miRNAs sounding better

By Tim Fulmer, Senior writer

Hearing loss is a field that has been totally refractory to drug treatment. Now, British and Spanish researchers have converged on microRNA-96, or elements downstream of it, as a potential therapeutic target for treating age-related hearing loss.^{1,2} The strategy could hold promise in a space that has little to offer patients beyond hearing aids, although effective and timely delivery of a therapy will be challenging.

Age-related hearing loss (presbycusis) affects about 25–40% of individuals over 65 years of age.³ The condition involves damage to or loss of auditory hair cells in the inner ear and is usually irreversible. The only commercial treatments are hearing aids and cochlear implants. On the therapeutic front, researchers have focused on ways

to induce formation or regeneration of new hair cells, including gene therapies, cell therapies and small molecules.^{3,4}

In one of the new papers, a large-scale screen of mouse mutants identified a deaf mouse strain the researchers dubbed *diminuendo*. The mice had either progressive loss or total absence of the Preyer reflex, an ear flick response to sound that is indicative of hearing ability.

Chromosomal mapping studies showed that a mutation in the *miR-96* gene, which encodes microRNA-96 (miRNA-96), was responsible for the phenotype. Microarray analysis of auditory hair cells showed that the *miR-96* mutation had wide-ranging downstream effects on protein expression, with 50 genes upregulated and 36 genes downregulated in the mutant mice compared with what was seen in wild-type controls ($p < 0.05$).

In the second paper, sequence analysis of two Spanish families with progressive hearing loss identified another mutation in the *miR-96* gene that was not detected in 462 unrelated normal-hearing Spanish controls, suggesting that a mutation in miRNA-96 could be the cause of the families' hearing impairment.

Both papers were published in *Nature Genetics*.

Confirming the target

Researchers polled by SciBX wanted to see additional proof in patient populations and on the molecular level that mutations in *miR-96* are

Box 1. So many miRNAs, so little time.

A team of researchers from **Tel Aviv University** and **Purdue University** is busy identifying microRNAs that are expressed in the cochlea and vestibule, the two compartments of the inner ear responsible for hearing and balance.

In their latest study published in *The Proceedings of the National Academy of Sciences*,⁷ the group confirmed the importance of miRNAs to the proper development of hearing by knocking out the Dicer enzyme in the inner ear hair cells of mice. Dicer is required for the production of mature and functional miRNAs. The resulting mice developed total deafness by day 38 and showed noticeable defects in the cochlea.

Next, the researchers analyzed miRNA expression in the cochlea and vestibule of newborn wild-type mice and found distinct patterns of expression for multiple miRNA genes in the inner ear, including *miR-15a*, *miR-18a*,

miR-30b, *miR-99a* and *miR-199a*. Subsequent sequence analysis revealed that potential targets of miRNA-15a included brain-derived neurotrophic factor (Bdnf), Claudin 12 (Cldn12) and solute carrier family 12 member 2 (Slc12a2).

"We plan to insert some of the identified microRNAs into cultured cells and into the inner ear of mouse models of human deafness to look for positive effects," said Karen Avraham of Tel Aviv University, corresponding author on the *PNAS* paper. She added that in the longer term, an option for decreasing hearing loss might be delivering antagomirs of specific miRNAs to activate pathways that induce regeneration of or decrease apoptosis of hair cells.

In either case, Avraham acknowledged the need to develop a way to deliver therapeutic miRNAs to the inner

ear. "This is completely untested territory. Systemically injected antagomirs may not reach the inner ear. Thus, they would have to be delivered directly into the ear while keeping the tissue intact," she said.

Karen Steel, head of the Genetics of Deafness program at the **Wellcome Trust Sanger Institute**, told SciBX that "the *PNAS* paper complements our own work and shows that miRNA-96 is probably not the only important miRNA involved in hearing loss. From a therapeutic perspective, the long-term challenge becomes sorting out which miRNAs are most worth targeting to treat disease."

Avraham, who is chair of the Department of Human Molecular Genetics and Biochemistry in Tel Aviv's Sackler School of Medicine, said the *PNAS* findings are not patented and are unavailable for licensing. — TF

indeed key contributors to hearing pathology.

A clear next step “would be to screen other families with a similar type of hearing loss for mutations in miRNA-96,” said Michael Hildebrand, a research fellow in the Department of Otolaryngology at **The University of Iowa**. Measuring the prevalence of the mutation across other, non-Spanish populations could also help determine the value of developing an miRNA-96-targeting therapeutic, he said.

It will also be important to study the relative importance of miRNA-96 among the other miRNAs that are known to play a role in the development of the architecture of inner ear cells, said Kirk Beisel, professor of biomedical sciences at the **Creighton University School of Medicine**. He suggested looking at how altering levels of miRNA-96 and other miRNAs correlate with changes in the transcriptome and proteome of healthy and diseased inner ear cells.

Beisel and colleague Garrett Soukup at Creighton have shown that multiple miRNAs have roles in hair cell development in vertebrates and invertebrates.^{5,6}

Several research groups are pursuing projects to identify miRNAs that play a role in the development and maintenance of inner ear cell architecture (see **Box 1**, “**So many miRNAs, so little time**”).

Miguel Ángel Moreno-Pelayo, corresponding author on the Menicia *et al.* paper, told *SciBX* that he and Karen Steel, corresponding author on the Lewis *et al.* paper, now want to generate knock-in mouse strains that express the *miR-96* mutant identified in the Spanish families. “Analysis of the hair cells from those mice should provide crucial information on what targets are directly and indirectly dysregulated as a result of the miRNA-96 mutation,” he said.

Moreno-Pelayo also plans to mimic the effects of the mutant miRNA-96 in cultured human auditory progenitor cells that can differentiate into hair cells. Analysis of gene expression for those cells and their comparison to the mouse miRNA-96 mutant should also provide insight into the downstream effects of miRNA-96 mutation, he said.

Moreno-Pelayo is an investigator at the **Hospital Ramon y Cajal** and an associate professor at the **University of Carlos III**.

Steel is professor and head of the Genetics of Deafness program at the **Wellcome Trust Sanger Institute**.

Stepping toward a therapy

Developing a therapeutic to target miRNA-96 will likely pose two main challenges: delivery to cells in the inner ear and avoidance of side effects associated with interfering with non-hearing-related genes potentially regulated by the miRNA.

Before either of those issues can be addressed, however, Elena Feinstein, CSO of **Quark Pharmaceuticals Inc.**, said a key next step is determining whether the hearing loss phenotype is caused by a loss-of-function mutation, in which the miRNA-96 mutant cannot bind its original targets, or a gain-of-function mutation, in which the miRNA-96 mutant binds a new target instead of or in addition to its original targets.

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**—Karen Steel,
Wellcome Trust Sanger Institute**

In addition, Quark president and CEO Daniel Zurr said controlled, local delivery of an miRNA-96-targeting therapeutic to the hair cells will be essential. “Because miRNAs can affect the expression of many target genes, there exists the potential for unwanted modulation of off-target genes in healthy tissue if delivery is not highly specific,” he said.

Quark has a small interfering RNA molecule in preclinical development against an undisclosed target to treat acute hearing loss associated with ototoxicity and acoustic trauma.

Jonathan Kil, president and CEO of **Sound Pharmaceuticals Inc.**, thinks targeting a single miRNA such as miRNA-96 to treat age-related hearing loss could be a tall order. “Age-related hearing loss is a multi-factorial

disease with a complex underlying biology, and it’s not clear that targeting a single miRNA like miRNA-96 is going to be sufficient to reduce or reverse disease,” he said.

Sound Pharmaceuticals has two orally available small molecules, both inducers of glutathione peroxidase (GPX) antioxidant activity, in Phase II testing: SPI-1005 to treat noise-induced hearing loss and SPI-3005 to treat chemotherapy-induced hearing loss.

The company’s SPI-5557, a locally injected siRNA against cyclin-dependent kinase inhibitor 1B (CDKN1B; p27; Kip1), is in preclinical development to induce regeneration of cochlear cells and treat deafness. Kil and colleagues have shown in mice that knocking out p27, a growth inhibitory protein, can induce cell proliferation and hair cell regeneration in the inner ear.

Regardless of therapeutic strategy, Thomas Meyer, founder and managing director of **Auris Medical AG**, said the indication itself poses multiple challenges. Age-related hearing is a slowly progressing condition that generally lacks an identifiable therapeutic window, he noted.

“Because age-related hearing loss usually progresses at an average rate of only one to two decibels per year, the patient is often unaware there’s a problem until the disease has advanced so far that any type of treatment may be too late,” Meyer said. “In such a situation, a preventive therapeutic strategy might *a priori* be more promising. However, it’s unclear whether people would accept long-term treatment for a problem that would materialize only in the distant future and not with certainty,” he added.

Auris is developing therapies for acute hearing loss. “Our focus is on acute hearing loss that results from acoustic trauma or other cochlear stress factors like viral infections and vascular disturbances. Those conditions are generally rarer than age-related hearing loss but potentially more amenable to treatment, especially in an emergency setting,” said Meyer.

The company’s AM-111, a cell-permeable peptide that inhibits the c-jun N-terminal kinase (JNK) pathway and is delivered by injection through the eardrum, is in a Phase IIb trial in Europe to treat acute hearing loss. Targeting the JNK pathway has an antiapoptotic effect and thus potentially prevents damaged hair cells from dying.

Steel noted that she isn’t married to any one therapeutic approach to modulating miRNA-96. “Our future work to elucidate the targets of miRNA-96 could provide us with genes and proteins that might be

targeted directly by small molecules to trigger regeneration and treat some forms of hearing loss,” she said. “In any case, the therapeutic goal remains to stop progressive degeneration of hair cells that can cause age-related and environmental hearing impairment.”

The patent and licensing status of both *Nature Genetics* papers is undisclosed.

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e-mail: kps@sanger.ac.uk
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Contact: Miguel Ángel Moreno-Pelayo, Hospital Ramon y Cajal, Madrid, Spain

e-mail: mmoreno.hrc@salud.madrid.org

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Contact: Karen Avraham, Tel Aviv University, Tel Aviv, Israel
e-mail: karena@post.tau.ac.il

COMPANIES AND INSTITUTIONS MENTIONED

Auris Medical AG, Basel, Switzerland
Creighton University School of Medicine, Omaha, Neb.
Hospital Ramon y Cajal, Madrid, Spain
Purdue University, West Lafayette, Ind.
Quark Pharmaceuticals Inc., Fremont, Calif.
Sound Pharmaceuticals Inc., Seattle, Wash.
University of Carlos III, Madrid, Spain
The University of Iowa, Iowa City, Iowa
Tel Aviv University, Tel Aviv, Israel
Wellcome Trust Sanger Institute, Hinxton, U.K.