Scientists use drug-like cocktail to regenerate hair cells in preclinical study

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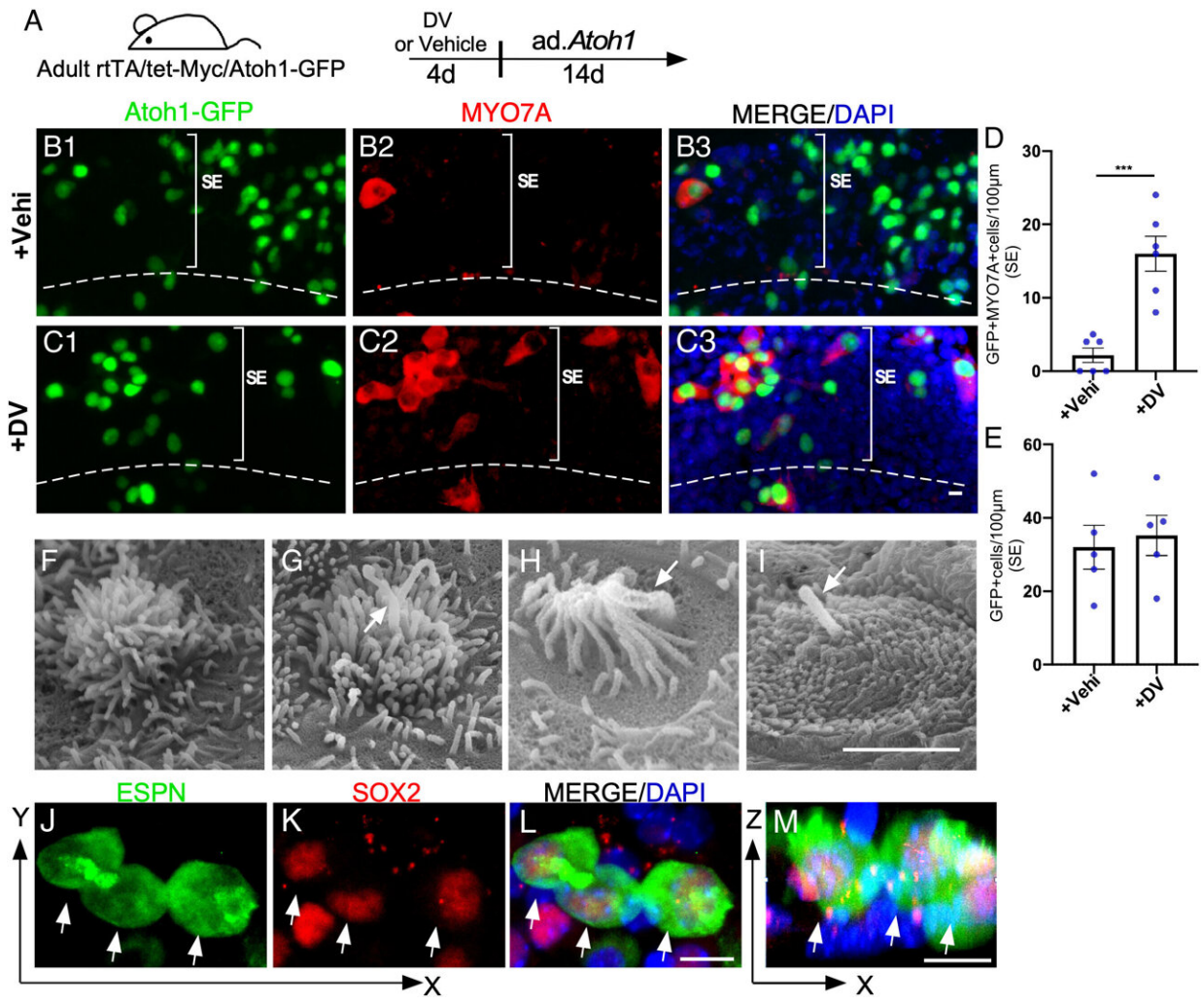

VPA/MYC synergistically reprogram adult SCs for HC-like cell regeneration. (*A*) A schematic diagram illustrating the experimental procedure of the cultured adult cochleae treated transiently by Dox to induce *Myc* and VPA to activate *NICD* (DV) and HC-like cell induction by Ad.*Atoh1*. (*B* and *C*) Vehicle (sterile

water)/Ad.*Atoh1* or Dox/VPA/Ad.*Atoh1*-treated adult (P30) rtTA/tet-*Myc*/Atoh1-GFP mice cochleae were labeled with MYO7A/GFP. Regenerated HC-like cells (MYO7A$^+$/Atoh1-GFP$^+$) were seen in the DV-treated group and occasional HCs were seen in the control sample. GFP$^+$ cells were Ad.*Atoh1* infected. (*D* and *E*) Quantification and comparison of regenerated HC-like cells and GFP$^+$ cells in the apical turn of the cultured cochleae between Dox/VPA/Ad.*Atoh1*-treated and vehicle/Ad.*Atoh1*-treated groups. Significantly more HC-like cells were seen in the DV-treated than in control samples (*D*) and a similar number of GFP$^+$ cells, i.e., the Ad.*Atoh1*-infected cells, were seen in the two groups (*E*). ***P t test. Error bar, mean ± SEM, n = 5. (*F*–*I*) Images of scanning electron microscopy (SEM) showing immature stereocilia from regenerated HC-like cells in rtTA/tet-*Myc*/Atoh1-GFP cochlea treated with Dox/VPA and Ad.*Atoh1* in vitro. Arrows point to kinocilia. (*J*–*M*) Regenerated HC-like cells (ESPN$^+$) co-labeled with SOX2 (arrows) were seen in a cultured adult (P30) rtTA/tet-*Myc* mouse cochlea treated with Dox/VPA/Ad.*Atoh1*. Arrows point to the SOX2$^+$/ESPN$^+$ double-positive HC-like cells. SE: sensory epithelial region. (Scale bar in *F*–*I*: 2 μm; *J*–*M*: 10 μm.). Credit: *Proceedings of the National Academy of Sciences* (2023). DOI: 10.1073/pnas.2215253120

Effective hearing loss treatments have eluded medicine because once sensory cells in the inner ear called hair cells are damaged or destroyed, they cannot be regenerated. This loss of hair cells, which can be caused by aging, noise exposure and other factors, renders an individual's hearing loss permanent.

Scientists at Mass Eye and Ear, a member of Mass General Brigham, are hopeful they've developed a solution to address this longstanding limitation. A research team led by Zheng-Yi Chen, DPhil, an associate scientist in the Eaton-Peabody Laboratories at Mass Eye and Ear, reports that they have created a drug-like cocktail of different molecules that successfully regenerated hair cells in a mouse model by reprogramming a series of genetic pathways within the inner ear.

They hope their novel findings, published in *Proceedings of the National Academy of Sciences (PNAS)*, could one day pave the way for clinical trials for a gene therapy that can be administered to people with hearing loss.

"These findings are extremely exciting because throughout the history of the hearing loss field, the ability to regenerate hair cells in an inner ear has been the holy grail," said Chen, who is also an associate professor of Otolaryngology–Head and Neck Surgery at Harvard Medical School. "We now have a drug-like cocktail that shows the feasibility of an approach that we can explore for future clinical applications."

New approach to achieve hearing loss treatment

Hearing loss impacts about 48 million Americans and 430 million people worldwide, numbers expected to grow with the aging population. More than 90 percent of these individuals have sensorineural hearing loss, which is caused by damage to the inner ear and the destruction of hair cells responsible for relaying sounds to the brain.

Hair cells cannot be regenerated in mammals including humans because unlike other cells in the body, any remaining hair cells in the inner ear cannot divide and other inner ear cells cannot convert themselves into new hair cells. Other species like fish, birds and reptiles, however, possess this ability.

Previously Chen's research team studied zebrafish and chickens to uncover which pathways were responsible for inducing the cell division required to regenerate new hair cells. They discovered two molecular signaling pathways, Myc and Notch, were crucial to this process. In a study published in 2019, they showed for the first time that when these pathways were activated in adult transgenic mice, remaining inner ear cells could divide and develop characteristics of hair cells. The new cells

contained transduction channels that relay sound signals and the ability to form connections with auditory neurons—processes essential to hearing.

While an exciting discovery at the time, such an approach was not directly translatable to people, according to Chen. Unlike transgenic mice, humans cannot have Myc and Notch pathways turned on like a light switch. A drug therapy, he explained, would have to be introduced to the inner ear to activate the Myc and Notch pathways.

Previous studies have shown that a chemical compound called valproic acid (VPA), can activate Notch, however no molecule exists to effectively activate Myc. That led the researchers to instead look for drug molecules that can alter the downstream pathways that turn on and off when Myc is activated.

Through single-cell RNA sequencing, they discovered that activating Myc and Notch led to a downstream effect in which two other pathways, Wnt and cAMP, became activated. Importantly they found chemical compounds that can directly activate Wnt and cAMP. They then used small biological molecules called small interfering RNAs (siRNA) to remove genes downstream that suppressed the activation of the Myc pathway.

"Think about a brake when driving a car," explained Chen. "If the brake is always engaged, you can't drive. We found an siRNA that could remove the brake in this genetic pathway."

The researchers then combined the chemical compounds and siRNA molecules into a drug-like cocktail. They delivered it to the inner ear of a normal adult mouse with damaged hair cells—an important distinction, as wildtype, non-transgenic mice would be more translatable to humans.

They further delivered the gene Atoh1 by a gene therapy approach that utilizes a harmless adenovirus into the cocktail-treated inner ear. Remarkably, they found this drug-like cocktail combined with adenovirus turned on Myc and Notch, which led to the regeneration of new hair cells. They verified that the hair cells were functional through advanced imaging and other techniques.

Regenerating hair cells through gene therapy approach

Studies like Chen's show the promise of gene therapy for treating incurable conditions like hearing loss. Last year, this research project was selected out of hundreds as one of the "Disruptive Dozen" gene and cell therapy technologies most likely to have a significant impact on health care over the next several years at the Mass General Brigham World Medical Innovation Forum. Mass General Brigham recently launched its Gene and Cell Therapy Institute to help translate scientific discoveries made by researchers like Chen into first-in-human clinical trials and, ultimately, life-changing treatments for patients.

The researchers are conducting ongoing studies and refinements to this treatment approach in larger animal models, which are necessary before applying to initiate clinical trials. They note that more research is needed to address limitations and challenges for delivering a treatment to the inner ear.

They are examining different gene therapy and surgical methods, including an approach previously honed at Mass Eye and Ear, in which a different viral vector called an adeno-associated virus (AAV) was able to precisely and safely deliver gene therapy to the inner ear through a novel surgery. Similar AAV-surgical approaches are currently used at Mass Eye and Ear in approved and experimental drug therapies for patients

with blinding inherited retinal disorders.

"My colleagues and I frequently are contacted by people with hearing loss who are desperate for effective treatments," said Chen. "If we can combine a surgical procedure with a refined gene therapy delivery method, we hope we can achieve our number one goal of bringing a new treatment into the clinic."

More information: Yi-Zhou Quan et al, Reprogramming by drug-like molecules leads to regeneration of cochlear hair cell-like cells in adult mice, *Proceedings of the National Academy of Sciences* (2023). DOI: 10.1073/pnas.2215253120

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