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Commentary

Molecular earplugs to protect the inner ear

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Inner ear gene therapy is a nascent therapeutic paradigm that may one day benefit some hearing loss patients. Robust preclinical data have demonstrated efficacy in several animal models of genetic hearing loss,[1] supporting the first clinical trials for inner ear gene replacement therapy, scheduled to commence in 2023. While genetic hearing loss has been at the forefront of recent inner ear gene therapy efforts, gene therapy for acquired hearing loss has taken a back seat. In the current issue of *Molecular Therapy Methods and Clinical Development*, Zhang, Fuchs, and colleagues[2] have pioneered a novel gene therapy approach that raises the prospects for protecting the inner ear from some forms of acquired hearing loss. Although further development will be required, the approach is a first of its kind and thus offers hope for protecting the inner ear against the most common form of hearing loss, caused by overexposure to loud sounds.

Noise-induced hearing loss can damage sensory hair cells, their mechanosensory hair bundles, their afferent synapses, which connect hair cells to eighth cranial nerve fibers, and can lead to death of hair cells and auditory neurons. Unfortunately, the mature mammalian cochlea lacks the capacity to generate new hair cells and neurons. Development of gene therapy strategies to regenerate auditory hair cells and neurons have thus far been met with limited success. However, protection of vulnerable inner ear structures and cells prior to cell damage or death may be a viable prophylactic strategy. Interestingly, the inner ear contains its own inherent mechanisms that offer some protection against overstimulation. The Zhang et al.[2] approach takes advantage of a novel gene therapy target and aims to ramp up the native protective mechanism in healthy ears, providing something analogous to molecular earplugs.

The activity of the cochlear epithelium and its sensory innervation are modulated by efferent feedback via the olivocochlear (OC) pathway. OC neurons contribute to two efferent subsystems: a lateral OC pathway innervating afferent dendrites associated with inner hair cells (IHCs) and a medial olivocochlear (MOC) pathway projecting to outer hair cells (OHCs).[3] When activated, MOC neurons reduce auditory sensitivity by decreasing OHC contributions to mechanical amplification within the cochlea.[4] This feedback inhibition is mediated by α9α10-containing nicotinic acetylcholine receptors (nAChRs) in the basolateral membranes of OHCs.[5] Influx of calcium through α9α10 receptors activates small conductance Ca^{2+}-dependent potassium channels (SK2), which leads to potassium efflux, thereby hyperpolarizing the OHC membrane potential.[6] Thus, activation of the MOC efferent pathway hyperpolarizes OHCs and reduces the OHC contribution to cochlear amplification.[7] This effect is implicated in frequency tuning, selective attention, improved hearing in noise, and notably, protection of the inner ear from acoustic trauma.[8]

Using a clever approach, Zhang et al.[2] targeted hair-cell-specific nAChRs in an effort to leverage the protective role of the MOC system against noise trauma. Gain-of-function knockin (Chrna9L9′T KI) mice carrying an α9 point mutation with higher calcium permeability exhibit enhanced inhibition to endogenous cholinergic feedback via MOC neurons.[9] Consequently, the increased magnitude of the MOC effect on OHCs results in greater tolerance to noise-induced trauma as measured by auditory brainstem responses (ABRs), a physiological measure of the summed electrical activity in the auditory nerve and brainstem.

Zhang et al.[2] reasoned that the genetically induced gain-of-function mutation in the hair-cell-specific nAChRs may ameliorate the damaging effects of acoustic overexposure. They generated adeno-associated viral (AAV) vectors encoding nAChR channels with higher calcium permeability (α9L9′T). The team injected the α9L9′T vectors into the inner ears of α9-null mice, previously shown to have qualitatively normal efferent innervation but no cochlear feedback inhibition.[10] Injected mice recovered ABR thresholds 2 weeks after noise exposure. Importantly, the group found that auditory sensitivity at the low frequency end of the spectrum was not affected 1 day after acoustic trauma, whereas control mice showed deterioration of auditory sensitivity following noise trauma. The data suggest that ears of α9L9′T-treated mice had better preservation of auditory function than those without.

Although auditory sensitivity recovered 2 weeks after noise exposure, α9L9′T-injected α9-null mice demonstrated changes in the ABR wave 1 amplitude both before and after noise trauma. The amplitude of wave 1 of the ABR represents the synchrony of firing in auditory nerve fibers and is highly correlated with the number of synapses between IHCs and auditory neurons. Although the baseline (pre-trauma) wave 1 amplitude was reduced in α9L9′T-injected α9-null mice relative to uninjected mice, the injected mice recovered wave 1 amplitudes 14 days after acoustic trauma, unlike controls. However, the difference in post-trauma wave 1 between injected and uninjected mice could not be explained by a difference in the number of afferent synapses between hair cells and nerve terminals.

Future studies will be needed to reveal the cellular and molecular mechanisms underlying the recovery of ABR wave 1 amplitudes in virally transduced mice. Perhaps changes

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in synaptic ribbon volume in IHCs may increase release probability evoking larger postsynaptic responses, evident as larger wave 1 amplitudes. Thus, the impact of noise could be further examined by quantifying afferent synaptic ribbon volumes in cochlear tissue excised from control and α9L9′T-injected mice. In addition, injection of α9L9′T vectors into the ears of wild-type mice will be important for demonstrating therapeutic efficacy in a model that more closely parallels the human condition.

While AAV-based gene therapies hold promise as a treatment for genetic and acquired hearing loss, targeting the optimal delivery window presents a looming challenge. For gene therapy to enhance efferent cochlear feedback and inner ear protection, anticipating when the system is likely to be stressed may help determine the therapeutic window for optimal outcomes. In addition to the timing of treatment, identifying the target patient population will be an important consideration. In a classic study from 1962, Rosen et al.[11] documented preservation of auditory sensitivity in human populations with limited noise exposure. Sudanese tribal people living in relatively quieter environments were compared with Americans of the same age living in industrialized regions. The results suggested noise exposure can exacerbate age-related hearing loss. This unique aging study was one of the first to examine the interaction between exposure to environmental noise and age-related hearing loss and now raises the question of whether the Zhang et al.[2] strategy might be advantageous for citizens of industrialized societies.

Alternatively, there may be vulnerable populations who might benefit more from enhanced efferent protection. The prevalence of noise-induced hearing loss among military service members is much greater than for the general public.[12] Conventional noise mitigation for military personnel or other noise-exposed occupations, such as construction workers, focuses on protective devices that dampen all auditory stimulation entering the inner ear. Engineering enhanced MOC inhibition could allow for normal hearing in low-noise environments while offering enhanced protection in noisy environments, modulated by the inner ear's native feedback pathways. While important unanswered questions remain, Zhang et al.[2] provide proof-of-concept evidence for genetic enhancement of efferent inhibition that may one day be used to protect the inner ear from noise trauma, motivating further exploration of this and other inner ear therapies.

DECLARATION OF INTERESTS

The authors declare no conflicts of interest.

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