MITOCHONDRIA, CELL DEATH, AND DEAFNESS: WILL IT BE POSSIBLE TO PREVENT PRESBYACUSIS?

James O. Pickles
Vision, Touch and Hearing Research Centre
School of Biomedical Sciences, University of Queensland
St. Lucia, 4072 Queensland, Australia.

Mitochondria are energy-producing structures within cells, using oxidation to produce the energy-rich compound ATP (adenosine triphosphate) which drives the cell's energy-consuming reactions. Mitochondria are also triggers of programmed cell death, called apoptosis. These two important aspects of cell function are linked: when energy production by the mitochondria fails, a set of biochemical reactions are initiated which lead to destruction of the cell. Some cells types are particularly vulnerable, including certain cells of the inner ear (e.g. outer hair cells and cells of the stria vascularis), leading to sensorineural deafness. It is argued here that this response may be an evolutionary maladaptation, that cell death may be sometimes be triggered unnecessarily, and therefore that some forms of sensorineural hearing loss such as that arising in old age might be preventable.

1. INTRODUCTION: MITOCHONDRIA, ENERGY AND CELL DEATH

Much of the hearing loss that occurs in old age, i.e. presbyacusis, is likely to be due to the long-term deterioration of the mitochondria in the cells of the cochlea.

Mitochondria are the main energy-producing structures of cells. In order to couple as much energy as possible for the production of ATP, high energy electrons, originally from the oxidation of glucose, are transferred to oxygen through a set of enzymes called the electron transport chain, where their energy level changes in many small steps so little energy is lost in heat, and where the energy made available at each step is coupled to pump protons into an intermembranous space within the mitochondria. In this way, the energy from each stage is summed. The resulting high concentration of protons gives rise to what is known as the mitochondrial potential, and is used to drive the production of ATP. The arrangement is thermodynamically very efficient. However because there are only small drops in energy level at each stage in the electron transport chain, electrons tend to be diverted out of the chain, where they can react with oxygen to make the highly reactive and damaging reactive oxygen species (ROS). Overproduction of ROS can among other actions damage the proteins and lipids of the mitochondria, interfering with the stages of the electron transport chain, and then induce further production of ROS, in a vicious cycle of damage.

Damaged mitochondria are a source of danger not only to themselves, but to the rest of the cell, because the highly reactive ROS are released generally into the cell body. There appears, however, to be a valuable evolutionary adaptation in that highly damaged mitochondria are degraded and removed from the cell. Once mitochondria are not functioning properly, the mitochondrial potential falls. As a result, a structure known as the permeability transition pore opens in the mitochondrial inner membrane. The pore is permeable to low molecular mass solutes which upset the osmotic balance of the inner and outer membranes of the mitochondrion, causing water to enter the mitochondrial matrix (central space). This then swells, which breaks the outer mitochondrial membrane, and leads to destruction of the mitochondrion. In this way, mitochondria that produce excessive ROS are removed from the cell (a process described as 'mitoptosis' by Skulachev [1]). This is undoubtedly a valuable adaptation in preserving the integrity of the cell. Given that there are a large number of mitochondria per cell (tens to thousands), other mitochondria will be available to replace those lost as a result of the mitoptosis, and there is evidence that if energy production becomes inadequate, the other mitochondria within the cell will be triggered to divide and increase in number.

The suicide of individual mitochondria, which preserves the integrity of the host cell, is also continued to the next level of organization. When mitochondria break open, they release apoptotic (cell-death) substances into the cell's cytoplasm (i.e. into the cell's interior). Destruction of only a few mitochondria will be insufficient to trigger the cell death pathway, but if larger numbers are destroyed the apoptotic substances accumulate to a sufficient level to trigger the programmed, systematic, destruction of the cell and its contents. In this way, if a cell has many sick mitochondria, the whole cell will be removed from the organism. Put in an alternative way, the whole cell now commits suicide and therefore the sick cell is unable to cause further damage to the organism [1].

2. MITOCHONDRIAL INTEGRITY

This process of self-sacrifice of the lowliest individuals in a colony (or organism) to preserve the life of the colony as a whole may work well at some levels of organization, but when applied to a whole human being has some negative consequences. If the damaged elements can be replaced without penalty, then these consequences may be avoided; however, at both the mitochondrial and cellular levels, replacement has problems. Mitochondria, like the bacteria from which they evolved, contain their own separate genetic system, and reproduce by dividing. The mitochondrial genome is copied

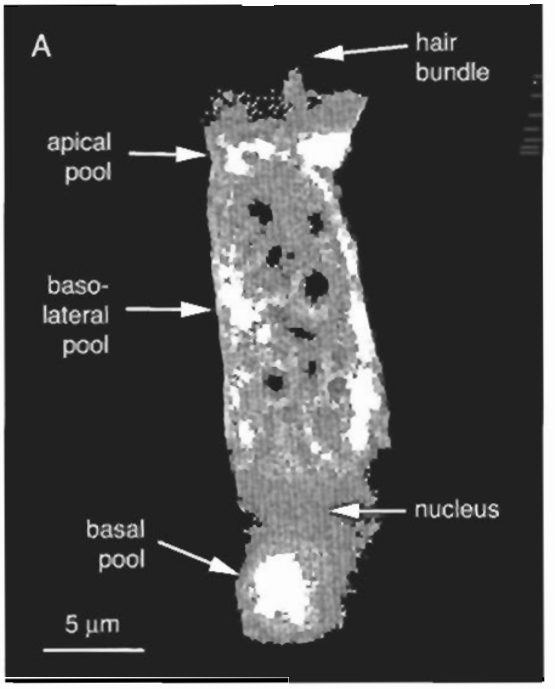

Figure 1. A. Functionally active mitochondria in a single outer hair cell of the guinea pig cochlea. The hair cell was stained with Mitotracker Red, a dye that is taken up by mitochondria with a normal mitochondrial potential. The active mitochondria are stained more intensely, against a less bright background stain, which allows the cell outline to be visible. Three pools of mitochondria are visible: (1) a pool just below the apical surface of the hair cell, (2) a pool around the basolateral walls of the hair cell, and (3) a pool just below the nucleus. The basolateral pool has a meshwork appearance. The image was reconstructed from multiple confocal images obtained at different depths in the specimen. For clarity, background signal unrelated to the cell was deleted in an image processing program.

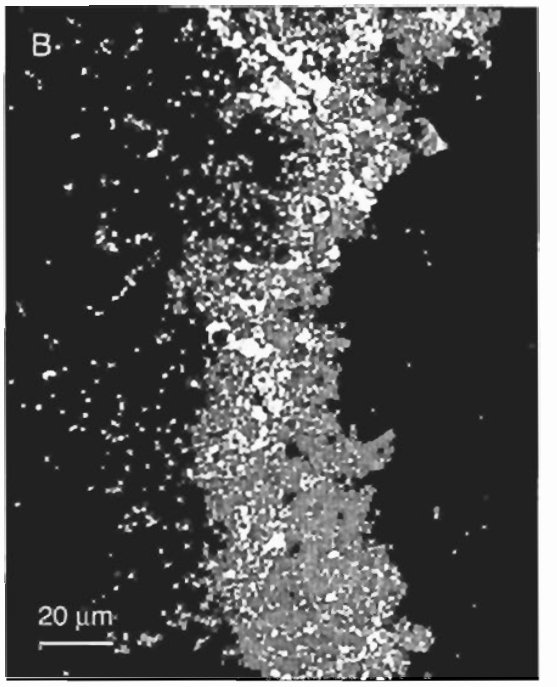

Figure 1. B. Active mitochondria in the stria vascularis of the mouse cochlea, shown by Mitotracker Red staining. The stria is the metabolically highly active secretory membrane that produces the endocochlear potential and secretes endolymph, which together provide energy for cochlear function. In this view, the stria is seen *en face*. Active mitochondria are brightly stained, against a lower level of background staining. The active mitochondria are scattered throughout the stria, but are found mainly in a thin layer in depth, corresponding to the basal ends of the marginal cells which contain many of the secretory enzymes. The mitochondria tend to be clustered around the cells' nuclei, which are non staining. Note difference in scale from Part A.

with each mitochondrial division. If a mitochondrion's DNA is mutated, then the daughter mitochondria will inherit the mutated DNA. If the DNA is only so slightly mutated that cell function is not normally compromised, then the mutated DNA can spread and populate many of the cell's mitochondria, to only reveal its effects when the cell is put under other, e.g. metabolic, stressors. Furthermore, if the trigger for reproduction in the cell is especially frequent because other, dysfunctional, mitochondria in the cell also contain mutated DNA, the mutated DNA will tend to be reproduced to a greater extent than in more normal cells. In other words, if the mutation is not so severe as to cause immediate mitoptosis of all damaged mitochondria, there is a danger that mutated DNA will come to dominate overall.

Preserving the integrity of mitochondrial DNA is a challenge for the organism. The approach inherited from bacteria, of jettisoning organelles with the damaged DNA, can have, as pointed out in the last paragraph, some negative consequences. Another approach inherited from bacteria is to have a safety factor by having many copies of the genome per mitochondrion, as against only two for eukaryotic cells (i.e. cells with nuclei). In addition, mitochondria have some DNA repair enzymes, although limited in function compared with the repair enzymes for the cells' main nuclear genomic DNA [2]. The enzyme that copies mitochondrial DNA, mitochondrial polymerase-A (pol-A) has some error-correction capability, although with a relatively high error rate compared with the corresponding enzyme for the cells' nuclear DNA [3]. While oxidative damage to mitochondrial DNA can be efficiently removed in vivo [2], other types of damage, such as the more complex of the nucleotide modifications, may be much less efficiently removed than with repair of nuclear DNA. The repair of mutated mitochondrial DNA is also dependent on the state of the cell: it is less efficient in cells with a low mitochondrial potential, but since these cells are also more likely to enter apoptosis, the result is that although their mitochondrial DNA is not error-corrected, it is also more likely to become excluded from the organism [4].

In spite of its disadvantages, preserving the integrity of mitochondrial DNA by killing cells that contain increased amounts of mutated mitochondrial DNA seems to work where the cells can be replaced afterwards. The removal of

mutated mitochondrial DNA from tissues that are renewed by mitosis (i.e. the production of new cells by cell division) may explain why inherited mutant loads have usually been found to *decrease* with age in renewable tissues such as epithelial cells and blood [5]. The removal of cells can therefore be advantageous if damaged cells are replaced by mitosis, and the mechanism is possibly an adaptation that has for this reason been favoured by evolution.

However, in some organs the cells cannot be renewed; in these cases the cells are entirely "postmitotic" – i.e. cannot undergo further rounds of cell division. Organs where the main cell types are postmitotic include neurones as in the brain, muscle cells, and many cells types of the inner ear. Here, the adaptation is definitely non-advantageous, and mitochondrially-induced apoptosis can eventually compromise the function of first the whole organ, and, in some cases, the entire organism. In terms of hearing, a consequence is the hearing loss of old age, or presbyacusis.

3. MITOCHONDRIA AND HEARING LOSS

What is the evidence that some at least of cochlear hearing loss is mitochondrially-induced? Evidence comes from two main sources:

1. Mitochondrial defects tend to affect cell types that are postmitotic, that have high energy requirements dependent on oxidative phosphorylation, and that are often heavily involved in ion pumping. Such cells in the ear are neurones of the spiral ganglion, the hair cells, and the cells of the stria vascularis. The latter cells provide the endocochlear potential, which is the battery that drives cochlear function. These are the cell types that degenerate first in many types of hearing loss.

2. Inherited mitochondrial diseases commonly first appear in the ear, brain, muscle, the eye, and kidney, and where there are known mitochondrial encephalomyopathies there is a high chance (42%) of associated hearing defects [6]. Inherited mitochondrial diseases usually occurs where mutated mitochondrial DNA is inherited from the mother (it is only from the mother, because all the mitochondria in the embryo come from the ovum; sperm contain no, or very few, mitochondria). The mutations may not be so severe so that the organism becomes non-viable and dies early in life; the mutated mitochondrial DNA comes to populates all or many cells in the organism, and shows its effects only later in life or when the organism is stressed. Many of these diseases show themselves in syndromes, where multiple organ types are affected: organs commonly involved are those with cells that are postmitotic, and have high energy requirements. Deafness is commonly a consequence [7 – 11], with degeneration particularly in the stria vascularis, spiral ganglion, and organ of Corti [9, 11].

Outer hair cells, which contain high levels of mitochondria, are among the first to degenerate in the inner ear. Why are these cells so vulnerable to energy disruption? It is not certain. A major function of outer hair cells is their active motile process, which generates the high sensitivity and sharp frequency tuning of the cochlea. However, the active process is thought to derive its energy from the ionic concentration and electrical potential of the endocochlear space (i.e. of the scala media) rather than from the outer hair cells themselves [12]. Outer hair cells are likely to have minimal neurotransmitter production and synaptic activation, since the type II afferent fibres which make synaptic connections with them have small, sparse synapses, and, as far as current information goes, do not generate a high rate of action potentials [13]. In contrast, in neurones, the energy requirements of synaptic activity are likely to be a major contributor to oxidative stress [14]. Mechanotransduction is another possible energy demand. However, the mechanotransducer current is mainly carried by K^+, which flows passively through the cell down its electrochemical gradient between scala media and scala tympani, and therefore should not make energetic demands on the cell [15]. Significant demand may be made by Ca^{2+}, which also enters through the mechanotransducer channels [48], and which is very far from its electrochemical equilibrium within the cell, and which therefore needs to be actively removed. The high concentration of mitochondria around the basolateral walls of the outer hair cells (Fig. 1A), through which the Ca^{2+} would have to be removed, suggests that this indeed is the major site of energy demand. However, there is no definitive evidence on this point.

The stria vascularis is a second important site for sensorineural hearing loss [50]. Cells such as the marginal cells and basal cells, which are heavily involved in ion pumping and which have a high concentration of mitochondria and a high energy consumption, become unable to generate the endocochlear potential. The latter forms the battery that drives the operation of the hair cells and the organ of Corti, and when the endocochlear potential falls, hearing loss is a consequence.

Reactive oxygen species (ROS) are responsible for some types of cochlear damage, with mitochondria and mitochondrial DNA among their possible targets. Knockout of an antioxidant enzyme called Gpx-1, which results in enhanced levels of ROS in tissues, elevates auditory thresholds and increases the susceptibility to acoustic trauma [16]. ROS induced by the application of paraquat to the inner ear causes hearing loss and loss of hair cells [49]. ROS is involved in some forms of ototoxicity: production of ROS in the cochlea is enhanced by both aminoglycoside antibiotics and by cisplatin, while anti-oxidants can provide some protection [e.g. 18 - 22]. The mitochondrial transition pore is involved in the apoptosis after aminoglycoside ototoxicity, since blocking the pore with cyclosporin A partially protects against the ototoxicity in culture [23]. Acoustic overstimulation causes oxidative damage to total cochlear DNA [17], while oxidative damage caused by knockout of the antoxidant enzyme SOD-1, produces deletions in mitochondrial DNA from whole cochleae [24]. Under the hypothesis presented here, the ROS-induced damage to the mitochondria causes a loss of efficiency of electron transport, so that still more ROS are produced, setting off the vicious cycle of degradation.

4. PROTECTION FROM HEARING LOSS INHIBITION OF THE CELL DEATH PATHWAYS

Loss of hair cells is a common result of ageing, acoustic trauma and ototoxicity, with apoptosis (otherwise known as programmed cell death) included as a mechanism [e.g. 25 - 27]. Proteins known as the caspases act as biochemical signals within cell death pathways; however, in the presence of caspase inhibitors, hair cells can survive doses of aminoglycosides or cisplatin that would otherwise be lethal [28 - 30]. Moreover, if members of another family of proteins called Bcl-2 are overexpressed, cell death is inhibited, and hair cells will survive an ototoxic insult that would otherwise be lethal to them [31]. This shows that in normal cells, i.e. those unprotected by the overexpressed Bcl-2, mitochondrially-triggered cell death occurs, although the cells otherwise might be capable of surviving the insult. The results suggest that apoptosis after insult may be an evolutionary adaptation which in the postmitotic cells of the cochlea, is not always necessary. It also suggests that were it possible to inhibit the cell death pathways, cells of the inner ear might survive and remain functional, where they otherwise might have degenerated.

One biochemical factor that may be involved in protecting hair cells, possibly by modulating the cell death pathways, is the heat shock protein Hsp70. This factor is induced in the cochlea by a wide variety of stresses including noise, hyperthermia and ototoxic drugs [34]. When Hsp70 is induced, there is protection from a subsequent noise that would normally cause a permanent hearing loss, and partial protection from ototoxic drugs [35, 36]. The same factors that induce Hsp70 also increase the level of a growth factor called glial cell line-derived neurotrophic factor (GDNF) in the cochlea. Moreover, GDNF, when applied to the cochlea, helps to protect the inner ear from acoustic trauma and ototoxicity, and in this it has similar effects to other diffusible growth factors such as neurotrophin-3 (NT-3) and the transforming growth factors TFG-α and TGF-β [37 – 39]. These growth factors may have direct effects on the cell death pathways and so promote cellular survival under stress [e.g. 40]. These latter results suggest that in the absence of such factors, the apoptosis that occurs after an insult may be an evolutionary adaptation which in the postmitotic cells of the cochlea, is not always necessary or indeed advantageous.

The arguments that apply to acoustic trauma or ototoxic damage also apply to ageing. One common change commonly found in ageing, and which is easy to detect, is what is known as the 'common ageing deletion'. It is a deletion of 4,977 base pairs from the mitochondrial genome that occurs as a result of anomalous annealing of the mitochondrial DNA during replication of the genome. Bai et al. [41] found that 14 out of 17 patients with presbyacusis had detectable levels of the 4,977-bp deletion in their cochlear tissues post mortem, but that the deletion was present in only 1 of 17 patients with normal audiograms. The 4,977-bp deletion was more common in the lymphocytes of patients with presbyacusis than in normal controls, and patients with higher degrees of hearing loss had a greater detection rate for the deletion [42]. This mutation is clearly associated with hearing loss, as shown in the unusual case of a family with inherited 4,977-bp mutations measurable in lymphocyte mtDNA. Even young members of this family could have profound hearing losses [43]. Sporadic mutations that arise randomly and are not inherited are also likely to contribute, being found at much higher rates - up to 14% in one case - in the spiral ganglion and membranous labyrinth of patients with presbyacusis than in normal controls [44].

Ageing may result in the production of increasing amounts of ROS as the mitochondria get progressively more damaged. Oxidative damage to the cochlea can be reduced by mitochondrial metabolites such as α-lipoic acid or acetyl-l-carnitine, both of which facilitate mitochondrial function. In a small-scale study, both compounds abolished further age-related hearing losses in aged Fisher rats, and reduced the amount of common-ageing deleted mitochondrial DNA extracted from stria vascularis and auditory nerve [45]. In a further small study, anti-oxidants were also found to reduce age-related hearing losses and mutations in mitochondrial DNA in rats, although dietary restriction had the most beneficial effect of all the treatments tried [46]. It is a common finding that basal hair cells are more vulnerable to ageing and to many insults than are apical hair cells, at least in part because they are more susceptible to ROS, associated with their lower levels of anti-oxidant protection [47].

5. CONCLUSION

Cells of the inner ear, like other cells, are vulnerable to disruption of their mitochondria. Some of the mechanisms that the body has evolved to protect itself against damaged mitochondria, many of which reflect the bacterial origin of mitochondria, have negative consequences. These include the programmed death of the vulnerable cells of the inner ear, leading to hearing loss. However, there is evidence that cells can otherwise survive the levels of damage at which the programmed cell death pathway normally kicks in. If this pathway can be inhibited in cells of the inner ear, there is the possibility that some of the most currently intractable forms of hearing loss, such as sensorineural hearing loss arising in old age, can be slowed or prevented.

ACKNOWLEDGEMENT

This work was supported by the Garnett Passe and Rodney Williams Memorial Foundation.

REFERENCES

1. Skulachev, V.P. "Mitochondria in the programmed death phenomena; A principle of biology: 'It is better to die than to be wrong'" *IUBMB Life* **49,** 365 - 373 (2000)
2. Shadel, G.S. and Clayton, D.A. "Mitochondrial DNA maintenance in vertebrates" Ann. Rev. Biochem. **66,** 409 - 435 (1997)
3. Kunkel, T.A. and Loeb, L.A. "Fidelity of mammalian DNA polymerases" *Science* **213,** 765 – 767 (1997).
4. Santos, J.H., Hunakova, L., Chen, Y., Bortner, C. and van Houten, B. "Cell sorting experiments link persistent mitochondrial DNA damage with loss of mitochondrial membrane potential and apoptotic cell death" J. Biol. Chem. **278,** 1728 - 1734. (2003)

5. Olsson, C., Johnsen, E., Nilsson, M., Wilander, E., Syvanen, A.C. and Lagerstrom-Fermer, M. "The level of the mitochondrial mutation A3243G decreases upon ageing in epithelial cells from individuals with diabetes and deafness" *Eur. J. Hum. Genet.* **9,** 917 – 921 (2003)
6. Zwirner, P. and Wilichowski, E. "Progressive sensorineural hearing loss in children with mitochondrial encephalomyopathies" *Laryngoscope* **111,** 515 - 521 (2001)
7. 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. "Mitochondrial ribosomal RNA mutation associated with both antibiotic-induced and non-syndromic deafness" *Nat. Genet.* **4,** 289 - 294 (1993)
8. Hutchin, T., Haworth, I., Higashi, K., Fischel-Ghodsian, N., Stoneking, M., Saha, N., Arnos, C. and Cortopassi, G., "A molecular basis for human hypersensitivity to aminoglycoside antibiotics" *Nucleic Acids Res.* **21,** 4174 - 4179 (1993).
9. Sue, C.M., Lipsett, L.J., Crimmins, D.S., Tsang, C.S., Boyages, S.C., Presgrave, C.M., Gibson, W.P., Byrne, E. and Morris, J.G., "Cochlear origin of hearing loss in MELAS syndrome" *Ann. Neurol.* **43,** 350 - 359 (1998)
10. Fischel-Ghodsian, N. "Mitochondrial RNA processing and translation: Link between mitochondrial mutations and hearing loss?" *Mol. Genet. Metab.* **65,** 97 - 104 (1998)
11. Nadol, J.B. Jr. and Merchant, S.N. "Histopathology and molecular genetics of hearing loss in the human" *Int. J. Pediatr. Otorhinolaryngol.* **61,** 1 - 15 (2001).
12. Ashmore, J. "Biophysics of the cochlea - Biomechanics and ion channelopathies" Brit. Med. Bull. **63,** 59 - 72 (2002)
13. Robertson, D. "Horseradish peroxidase injection of physiologically characterised afferent and efferent neurones in the guinea pig spiral ganglion" *Hear. Res.* **15,** 113 - 121 (1984)
14. Mattson, M.P. and Liu, D. "Energetics and oxidative stress in synaptic plasticity and neurodegenerative disorders" *Neuromolecular Med.* **2,** 215 - 231 (2002)
15. Pickles, J.O., *An Introduction to the Physiology of Hearing*, London, Academic Press, London (1988)
16. Ohlemiller, K.K., McFaddlen, S.L., Ding, D.L, Lear, P.M. and Ho, Y.S, "Targeted mutation of the gene for cellular glutathione peroxidase (Gpx1) increases noise-induced hearing loss in mice" *J. Assoc. Res. Otolaryngol.* **1,** 243 - 354 (2000).
17. Van Campen, L.E., Murphy, W.J., Franks, J.R., Mathias, P.I. and Toraason, M.A., "Oxidative DNA damage is associated with intense noise exposure in the rat" *Hear. Res.* **164,** 29 - 38 (2002)
18. Kopke, R.D., Liu, W., Gabaizadeh, R., Jacono, A., Feghali, J., Spray, D., Garcia, P., Steinman, H., Malgrange, B., Ruben, R.J., Rybak, L. and Van de Water, T.R., "Use of organotypic cultures of Corti's organ to study the protective effects of antioxidant molecules on cisplatin-induced damage of auditory hair cells" *Am. J. Otol.* **18,** 559 - 571 (1997)
19. Lopez-Gonzalez, M.A., Delgado, F. and Lucas, M., "Aminoglycosides activate oxygen metabolites production in the cochlea of mature and developing rats" *Hear. Res.* **136,** 165 - 168 (1999)
20. Lopez-Gonzalez, M.A., Guerrero, J.M., Torronteras, R,, Osuna, C. and Delgado, F., "Ototoxicity caused by aminoglycosides is ameliorated by melatonin without interfering with the antibiotic capacity of the drugs" *J. Pineal Res.* **28,** 26 - 33 (2000)
21. Sha, S.H. and Schacht, J., "Antioxidants attenuate gentamicin-induced free radical formation in vitro and ototoxicity in vivo: D-Methionine is a potential protectant" *Hear. Res.* **142,** 34 - 40 (1999)
22. Sha, S.H., Taylor, R., Forge, A. and Schacht, J., "Differential vulnerability of basal and apical hair cells is based on intrinsic susceptibility to free radicals" *Hear. Res.* **155,** 1 - 8 (2000)
23. Dehne, N., Rauen, U., de Groot, H. and Lautermann, J., "Involvement of the mitochondrial permeability transition in gentamicin ototoxicity" *Hear. Res.* **169,** 47 - 55 (2002)
24. Zhang, X., Han, D., Ding, D., Dai, P., Yang, W., Jiang, S. and Salvi, R.J., "Deletions are easily detectable in cochlear mitochondrial DNA of Cu/Zn superoxide dismutase gene knockout mice" *Chin. Med. J.* **115,** 258 - 263 (2002)
25. Zheng, Y., Ikeda, K., Nakamura, M. and Takasaka, T., "Endonuclease cleavage of DNA in the aged cochlea of Mongolian gerbil" *Hear. Res.* **126,** 11 - 18 (1998)
26. Hu, B.H., Henderson, D. and Nicotera, T.M., "Involvement of apoptosis in progression of cochlear lesion following exposure to intense noise" *Hear. Res.* **166,** 62 - 71 (2002)
27. Nakagawa, T., Kim, T.S., Murai, N., Endo, T., Iguchi, F., Tateya, I., Yamamoto, N., Naito, Y. and Ito, J., "A novel technique for inducing local inner ear damage" *Hear. Res.* **176,** 122 - 127 (2003)
28. Forge, A. and Li, L., "Apoptotic death of hair cells in mammalian vestibular sensory epithelia" *Hear. Res.* **139,** 97 - 115 (2000)
29. Matsui, J.I., Ogilvie, J.M. and Warchol, M.E., "Inhibition of caspases prevents ototoxic and ongoing hair cell death" *J. Neurosci.* **22,** 1218 - 1227 (2002)
30. Wang, J., Ladrech, S., Pujol, R., Brabet, P., Van De Water, T.R. and Puel, J.L., "Caspase inhibitors, but not c-Jun NH2-terminal kinase inhibitor treatment, prevent cisplatin-induced hearing loss" *Cancer Res.* **64,** 9217 - 9224 (2004)
31. Cunningham, L.L., Matsui, J.I., Warchol, M.E. and Rubel, E.W, "Overexpression of Bcl-2 prevents neomycin-induced hair cell death and caspase-9 activation in the adult mouse utricle in vitro" *J. Neurobiol.* **60,** 89 - 100 (2004)
34. Altschuler, R.A., Fairfield, D., Cho, Y., Leonova, E., Benjamin, I.J., Miller, J.M. and Lomax, M.I., "Stress pathways in the rat cochlea and potential for protection from acquired deafness" *Audiol. Neurootol.* **7,** 152 - 156 (2002)
35. Mikuriya, T., Sugahara, K., Takemoto, T., Tanaka, K., Takeno, K., Shimogori, H., Nakai, A. and Yamashita, H., "Geranylgeranylacetone, a heat shock protein inducer, prevents acoustic injury in the guinea pig" *Brain Res.* **1065,** 107 - 114 (2005)
36. Takumida, M. and Anniko, M., "Heat shock protein 70 delays gentamicin-induced vestibular hair cell death" *Acta Otolaryngol.* **125,** 23 - 28 (2005)
37. Kawamoto, K., Yagi, M., Stover, T., Kanzaki, S. and Raphael, Y., "Hearing and hair cells are protected by adenoviral gene therapy with TGF-beta1 and GDNF" *Mol. Ther.* **7,** 484 - 492 (2003)
38. Bowers, W.J., Chen, X., Guo, H., Frisina, D.R., Federoff, H.J. and Frisina R,D., "Neurotrophin-3 transduction attenuates cisplatin spiral ganglion neuron ototoxicity in the cochlea" *Mol. Ther.* **6,** 12 - 18 (2002)
39. Chen, X., Frisina, R.D., Bowers, W.J., Frisina, D.R. and Federoff, H.J., "HSV amplicon-mediated neurotrophin-3 expression protects murine spiral ganglion neurons from cisplatin-induced damage" *Mol. Ther.* **3,** 958 - 963 (2001)
40. Subramaniam, S., Shahani, N., Strelau, J., Laliberte, C., Brandt, R., Kaplan, D. and Unsicker, K., "Insulin-like growth factor 1 inhibits extracellular signal-regulated kinase to promote neuronal survival via the phosphatidylinositol 3-kinase/protein kinase A/c-Raf pathway" *J Neurosci.* **25,** 2838 - 2852 (2005)
41. Bai, U., Seidman, M.D., Hinojosa, R. and Quirk, W.S., "Mitochondrial DNA deletions associated with aging and possibly presbycusis: A human archival temporal bone study" *Am. J. Otol.* **18,** 449 - 453 (1997)

42. Ueda, N., Oshima, T., Ikeda, K., Abe, K., Aoki, M. and Takasaka, T., "Mitochondrial DNA deletion is a predisposing cause for sensorineural hearing loss" *Laryngoscope* **108,** 580 - 584 (1998)
43. Bai, U. and Seidman, M.D., "A specific mitochondrial DNA deletion (mtDNA4977) is identified in a pedigree of a family with hearing loss" *Hear. Res.* **154,** 73 - 80 (2001)
44. Fischel-Ghodsian, N., Bykhovskaya, Y, Taylor, K., Kahen, T., Cantor, R., Ehrenman, K., Smith, R. and Keithley, E., "Temporal bone analysis of patients with presbycusis reveals high frequency of mitochondrial mutations" *Hear. Res.* **110,** 147 - 154 (1997)
45. Seidman, M.D., Khan, M.J., Bai, U., Shirwany, N. and Quirk, W.S., "Biologic activity of mitochondrial metabolites on aging and age-related hearing loss" *Am. J. Otol.* **21,** 161-167 (2000)
46. Seidman, M.D., "Effects of dietary restriction and antioxidants on presbyacusis" *Laryngoscope* **110,** 727 - 738 (2000)
47. Sha, S.H., Taylor, R., Forge, A. and Schacht, J., "Differential vulnerability of basal and apical hair cells is based on intrinsic susceptibility to free radicals" *Hear. Res.* **155,** 1 - 8 (2001)
48. Ricci, A.J., Crawford, A.C. and Fettiplace, R., "Tonotopic variation in the conductance of the hair cell mechanotransducer channel" *Neuron* **40,** 983–990 (2003)
49. Bielefeld, E.C., Hu, B.H., Harris, K.C. and Henderson, D., "Damage and threshold shift resulting from cochlear exposure to paraquat-generated superoxide" *Hear. Res.* **207,** 35-42 (2005)
50. Spicer, S.S. and Schulte, B.A., "Pathologic changes of presbycusis begin in secondary processes and spread to primary processes of strial marginal cells" *Hear. Res.* **205,** 225-240 (2005)