochondria (Erster et al., 2004) and directly binds to Bak and induces its oligomerization, leading to cytochrome *c* release and eventually to cell death (Leu et al., 2004; Mihara et al., 2003). We have previously reported that p53-induced apoptotic genes are induced in multiple tissues with aging (Edwards et al., 2007). In agreement with the role for p53 in aging, p53 is activated in hair cells following ototoxic drug exposure (Zhang et al., 2003), while deletion of *p53* protects hair cells from the same drug-induced cell death (Cheng et al., 2005). Therefore, we propose that in response to oxidative DNA damage caused by mitochondria-derived ROS in the aged cochlea and other target tissues, p53 may translocate to mitochondria and activate Bak, leading to Bak-mediated mitochondrial apoptosis (Fig. 2).

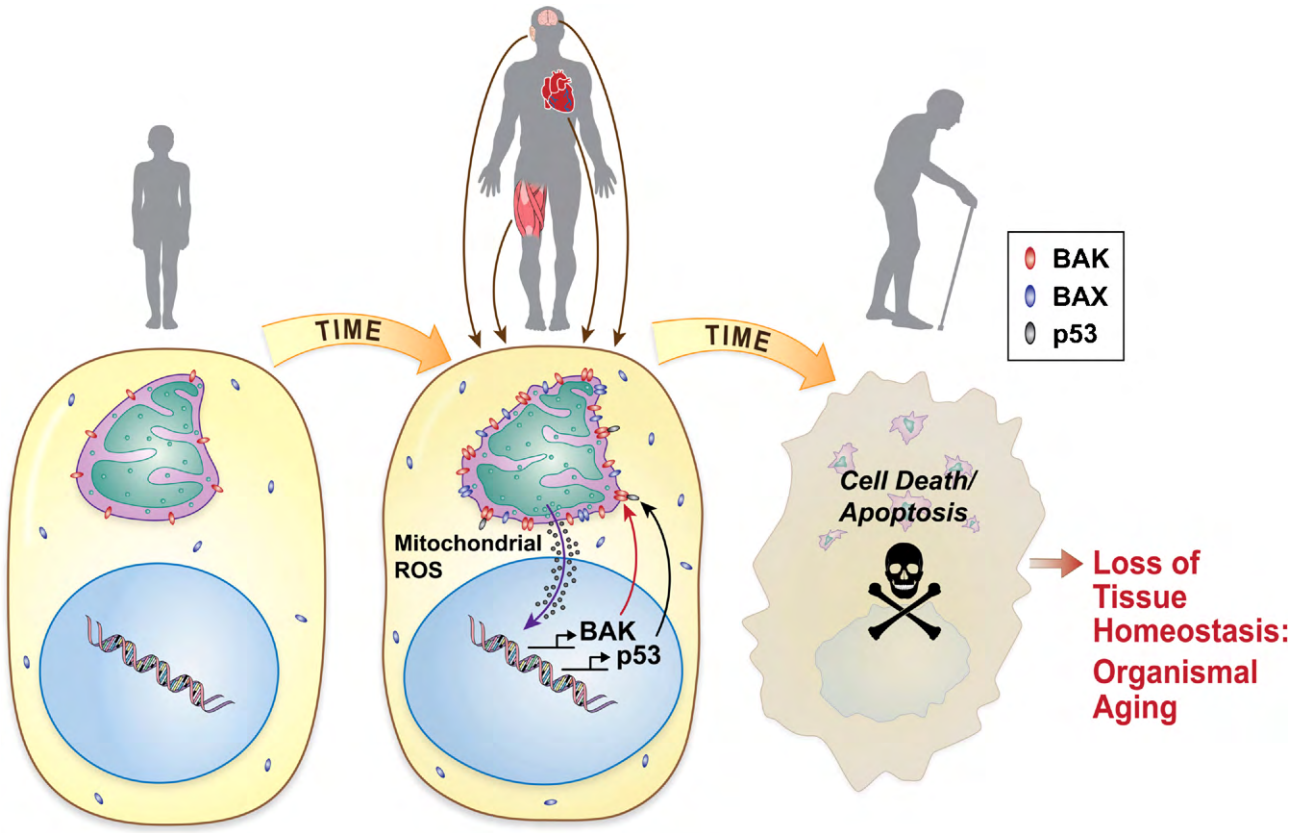

Fig. 2. Potential role of mitochondrial apoptosis in aging of long-lived cells. During aging, mitochondrial ROS production steadily increases, leading to DNA damage and the activation of a p53-mediated transcriptional response. p53 transcriptional targets include pro-apoptotic genes such as *Bak* and *Bax*. p53 also directly triggers mitochondrial apoptosis by binding to and promoting the oligomerization of pro-apoptotic Bak protein in the outer mitochondrial membrane. Chronic activation of this pathway is likely to negatively impact tissues dependent on non-regenerating long-lived cells, such as the cochlea, brain, and heart.

An important conclusion derived from our studies of the role of ROS and mitochondrial apoptosis in AHL is that cells may not need to be irreversibly damaged by ROS in order to enter the mitochondrial apoptotic program. This key conclusion is supported by the observation that $Bak^{-/-}$ mice do not display cochlear cell loss and display normal hearing at middle age, despite the fact that these animals have no evidence of reduced ROS (Someya et al., 2009). Presumably, the level of ROS that is produced in cochlear cells during aging is sufficient to trigger the Bak-mediated apoptotic program, but not sufficient to impair cellular function. Thus, the cell loss associated with AHL is an active process that can be blocked by Bak inhibition, in the absence of deleterious effects to the target tissue. If this paradigm is applicable to other tissues impacted by cell loss during aging, a significant component of the aging process may be pharmacologically blocked by improving the mitochondrial antioxidant defense system and by blocking mitochondrial apoptosis.

References

Administration on Aging, A.o., 2009. Administration on Aging, Aging Statistics Web Site.

Ahn, J.H., Kang, H.H., Kim, T.Y., Shin, J.E., Chung, J.W., 2008. Lipoic acid rescues DBA mice from early-onset age-related hearing impairment. Neuroreport 19, 1265–1269.

Amr, S., Heisey, C., Zhang, M., Xia, X.J., Shows, K.H., Ajlouni, K., Pandya, A., Satin, L.S., El-Shanti, H., Shiang, R., 2007. A homozygous mutation in a novel zinc-finger protein, ERIS, is responsible for Wolfram syndrome 2. Am. J. Hum. Genet. 81, 673–683.

Balaban, R.S., Nemoto, S., Finkel, T., 2005. Mitochondria, oxidants, and aging. Cell 120, 483–495.

Banaclocha, M.M., 2001. Therapeutic potential of N-acetylcysteine in age-related mitochondrial neurodegenerative diseases. Med. Hypotheses 56, 472–477.

Beckman, K.B., Ames, B.N., 1998. The free radical theory of aging matures. Physiol. Rev. 78, 547–581.

Chen, Y.F., Kao, C.H., Chen, Y.T., Wang, C.H., Wu, C.Y., Tsai, C.Y., Liu, F.C., Yang, C.W., Wei, Y.H., Hsu, M.T., et al., 2009. Cisd2 deficiency drives premature aging and causes mitochondria-mediated defects in mice. Genes Dev. 23, 1183–1194.

Cheng, A.G., Cunningham, L.L., Rubel, E.W., 2005. Mechanisms of hair cell death and protection. Curr. Opin. Otolaryngol. Head Neck Surg. 13, 343–348.

Chinnery, P.F., Elliott, C., Green, G.R., Rees, A., Coulthard, A., Turnbull, D.M., Griffiths, T.D., 2000. The spectrum of hearing loss due to mitochondrial DNA defects. Brain 123 (Pt 1), 82–92.

Cohen, H.Y., Miller, C., Bitterman, K.J., Wall, N.R., Hekking, B., Kessler, B., Howitz, K.T., Gorospe, M., de Cabo, R., Sinclair, D.A., 2004. Calorie restriction promotes mammalian cell survival by inducing the SIRT1 deacetylase. Science 16, 305.

Crimi, M., Galbiati, S., Perini, M.P., Bordoni, A., Malferrari, G., Sciacco, M., Biunno, I., Strazzer, S., Moggio, M., Bresolin, N., Comi, G.P., 2003. A mitochondrial tRNA (His) gene mutation causing pigmentary retinopathy and neurosensorial deafness. Neurology 8, 1200.

Culmsee, C., Mattson, M.P., 2005. p53 in neuronal apoptosis. Biochem. Biophys. Res. Commun. 331, 761–777.

Darrat, I., Ahmad, N., Seidman, K., Seidman, M.D., 2007. Auditory research involving antioxidants. Curr. Opin. Otolaryngol. Head Neck Surg. 15, 358–363.

Derin, A., Agirdir, B., Derin, N., Dinc, O., Guney, K., Ozcaglar, H., Kilincarslan, S., 2004. The effects of L-carnitine on presbyacusis in the rat model. Clin. Otolaryngol. Allied Sci. 29, 238–241.

Deschauer, M., Muller, T., Wieser, T., Schulte-Mattler, W., Kornhuber, M., Zierz, S., 2001. Hearing impairment is common in various phenotypes of the mitochondrial DNA A3243G mutation. Arch. Neurol. 58, 1885–1888.

Dirks, A.J., Hofer, T., Marzetti, E., Pahor, M., Leeuwenburgh, C., 2006. Mitochondrial DNA mutations, energy metabolism and apoptosis in aging muscle. Ageing Res. Rev. 5, 179–195.

Dirks, A.J., Leeuwenburgh, C., 2004. Aging and lifelong calorie restriction result in adaptations of skeletal muscle apoptosis repressor, apoptosis-inducing factor, X-linked inhibitor of apoptosis, caspase-3, and caspase-12. Free Radic. Biol. Med. 36, 27–39.

Edwards, M.G., Anderson, R.M., Yuan, M., Kendziorski, C.M., Weindruch, R., Prolla, T.A., 2007. Gene expression profiling of aging reveals activation of a p53-mediated transcriptional program. BMC Genomics 8, 80.

Erster, S., Mihara, M., Kim, R.H., Petrenko, O., Moll, U.M., 2004. In vivo mitochondrial p53 translocation triggers a rapid first wave of cell death in response to DNA

damage that can precede p53 target gene activation. Mol. Cell. Biol. 24, 6728–6741.
Fei, Q., McCormack, A.L., Di Monte, D.A., Ethell, D.W., 2008. Paraquat neurotoxicity is mediated by a Bak-dependent mechanism. J. Biol. Chem. 283, 3357–3364.
Filosto, M., Mancuso, M., Nishigaki, Y., Pancrudo, J., Harati, Y., Gooch, C., Mankodi, A., Bayne, L., Bonilla, E., Shanske, S., et al., 2003. Clinical and genetic heterogeneity in progressive external ophthalmoplegia due to mutations in polymerase gamma. Arch. Neurol. 60, 1279–1284.
Finkel, T., Holbrook, N.J., 2000. Oxidants, oxidative stress and the biology of ageing. Nature 408, 239–247.
Fischel-Ghodsian, N., 2003. Mitochondrial deafness. Ear Hear. 24, 303–313.
Fortunato, G., Marciano, E., Zarrilli, F., Mazzaccara, C., Intrieri, M., Calcagno, G., Vitale, D.F., La Manna, P., Saulino, C., Marcelli, V., Sacchetti, L., 2004. Paraoxonase and superoxide dismutase gene polymorphisms and noise-induced hearing loss. Clin. Chem. 50, 2012–2018.
Gates, G.A., Mills, J.H., 2005. Presbycusis. Lancet 366, 1111–1120.
Harman, D., 1956. Aging: a theory based on free radical and radiation chemistry. J. Gerontol. 11, 298–300.
Hart, A.M., Terenghi, G., Kellerth, J.O., Wiberg, M., 2004. Sensory neuroprotection, mitochondrial preservation, and therapeutic potential of N-acetyl-cysteine after nerve injury. Neuroscience 125, 91–101.
Hudson, G., Amati-Bonneau, P., Blakely, E.L., Stewart, J.D., He, L., Schaefer, A.M., Griffiths, P.G., Ahlqvist, K., Suomalainen, A., Reynier, P., et al., 2008. Mutation of OPA1 causes dominant optic atrophy with external ophthalmoplegia, ataxia, deafness and multiple mitochondrial DNA deletions: a novel disorder of mtDNA maintenance. Brain 131, 329–337.
Hunter, K.P., Willott, J.F., 1987. Aging and the auditory brainstem response in mice with severe or minimal presbycusis. Hear. Res. 30, 207–218.
Jacono, A.A., Hu, B., Kopke, R.D., Henderson, D., Van De Water, T.R., Steinman, H.M., 1998. Changes in cochlear antioxidant enzyme activity after sound conditioning and noise exposure in the chinchilla. Hear. Res. 117, 31–38.
Jiang, H., Talaska, A.E., Schacht, J., Sha, S.H., 2007. Oxidative imbalance in the aging inner ear. Neurobiol. Aging 28, 1605–1612.
Johnson, K.R., Zheng, Q.Y., Bykhovskaya, Y., Spirina, O., Fischel-Ghodsian, N., 2001. A nuclear-mitochondrial DNA interaction affecting hearing impairment in mice. Nat. Genet. 27, 191–194.
Keithley, E.M., Canto, C., Zheng, Q.Y., Fischel-Ghodsian, N., Johnson, K.R., 2004. Age-related hearing loss and the ahl locus in mice. Hear. Res. 188, 21–28.
Kitamura, Y., Shimohama, S., Kamoshima, W., Ota, T., Matsuoka, Y., Nomura, Y., Smith, M.A., Perry, G., Whitehouse, P.J., Taniguchi, T., 1998. Alteration of proteins regulating apoptosis, Bcl-2, Bcl-x, Bax, Bak, Bad, ICH-1 and CPP32, in Alzheimer's disease. Brain Res. 780, 260–269.
Kokotas, H., Petersen, M.B., Willems, P.J., 2007. Mitochondrial deafness. Clin. Genet. 71, 379–391.
Kujoth, G.C., Bradshaw, P.C., Haroon, S., Prolla, T.A., 2007. The role of mitochondrial DNA mutations in mammalian aging. PLoS Genet. 3, e24.
Kujoth, G.C., Hiona, A., Pugh, T.D., Someya, S., Panzer, K., Wohlgemuth, S.E., Hofer, T., Seo, A.Y., Sullivan, R., Jobling, W.A., et al., 2005. Mitochondrial DNA mutations, oxidative stress, and apoptosis in mammalian aging. Science 309, 481–484.
Laloi-Michelin, M., Meas, T., Ambonville, C., Bellanne-Chantelot, C., Beaufils, S., Massin, P., Vialettes, B., Gin, H., Timsit, J., Bauduceau, B., et al., 2009. The clinical variability of maternally inherited diabetes and deafness is associated with the degree of heteroplasmy in blood leukocytes. J. Clin. Endocrinol. Metab. 94, 3025–3030.
Leu, J.I., Dumont, P., Hafey, M., Murphy, M.E., George, D.L., 2004. Mitochondrial p53 activates Bak and causes disruption of a Bak-Mcl1 complex. Nat. Cell Biol. 6, 443–450.
Leveque, M., Marlin, S., Jonard, L., Procaccio, V., Reynier, P., Amati-Bonneau, P., Baulande, S., Pierron, D., Lacombe, D., Duriez, F., et al., 2007. Whole mitochondrial genome screening in maternally inherited non-syndromic hearing impairment using a microarray resequencing mitochondrial DNA chip. Eur. J. Hum. Genet. 15, 1145–1155.
Liguori, M., La Russa, A., Manna, I., Andreoli, V., Caracciolo, M., Spadafora, P., Cittadella, R., Quattrone, A., 2008. A phenotypic variation of dominant optic atrophy and deafness (ADOAD) due to a novel OPA1 mutation. J. Neurol. 255, 127–129.
Lindsten, T., Ross, A.J., King, A., Zong, W.X., Rathmell, J.C., Shiels, H.A., Ulrich, E., Waymire, K.G., Mahar, P., Frauwirth, K., et al., 2000. The combined functions of proapoptotic Bcl-2 family members bak and bax are essential for normal development of multiple tissues. Mol. Cell 6, 1389–1399.
Liu, X.Z., Yan, D., 2007. Ageing and hearing loss. J. Pathol. 211, 188–197.
Malik, S.G., Pieter, N., Sudoyo, H., Kadir, A., Marzuki, S., 2003. Prevalence of the mitochondrial DNA A1555G mutation in sensorineural deafness patients in island Southeast Asia. J. Hum. Genet. 48, 480–483.
Mancuso, M., Filosto, M., Bellan, M., Liguori, R., Montagna, P., Baruzzi, A., DiMauro, S., Carelli, V., 2004. POLG mutations causing ophthalmoplegia, sensorimotor polyneuropathy, ataxia, and deafness. Neurology 62, 316–318.
Marazita, M.L., Ploughman, L.M., Rawlings, B., Remington, E., Arnos, K.S., Nance, W.E., 1993. Genetic epidemiological studies of early-onset deafness in the U.S. school-age population. Am. J. Med. Genet. 46, 486–491.
Mattson, M.P., 2000. Apoptosis in neurodegenerative disorders. Nat. Rev. Mol. Cell Biol. 1, 120–129.
McFadden, S.L., Ding, D., Reaume, A.G., Flood, D.G., Salvi, R.J., 1999. Age-related cochlear hair cell loss is enhanced in mice lacking copper/zinc superoxide dismutase. Neurobiol. Aging 20, 1–8.
Mihara, M., Erster, S., Zaika, A., Petrenko, O., Chittenden, T., Pancoska, P., Moll, U.M., 2003. p53 has a direct apoptogenic role at the mitochondria. Mol. Cell 11, 577–590.
Nicotera, T.M., Ding, D., McFadden, S.L., Salvemini, D., Salvi, R., 2004. Paraquat-induced hair cell damage and protection with the superoxide dismutase mimetic m40403. Audiol. Neurootol. 9, 353–362.
Noben-Trauth, K., Zheng, Q.Y., Johnson, K.R., 2003. Association of cadherin 23 with polygenic inheritance and genetic modification of sensorineural hearing loss. Nat. Genet. 35, 21–23.
Obonai, T., Mizuguchi, M., Takashima, S., 1998. Developmental and aging changes of Bak expression in the human brain. Brain Res. 783, 167–170.
Ohlemiller, K.K., 2006. Contributions of mouse models to understanding of age- and noise-related hearing loss. Brain Res. 1091, 89–102.
Ohlemiller, K.K., McFadden, S.L., Ding, D.L., Lear, P.M., Ho, Y.S., 2000. Targeted mutation of the gene for cellular glutathione peroxidase (Gpx1) increases noise-induced hearing loss in mice. J. Assoc. Res. Otolaryngol. 1, 243–254.
Ohlemiller, K.K., Wright, J.S., Dugan, L.L., 1999. Early elevation of cochlear reactive oxygen species following noise exposure. Audiol. Neurootol. 4, 229–236.
Palaniappan, A.R., Dai, A., 2007. Mitochondrial ageing and the beneficial role of alpha-lipoic acid. Neurochem. Res. 32, 1552–1558.
Prezant, T.R., Agapian, J.V., Bohlman, M.C., Bu, X., Oztas, S., Qiu, W.Q., Arnos, K.S., Cortopassi, G.A., Jaber, L., Rotter, J.I., et al., 1993. Mitochondrial ribosomal RNA mutation associated with both antibiotic-induced and non-syndromic deafness. Nat. Genet. 4, 289.
Runko, A.P., Griswold, A.J., Min, K.T., 2008. Overexpression of frataxin in the mitochondria increases resistance to oxidative stress and extends lifespan in Drosophila. FEBS Lett. 582, 715–719.
Schriner, S.E., Linford, N.J., Martin, G.M., Treuting, P., Ogburn, C.E., Emond, M., Coskun, P.E., Ladiges, W., Wolf, N., Van Remmen, H., et al., 2005. Extension of murine life span by overexpression of catalase targeted to mitochondria. Science 308, 1909–1911.
Seidman, M.D., 2000. Effects of dietary restriction and antioxidants on presbyacusis. Laryngoscope 110, 727–738.
Seidman, M.D., Khan, M.J., Bai, U., Shirwany, N., Quirk, W.S., 2000. Biologic activity of mitochondrial metabolites on aging and age-related hearing loss. Am. J. Otol. 21, 161–167.
Shelke, R.R., Leeuwenburgh, C., 2003. Lifelong caloric restriction increases expression of apoptosis repressor with a caspase recruitment domain (ARC) in the brain. Faseb J. 17, 494–496.
Shigenaga, M.K., Hagen, T.M., Ames, B.N., 1994. Oxidative damage and mitochondrial decay in aging. Proc. Natl. Acad. Sci. U.S.A. 91, 10771–10778.
Sohal, R.S., Forster, M.J., 2007. Coenzyme Q, oxidative stress and aging. Mitochondrion 7 (Suppl.), S103–S111.
Sohal, R.S., Weindruch, R., 1996. Oxidative stress, caloric restriction, and aging. Science 273, 59–63.
Someya, S., Xu, J., Kondo, K., Ding, D., Salvi, R.J., Yamasoba, T., Rabinovitch, P.S., Weindruch, R., Leeuwenburgh, C., Tanokura, M., Prolla, T.A., 2009. Age-related hearing loss in C57BL/6J mice is mediated by Bak-dependent mitochondrial apoptosis. Proc. Natl. Acad. Sci. U.S.A..
Someya, S., Yamasoba, T., Kujoth, G.C., Pugh, T.D., Weindruch, R., Tanokura, M., Prolla, T.A., 2008. The role of mtDNA mutations in the pathogenesis of age-related hearing loss in mice carrying a mutator DNA polymerase gamma. Neurobiol. Aging 29, 1080–1092.
Someya, S., Yamasoba, T., Prolla, T.A., Tanokura, M., 2007a. Genes encoding mitochondrial respiratory chain components are profoundly down-regulated with aging in the cochlea of DBA/2J mice. Brain Res. 1182, 26–33.
Someya, S., Yamasoba, T., Weindruch, R., Prolla, T.A., Tanokura, M., 2007b. Caloric restriction suppresses apoptotic cell death in the mammalian cochlea and leads to prevention of presbycusis. Neurobiol. Aging 28, 1613–1622.
Staecker, H., Zheng, Q.Y., Van De Water, T.R., 2001. Oxidative stress in aging in the C57B16/J mouse cochlea. Acta Otolaryngol. 121, 666–672.
Sun, J., Folk, D., Bradley, T.J., Tower, J., 2002. Induced overexpression of mitochondrial Mn-superoxide dismutase extends the life span of adult *Drosophila melanogaster*. Genetics 161, 661–672.
Tadros, S.F., D'Souza, M., Zhu, X., Frisina, R.D., 2008. Apoptosis-related genes change their expression with age and hearing loss in the mouse cochlea. Apoptosis 13, 1303–1321.
Takeuchi, O., Fisher, J., Suh, H., Harada, H., Malynn, B.A., Korsmeyer, S.J., 2005. Essential role of BAX,BAK in B cell homeostasis and prevention of autoimmune disease. Proc. Natl. Acad. Sci. U.S.A. 102, 11272–11277.
Usami, S., Takumi, Y., Fujita, S., Shinkawa, H., Hosokawa, M., 1997. Cell death in the inner ear associated with aging is apoptosis? Brain Res. 747, 147–150.
Van Eyken, E., Van Camp, G., Van Laer, L., 2007. The complexity of age-related hearing impairment: contributing environmental and genetic factors. Audiol. Neurootol. 12, 345–358.
Vermulst, M., Bielas, J.H., Kujoth, G.C., Ladiges, W.C., Rabinovitch, P.S., Prolla, T.A., Loeb, L.A., 2007. Mitochondrial point mutations do not limit the natural lifespan of mice. Nat. Genet. 39, 540–543.
Walford, R.L., Harris, S.B., Weindruch, R., 1987. Dietary restriction and aging: historical phases, mechanisms and current directions. J. Nutr. 117, 1650–1654.
Wallace, D.C., 2005. A mitochondrial paradigm of metabolic and degenerative diseases, aging, and cancer: a dawn for evolutionary medicine. Annu. Rev. Genet. 39, 359–407.
Weindruch, R., Sohal, R.S., 1997. Seminars in medicine of the Beth Israel Deaconess Medical Center. Caloric intake and aging. N. Engl. J. Med. 337, 986–994.

Yamasoba, T., Someya, S., Yamada, C., Weindruch, R., Prolla, T.A., Tanokura, M., 2007. Role of mitochondrial dysfunction and mitochondrial DNA mutations in age-related hearing loss. Hear. Res. 226, 185–193.

Youle, R.J., Strasser, A., 2008. The BCL-2 protein family: opposing activities that mediate cell death. Nat. Rev. Mol. Cell Biol. 9, 47–59.

Zhang, M., Liu, W., Ding, D., Salvi, R., 2003. Pifithrin-alpha suppresses p53 and protects cochlear and vestibular hair cells from cisplatin-induced apoptosis. Neuroscience 120, 191–205.

Zheng, Q.Y., Ding, D., Yu, H., Salvi, R.J., Johnson, K.R., 2009. A locus on distal chromosome 10 (ahl4) affecting age-related hearing loss in A/J mice. Neurobiol. Aging 30, 1693–1705.

Zheng, Q.Y., Johnson, K.R., Erway, L.C., 1999. Assessment of hearing in 80 inbred strains of mice by ABR threshold analyses. Hear. Res. 130, 94–107.

Zheng, Y., Ikeda, K., Nakamura, M., Takasaka, T., 1998. Endonuclease cleavage of DNA in the aged cochlea of Mongolian gerbil. Hear. Res. 126, 11–18.