PROSPECTS FOR GENE THERAPY IN HEARING LOSS

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ABSTRACT

Deafness is the most common form of sensory impairment in humans. Depending on the age of onset, hearing impairment can affect oral language acquisition, cognitive development and psychosocial development /1/. Here, we cover the latest advances in gene therapy for alleviating or preventing hearing loss. This review is not meant to be comprehensive, but to highlight some of the most recent developments in the field. Several recent reviews have described potential therapeutic approaches /2-4/.

KEY WORDS

inner ear, cochlea, hair cells, *Math1*, retroviruses, adenoviruses

INTRODUCTION

Gene therapy is a technology based on the introduction of a foreign gene into the body aimed at synthesis of the gene product to replace a missing or defective gene, or to elicit a therapeutic response. This technology is, at best, still in its preliminary stages. Though there have been successes in the past, the negative impact generated by the few failures has generated a great deal of caution. Despite these reservations, many are forging ahead to attempt to find new ways to perform gene therapy in order to cure genetic diseases. For example, the

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combination of therapeutic cloning and gene therapy has been suggested and tested on immune-deficient mice /5/. Successes in humans have occurred, specifically for X-linked severe combined immunodeficiency (SCID) /6/. Much promise is held in stem cells, pluripotent cells derived from the inner cell mass of the early pre-implantation embryo, for a multitude of diseases, including Parkinson's and Alzheimer's, though this area is mired in debate /7,8/. Recent achievements in the handling of stem cells provide hope for their therapeutic use, as, for example, in the report describing stable genetic modification of stem cells that was achieved with lentiviral vectors /9/.

Attempts to implement gene therapy for treatment of hearing loss are also underway, using guinea-pigs, mice and rats as models for human deafness. The small size and relative isolation of the cochlea offer several advantages for gene therapy. Hearing loss is a clinically heterogeneous disorder, with differences in age of onset, severity, and site of lesion. Hereditary disease, ototoxic drugs, acoustic trauma, viral or bacterial infections are some factors leading to hearing loss. Usually, sensorineural hearing loss is associated with a severe, if not complete, loss of sensory hair cells, the mechanoreceptors of the inner ear. Any attempts at gene therapy must center on the prevention of cell death, or alternatively, replacement by newly generated hair cells. Furthermore, considerations regarding the method of delivery and choice of vector are an essential component.

PREVENTION OF CELL DEATH

Prior to damage of hair cells, several potential treatments are available to prevent subsequent loss, using neurotrophic factors, anti-oxidants, or anti-apoptotic agents. This strategy may succeed in cases of progressive hereditary hearing loss, where diagnostics can be used to predict that hearing loss will occur prior to clinical signs.

Neurotrophic factors, expressed by hair cells, are an essential component of the central and peripheral nervous systems and are involved in the development and survival of hair cells and neurons. Brain-derived neurotrophic factor (BDNF), delivered by a replication defective herpes simplex-1 vector, allowed for the survival and prevention of auditory neuron loss /10/. Glial cell line-derived neurotrophic factor (GDNF) inoculation in an adenovirus transgene was able to rescue

vestibular and auditory hair cells, and most compelling, convert severe auditory brainstem response (ABR) thresholds to near normal ones /11/. Protection against aminoglycoside ototoxicity was enhanced by the use of TGF-, in conjunction with adenoviral delivery of GDNF /12/.

Several apoptotic pathways are known to be involved in cisplatin- and gentamicin-induced hearing loss. Anti-apoptotic agents may be particularly effective in cases of cochlear implantation, in which insertion of electrodes may generate oxidative stress, thereby leading to further cell death. The JNK/c-Jun cascade has very recently been shown to be a significant initiator of apoptosis in damaged auditory neurons /13,14/. Neurons treated with a c-jun oligonucleotide were protected relative to non-treated cells. Caspases, major players in the apoptotic pathway, have been implicated in inner ear cell death. Caspase inhibitors have been demonstrated to protect against ototoxic-induced hearing loss /15/.

Reactive oxygen species (ROS) have been implicated in both noise-induced hearing loss and that of aging, paving the way for anti-oxidants to be used to protect hair cells. For example, superoxide dismutase protects against aminoglycoside ototoxicity delivered as gentamicin in mouse inner ears /16/. Other anti-oxidants have been used, such as L-NAC and salicylate, to protect against noise-induced hearing loss /17/.

REPLACEMENT BY NEWLY GENERATED HAIR CELLS

Perhaps the most promising avenue for replacement of hair cells has been the recent use of *Math1* and *Hath1*, the mouse and human, respectively, basic helix-loop-helix transcription factor atonal homologs /18,19/. *Math1*, in an adenoviral vector, has recently been successfully inoculated into the endolymph of mature guinea-pig cochleas, leading to generation of new hair cells in the interdental cells, inner sulcus, and Hensen cell regions (Fig. 1) /19/. Most significantly, axons from the auditory nerves reached towards some of the new hair cells, suggesting that even though displaced, these cells may attract auditory neurons. Due to the presence of endogenous hair cells in the organ of Corti, it was impossible to determine whether new hair cells were formed in this region. *Hath1* induced new hair cells

in mature mammalian inner ears, and specifically, in rat utricular maculae. Mammalian utricular hair cell regeneration is not new /20,21/, although the addition of *Hath1* appears to have dramatically improved the quantity of newly formed hair cells /18/. Both studies provide proof-of-principal that generating a large number of hair cells in the mammalian inner ear is indeed possible, and further improvements should lead to enhanced efficacy of these methods. Most striking, this is the first demonstration of regeneration of cochlear hair cells in mammals.

Fig. 1: Scanning electron microscopy of the cochlea after *in vivo* inoculation of an adenovirus with the *Math1* gene insert (Ad.*Math*1.11D) into the endolymph of a mature guinea-pig cochlea /12/. This ectopic hair cell lies in the interdental cell area with a well-developed stereocilia bundle. Scale bar, 2 μm. Reprinted with permission from /19/. © 2003 by the Society of Neuroscience.

METHOD OF DELIVERY

The major avenue for gene transfer today is via viral vectors, including adenovirus vectors (AV), adeno-associated virus vectors (AAV), herpes simplex virus vectors (HSSV), lentivirus vectors and vaccinia virus vectors (VV). Given the small volume required for introduction of viruses into the inner ear, producing adequate quantities of high titer virus should not be problematic. Moreover, the side effects often associated with systemic introduction of viruses would be minimized by introduction of viruses directly into the cochlea, given its physical isolation. Non-viral vectors, such as cationic liposome vectors, can also be used to shuttle genes into cells /22/. Low efficiency is the main disadvantage of non-viral vectors, but their non-toxic and non-immunogenic characteristics hold promise for their future value in gene therapy.

AN OUTLOOK TO THE FUTURE FOR INNER EAR GENE THERAPY

Genes that regulate inner ear development and genes that cause inner ear disease when mutated are constantly being discovered (see the Hereditary Hearing Loss Homepage, URL: http://dnalab-www.uia.ac.be/dnalab/hhh/). Along with advances in the technology for delivering genes into the inner ear, the knowledge of the genes will facilitate the development of gene therapy for inner ear disease. Proof of the principal that insertion of the wild-type gene can rescue an inner ear phenotype was demonstrated in the shaker 2 mouse, in which insertion of a BAC with the wild-type gene insert reversed the deafness and balance deficits in these animals, and more recently, in whirler mice /23,24/. This raises the hope that wild-type genes can be inserted using gene therapy to prevent or reverse hereditary inner ear disease.

Future vectors for gene therapy will need to keep improving to include at least some of the following desirable features. Vectors will need to have minimal toxicity and immune response. Ideally, vectors should only deliver the gene into the target cell, by using a cell-specific receptor. Finally, gene expression should be regulated in its duration and extent, using dietetically-regulated promoters.

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