Gene discoveries may lead to regeneration of cells needed for hearing

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School of Medicine scientists have discovered biological mechanisms that appear to play a role in the regeneration of cells in the inner ear.

Over a lifetime, these cells often are damaged or die due to oxidative stress, excessive noise exposure or toxic drugs. The accumulated loss can significantly compromise hearing. Nearly one in four people ages 65-74, and half who are 75 or older, are candidates for hearing aids because of disabling hearing loss.

The discoveries could lead to new ways of evaluating, in animal models, experimental drug treatments intended to prevent hearing loss or restore hearing, and might even lead to methods for regenerating vital cells that have been lost, said Stefan Heller, PhD, professor of otolaryngology.

A paper describing the findings, as well as new methods to quickly link changes in cell function during development to molecular changes within cells, was published June 9 in *Cell Reports*. Heller is the senior author of the paper. Postdoctoral scholars Jöerg Waldhaus, PhD, and Robert Durruthy-Durruthy, PhD, share the lead authorship.

Sound waves striking the eardrum cause vibrations that are transmitted through tiny bones in the middle ear to fluid within the snail-shell-shaped cochlea of the inner ear. Specialized cochlear cells in a region called the organ of Corti use hairlike sensors to detect the vibrations in cochlear fluid and then trigger nerve signals that are sent to the brain.

"Compared to other senses, we know very little about how hearing works," Heller said. "The cells are rare. We have to crack open a bone to get to them. They perish quickly, so we must work fast." There are 120 million retinal cells in a mouse eye, Heller said, but only 3,200 hair cells in a mouse ear.

By using new techniques to rapidly and deeply probe individual cells, Heller's team has begun to close the knowledge gap.

Molecular mysteries

Many of the biophysical properties of hair cells are understood. Different hair cells along the cochlear spiral are tuned to respond to distinct ranges of sound frequency based on differences in their electrical properties. Frequency is encoded by the place and the properties of the cells' locations in the cochlea. This understanding has led to the development of cochlear implants to restore hearing in deaf people.

However, little is known about the molecular biology that determines how hair cells develop at specific locations and how different electrical properties arise among hair cells specialized to detect different frequencies. This makes it difficult for scientists to envision strategies to regenerate the specialized cells or to prevent their death, particularly in the high-frequency region of the cochlea, where cells are more susceptible to injury.

Once hair cells die in a mature mammal, they are not replaced. But scientists have recently determined that a supporting cell type, called the inner pillar cell, has the potential to regenerate hair cells in newborn mice.

In its new study of 2-day-old mice, Heller's lab team measured the

activity of 192 genes. The researchers determined which genes were turned on, or "expressed," in each of 808 hair cells and supporting cells from either the apex or base of the organ of Corti. They quantified this gene expression by measuring the amount of RNA produced from each gene.

The researchers identified patterns of gene expression that may determine whether inner pillar cells can give rise to new hair cells. Similarly, they discovered gradual changes in the expression of specific genes across cells that span the organ of Corti from its base to its apex that may be crucial for the establishment and maintenance of a population of hair cells that responds to a range of sound frequencies.

Crunching the data

Using powerful number-crunching software to analyze the large amount of genetic data, Heller's lab team accurately identified the two known types of hair cells and the seven known types of supporting cells and created a computer-generated map of their locations within the organ of Corti. They did this using only the genetic data, but then used other previously known DNA sequences to independently verify the accuracy of the cell identification and mapping.

The strategy the researchers used to predict the spatial location of cells within the organ of Corti from gene-expression data also should prove useful to biologists who study other types of cells in different organs, Heller said.

Rapid advances in single-cell gene-expression analysis are likely to supplant a standard technique called in-situ hybridization, according to Heller. The standard technique relies on labeled genetic probes to target individual genes one by one in order to identify specific cell types. The new approach of measuring hundreds of genes in parallel and

reconstructing the organs in the computer appears to be more accurate and powerful.

"Molecular gradients play a key role in developmental biology, but in the past researchers depended on identifying gradients in one molecule at a time," Heller said. "With these new techniques, we are identifying cells that, for example, have molecular characteristics of stem cells, by analyzing the expression of many genes all at once, and we know precisely where they are located."

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