

Knowles Hearing Center

Northwestern University

Hearing Restoration: Gene therapy, stem cells, regeneration

SPEAKER ABSTRACTS

Towards understanding the generation of inner vs outer hair cells in the cochlea

Jaime García-Añoveros, Ph.D., Northwestern University

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The leading cause of hearing loss is the death of cochlear outer hair cells (OHCs), and since these cells are not produced after birth, current attempts to restore hearing involve stem cell or related technologies to replace lost OHCs. However, we presently have no knowledge of the critical factors required to generate an OHC as opposed to an inner hair cell (IHC), the other hair cell type in the cochlea. Our studies are leading to the identification of the genes that are differentially expressed in nascent outer vs inner hair cells. Among these some encode transcription factors that we suspect may instruct the generation of either subtype of hair cell. These studies could be instrumental in the development of regenerative approaches aimed at reversing loss of hearing due to death of OHCs.

Regeneration of the mammalian vestibular system: does function follow form?

Alan G. Cheng, M.D., Stanford Medicine

Zahra Sayyid, Tian Wang, Sherri Jones, Alan G. Cheng

As mechanoreceptors, sensory hair cells are required by vestibular organs to detect head motion. Like other vestibular organs in non-mammalian species, the macula utricle organ

robustly regenerates lost hair cells to regain function close to a pre-injury state. In contrast, the mammalian utricle incompletely regains lost hair cells after aminoglycoside-induced damage or hair cell ablation via a transgenic approach. Although few reports have demonstrated an improvement of vestibular function over time as determined by behavior assays, it is unclear whether the degree of regeneration and the maturity of regenerated hair cells correlate with improved vestibular function.

My research focuses on characterizing the mechanisms of hair cell regeneration and how they relate to functional recovery. I will present our recent work on the regenerating mammalian utricle. After ablation of utricular hair cells in adult mice, we have quantified the loss and recovery of vestibular function by measuring vestibular sensory evoked potentials 1 week to 6 months after injury, when regenerated hair cells have been observed. At these time points, individual regenerated hair cells are identified via fate-mapping of supporting cells, and their degree of maturity is analyzed and compared to the vestibular functions over these periods.


Development of gene therapy for hearing loss and dizziness

Wade W. Chien, M.D., National Institutes of Health

Hearing loss and dizziness are amongst the most common disabilities affecting the world's population. Many forms of hearing loss and dizziness are associated with mutations affecting genes which are important for inner ear development. In this talk, the current progress of inner ear gene therapy development will be summarized. Applications of gene therapy to animal models of hearing loss and dizziness will be discussed.

Coordinated regulation of Atoh1 in cochlear progenitor cell differentiation to hair cells

Albert Edge, Ph.D., Harvard Medical School, Massachusetts Eye and Ear

Control of the expression of Atoh1 is important for hair cell (HC) differentiation in the cochlea. Several overlapping pathways have been implicated in the control of Atoh1 expression. Wnt signaling is required for the establishment of the prosensory domain in the cochlea and for the differentiation of HCs. Sox2 is also required for both prosensory domain establishment and the differentiation of HCs. Overlapping control of these pathways is regulated by binding to consensus sequences in the Atoh1 3' enhancer.  Catenin and Sox2 interact with the Atoh1

enhancer and are both needed to initiate the expression of Atoh1. In addition to the regulation of Atoh1 gene expression, the level of Atoh1 must be accurately controlled by degradation of the protein to achieve normal cellular patterning during development of the cochlear sensory epithelium. The stability of Atoh1 was regulated by the ubiquitin proteasome system through the action of Huwe1, a HECT-domain, E3 ubiquitin ligase. Transfer of a polyubiquitin chain to Atoh1 by Huwe1 could be demonstrated both in intact cells and in a cell-free system, and proteasome inhibition or Huwe1 silencing increased Atoh1 levels.

Limitations of today's hearing technology: do hearing devices really restore hearing?

Tina Grieco-Calub, Ph.D., Northwestern University

Advances in hearing device technology have resulted in a wide range of hearing loss solutions for patients across the lifespan. Although today's hearing devices provide significant advantages over older technology, there continue to be limitations. Patients often report dissatisfaction with their devices, including poor speech understanding in noise, poor music perception, and, in children, delayed language acquisition. This presentation will be an overview the advances in hearing device technology as well as highlight areas where today's devices underperform.

A Novel Therapeutic Approach for the Prevention of Hearing Loss

Michelle L. Hastings, Ph.D., Chicago Medical School, Rosalind Franklin University of Medicine and Science, North Chicago, IL, USA

Drug discovery for the treatment and prevention of hearing loss has largely focused on small molecule drugs and gene-delivery approaches. In this talk, I will discuss our recent development of a novel therapeutic that we have used to effectively prevent profound deafness in animals. We focused on a gene mutation that causes Usher syndrome, the leading genetic cause of combined deafness and blindness and an associated balance dysfunction. Our therapeutic is a modified nucleic acids that can bind to gene products within the cell and, in so doing, alter the output from the gene in a highly specific and predictable manner. These, modified nucleic acids, referred to as antisense oligonucleotides (ASOs), were selectively designed to target the mutation causing Usher syndrome. When mice modelled to have Usher

syndrome were treated with a single injection ASO, they exhibited improvements in hearing and spatiotemporal behavior associated with balance that lasted for many months. Our successful treatment of hearing loss and balance deficits is a striking demonstration of the potential for this therapeutic as a treatment for this form of Usher syndrome, and also suggests the potential of this type of therapeutic in the treatment of other forms of hearing loss.

1This work was supported by the NIH/NIDCD, Hearing Health Foundation, Foundation Fighting Blindness, Midwest Eye Banks, Capita Foundation and the National Organization for Hearing Research

2 Michelle Hastings receives funding from Ionis Pharmaceuticals.

Transcriptional profiling of cochlear development using single cell RNA-Seq

Matthew W. Kelley, Ph.D., National Institutes of Health

The appearance of specialized cell types was a key step in the evolution of multicellular organisms. Over time, continued refinement led to the formation of organs or organ systems comprised of cells with highly unique functions such as red blood cells, liver hepatocytes, or neurons. Some of the most striking examples of cellular specialization are found in the mammalian inner ear. For instance, sound and movement are perceived through different types of mechanosensory hair cells while ionic homeostasis is maintained through unique cell types within the stria vascularis and endolymphatic duct. Moreover, even within a single organ, such as the auditory cochlea, two different types of hair cells, Inners and Outers, are known to be present, and even greater levels of diversity may exist within the hair cell population. A greater understanding of the degree of cellular diversity within the inner ear will be crucial to any efforts to repair or restore hearing.

Among the most significant road blocks in efforts to reveal and characterize cellular diversity within the inner ear is its small size relative to the number of unique cell types. Previous technologies lacked sufficient sensitivity to generate genetic expression profiles for individual cells, requiring pooling of populations of similar cells, ultimately resulting in the masking of differences between largely similar cells. To circumvent this challenge, we have developed techniques to isolate and characterize individual inner ear cells using RNA-seq. Initial studies examining differences in gene expression profiles between single hair cells and supporting cells isolated from the post-natal utricle and cochlea revealed unexpected and previously unknown specializations within each cellular population. As we expand our data sets to include additional time points and increased sample sizes, we hope to identify rarer cell populations

and to gain a greater understanding of the developmental and evolutionary processes that create a functioning inner ear.

Screening for Neurite Promoting Factors on Spiral Ganglion Neurons

Donna S. Whitlon, Ph.D.

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Exposure to high decibel noise can cause dysfunction or degeneration of cochlear hair cells, degeneration of synapses, retraction of peripheral fibers of spiral ganglion neurons and neuronal cell death. Because the mechanisms of damage and repair of these dissimilar physiologies are likely to be different, effective drugs for hearing loss may eventually be cocktails of compounds aimed at an assortment of molecular targets. However, the contributing molecular mechanisms to target in the auditory system are largely unknown.

To begin to address this lack of information, we have narrowed down the larger problem of hearing protection and repair to a single aspect of neural physiology: regeneration of neurite length from retracted peripheral fibers of spiral ganglion neurons. We developed, characterized and validated an approach to phenotypic screening of chemical compound libraries on primary dissociated cultures of newborn mouse spiral ganglia. Using manual dissections, plating and immunolabeling, automated imaging and computer assisted determinations of neurite lengths, we have found this assay to be highly reproducible, consistent with manual measurements of neurite lengths, and able to assay in quadruplicate 3 controls and 45 compounds with each dissection. We used this assay to screen 440 compounds of the NIH Clinical Collection, a library of compounds with a history of use in clinical trials. The assay returned one positive result or “hit” that increased neurite length: the HMG-CoA reductase inhibitor cerivastatin. Dose response curves carried out with similar inhibitors showed that most also increased neurite length with sensitivities in the order: cerivastatin (1 μ M)=fluvastatin (1 μ M) >simvastatin (5 μ M)=lovastatin (5 μ M)>atorvastatin (10 μ M). Neither pravastatin nor rosuvastatin increased neurite length when tested up to 25 μ M. Geranylgeraniol (10 μ M) blocked the neurite elongation promoting activity when tested against cerivastatin and fluvastatin, but not when tested against the Rho Kinase inhibitor H1152, which is used in the assay as a positive control. These data indicate that inhibition of cholesterol synthesis is not the mechanism by which

statins increase neurite length, but rather the mechanism involves the depletion of the geranylgeranyl-pp arm of the mevalonate pathway. Elevation of one statin to evaluation in guinea pigs exposed to high decibel noise revealed a protective effect on noise induced threshold elevation.

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