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Research Report

Manipulating gene expression in the mature inner ear

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ABSTRACT

It is possible to manipulate gene expression in cochlear tissue, but technical issues have made this challenging in the mature in vivo inner ear. Generally, the most common reasons for such manipulations involve basic science or therapeutic quests. Examples of experimental studies are those designed to elucidate the role of a specific gene or a gene expression cascade or to understand the function of a particular cell type. Therapeutic goals may include replacing a defective gene or enhancing tissue protection, repair, or regeneration. This review summarizes the main technical approaches that are viable options for in vivo manipulation of gene expression in the mature inner ear, as well as major research and clinical issues likely to benefit from such genetic manipulations.

1. Vectors

Large nucleic acid molecules, which make up genes, are not readily taken up by cells when present in the extracellular environment. To be inserted into a cell, delivered genes usually need to be helped across the plasma membrane. This can be accomplished by packaging the gene into certain types of vectors, usually classed as viral or non-viral. The main advantage of non-viral vectors is their safety and lack of side effects. However, low efficiency of gene transfer has limited them to in vitro use, where cells can be exposed to virtually unlimited amounts of vector-containing fluid. Improvements in efficiency of non-viral vectors may make them prime candidates for clinical gene therapy. Examples of newer generation non-viral vectors include cell-penetrating peptides (El-Andaloussi et al., 2005) and "Smart" molecules that respond to cellular cues (Hart, 2005). Viral vectors are more efficient at gene transfer but tend to have more side effects. Important variables to consider when using viral vectors include ability to transduce (or transfect) a specific target cell type, ability to express the transgene in quiescent cells (versus need for genomic integration during mitosis), degree of toxicity and immune response, duration of gene expression and ability to regulate it, the size of gene insert, and the ease of producing high-titer suspension of the vector. Each of the existing viral vectors has some of the desirable characteristics along with at least one disadvantage.

Current efforts are focused at improving viral vectors by further enhancing efficiency and specificity and reducing side effects. For example, the specificity of adenoviruses to a target cell is being addressed by modification of the viral proteins that recognize the target (Noureddini and Curiel, 2005). The ability to package larger genes into vectors is enhanced by eliminating viral genes, to the extreme of generating "gutted" adenoviruses (Amalfitano, 2003). Reduced immune response is also accomplished by modification of viral proteins (Brough et al., 1997). Similar enhancements of viral vector performance are being developed for other vectors such as lentivirus,

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adeno-associated virus (AAV) and herpes simplex virus (HSV) (Burton et al., 2005; White et al., 1999; Wilson, 2004). Most published work on gene transfer in the inner ear describes work with adenovirus, adeno-associated virus, herpes simplex virus, and lentivirus (Avraham and Raphael, 2003; Lalwani and Mhatre, 2003; Patel et al., 2004; Stone et al., 2005).

2. Choice of genes

With the completed mapping of the mouse and human genomes, the choice of genetic targets is wide open and holds the potential to be tailored to specific pathological conditions or experimental aims. When a candidate gene is identified for a particular experimental or therapeutic purpose, it must be determined if up- or down-regulation of the gene is required. Vectors can be used to deliver transgenes to manipulate the target gene's expression. A transgene can be either a version of the target gene itself (e.g., to correct a null or hypomorphic allele or to overexpress a protein), or the means of manipulating the target gene and its product (usually to knock down the expression of a gene). Size of the transgene is the primary limitation in the selection of candidate gene targets because of the packaging constraints imposed by many of the vectors. This limitation is especially relevant for inner ear applications because mutations in myosin and connexin genes are prevalent in deafness (Kitamura et al., 2000), and these genes are often very large (Liang et al., 1998; Probst et al., 1998).

3. Manipulation techniques

Use of gene therapy for treating hereditary disease would usually involve replacing a defective gene with a functional (wild-type) copy. This is preferably done in a cell type that normally expresses the gene of interest. However, for some mutations, it would be sufficient to express the gene in another cell type. For example, with mutations in genes encoding secreted proteins, paracrine therapy may be sufficient. This strategy uses inner ear cells as "factories" to secrete proteins, like growth factors, into the extracellular environment (Kawamoto et al., 2003a,b; Yagi et al., 1999). Additionally, in some cases, the defective cell type is missing due to a pathological process, so it is beneficial to express the transgene in a different cell.

When size limitations preclude the inclusion of a full-length transgene into a vector, a recent advance, called spliceosome-mediated RNA trans-splicing (SMaRT (Liu et al., 2005; Puttaraju et al., 1999)), could provide a way to circumvent this problem. The most common mutation causing cystic fibrosis involves deletion of 3 bp in the CFTR gene (Kerem et al., 1989). Researchers have been able to perform SMaRT using adenovirus or AAV vectors to replace the mutated segment of the mRNA with functional exons in cystic fibrotic airway epithelia, resulting in partial restoration of CFTR activity (Liu et al., 2002, 2005). For the inner ear, a parallel can be drawn between the CFTR and connexin 26. Many cases of congenital deafness are caused by small mutations in GJB2, the gene for connexin 26 (Rabionet et al., 2002). Most are recessive, but some are dominant with dominant-negative interactions at the protein level (Martin et al., 1999; Rouan et al., 2001). Virally driven SMaRT has the dual potential of restoring functional proteins to mutated connexin alleles and preventing production of aberrant connexin proteins, possibly overcoming both recessive and dominant-negative mutations.

In other situations, it might be desirable to decrease or eliminate the expressed function of a particular gene. For this purpose, dominant-negative versions of a gene of interest can be introduced to block function at the protein level. This works well with proteins that normally form homotypic dimers or oligomers (e.g., some ion channel subunits) but can lead to non-specific effects if the dominant-negative protein binds to other subunits. RNA interference (RNAi), a recently discovered endogenous mechanism for shutting off gene expression, offers more specificity. RNAi relies on short (~20 bp) sequences of double-stranded RNA (dsRNA), called short interfering RNAs (siRNAs), that trigger a cell to degrade RNA sharing the same sequence (Caplen, 2003; Manoharan, 2003). TRPA1, the putative hair cell transduction channel, has been knocked down in mouse utricular hair cells in vitro using adenovirus to deliver DNA coding for the siRNA (Corey et al., 2004). AAV has been used successfully in other cells and tissues as a vector to initiate RNAi (Babcock et al., 2005; Tomar et al., 2003; Xia et al., 2004). The applicability of RNAi technology to the in vivo inner ear has recently been demonstrated (Maeda et al., 2005).

In the first era of gene transfer experiments, gene expression was unregulated. The outcome of gene therapy will likely be enhanced by regulating the duration of expression of the transgene. Ideally, gene expression would be repressed and/or enhanced at will. Several methods have been developed for regulating gene expression with potential for human clinical therapy. The most widely used system involves diet-controlled activation or repression of the transgene promoter using tetracycline-sensitive transcriptional repressors (Lee et al., 2005; Nakagawa et al., 2001; Rossi and Blau, 1998).

Site-specific modification of mammalian gene function is also possible using Cre/loxP and Flp/FRT approaches (Kos, 2004; Nagy, 2000; Rodin and Georgiev, 2005). With these methods, it is possible to disrupt expression of a specific gene in a single cell type, in vivo (Branda and Dymecki, 2004; Kos, 2004; Sorrell and Kolb, 2005). These approaches are currently limited for use in the mouse because of the need for germ-line homologous recombination. At present, the ability to perform such disruption in somatic cells of the living animal is limited, especially in quiescent cells. However, in the mouse inner ear, Cre/loxP approaches have been used for studying the role of specific genes during development. The retinoblastoma gene pRb maintains quiescence of the auditory epithelium (Sage et al., 2005). To achieve disruption of Rb1 in the inner ear, collagen1A1 (Col1A1)-Cre mice were bred with floxed Rb1 mice and resultant Cre;Col1A1-pRb$^{-/flox}$ ears exhibited significantly increased numbers of auditory and vestibular hair cells. In another study, site-specific modification of gene function was used to study the role of Pax2 in the development of the inner ear and other organs (Ohyama and Groves, 2004).