LETTER

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Treatment of autosomal dominant hearing loss by *in vivo* delivery of genome editing agents

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Although genetic factors contribute to almost half of all cases of deafness, treatment options for genetic deafness are limited[1–5]. We developed a genome-editing approach to target a dominantly inherited form of genetic deafness. Here we show that cationic lipid-mediated *in vivo* delivery of Cas9–guide RNA complexes can ameliorate hearing loss in a mouse model of human genetic deafness. We designed and validated, both *in vitro* and in primary fibroblasts, genome editing agents that preferentially disrupt the dominant deafness-associated allele in the *Tmc1* (transmembrane channel-like gene family 1) Beethoven (*Bth*) mouse model, even though the mutant $Tmc1^{Bth}$ allele differs from the wild-type allele at only a single base pair. Injection of Cas9–guide RNA–lipid complexes targeting the $Tmc1^{Bth}$ allele into the cochlea of neonatal $Tmc1^{Bth/+}$ mice substantially reduced progressive hearing loss. We observed higher hair cell survival rates and lower auditory brainstem response thresholds in injected ears than in uninjected ears or ears injected with control complexes that targeted an unrelated gene. Enhanced acoustic startle responses were observed among injected compared to uninjected $Tmc1^{Bth/+}$ mice. These findings suggest that protein–RNA complex delivery of target gene-disrupting agents *in vivo* is a potential strategy for the treatment of some types of autosomal-dominant hearing loss.

Although about 100 deafness-associated alleles have been identified, few treatments are available to slow or reverse genetic deafness[4,5]. Complementation of wild-type alleles, or silencing of dominant-negative mutant alleles, have shown promising results in animal models[6,7]. Nonetheless, current approaches face potential challenges including immunogenicity, oncogenicity, and limitations of viral vectors[8,9].

Cas9-based genome editing agents can mediate targeted gene disruption or repair[10–13]. For applications that seek a one-time, permanent modification of genomic DNA, the delivery of non-replicable, transient Cas9–single guide RNA (sgRNA) ribonucleotide protein (RNP) complexes *in vivo* offers improved DNA specificity and potentially greater safety and applicability[14,15], compared with methods that introduce DNA expressing these agents. Approximately 20% of alleles associated with genetic deafness are dominantly inherited[1]. As Cas9–sgRNA complexes can efficiently disrupt genes through end-joining processes, we sought to design Cas9–sgRNA complexes that selectively disrupt dominant alleles associated with hearing loss.

Many genes linked to genetic hearing loss affect the function of sensory hair cells, which transduce acoustic vibrations into electrical nerve signals. TMC1 is an essential component of mechanotransduction channels in mammalian hair cells[16]. Mutations in *TMC1* have been linked to recessive and dominant genetic deafness in humans[17]. A dominant-negative missense mutation in *TMC1* (p.M418K, c.T1253A) causes reduced single-channel current levels and calcium permeability[16] in hair cells, and progressive post-lingual sensorineural hearing loss in humans[18–20]. The $Tmc1^{Bth/+}$ mouse model carries the orthologous missense mutation (p.M412K, c.T1235A) in the mouse *Tmc1* gene and exhibits progressive elevation of the auditory response threshold and progressive hair cell loss beginning at one month of age[21]. As the orthologous mutations in human and mouse both cause progressive, profound hearing loss, the $Tmc1^{Bth/+}$ mouse is a promising model for the development of treatment strategies[21].

We began by developing a genome editing strategy that preferentially disrupts the mouse mutant $Tmc1^{Bth}$ allele. To distinguish the mutant and wild-type alleles, we identified sgRNAs that target *Tmc1* at sites that include the T1235A mutation and a nearby NGG protospacer-adjacent motif (PAM) sequence required by *Streptococcus pyogenes* Cas9. We identified three candidate sgRNAs (Tmc1-mut1, Tmc1-mut2 and Tmc1-mut3) that place the *Bth* mutation at position 11, 12, and 15, respectively, of the spacer, counting the PAM as positions 21–23 (Fig. 1a). Mismatches between the sgRNA and genomic DNA that are close to the PAM are poorly tolerated by Cas9[10], increasing the likelihood that the *Bth* mutant allele will be selectively edited. A fourth sgRNA, Tmc1-mut4, is a truncated version of Tmc1-mut3 designed to increase genome editing DNA specificity[22]. We evaluated the ability of these four sgRNAs in complex with Cas9 to cleave either the wild-type *Tmc1* or the $Tmc1^{Bth}$ allele *in vitro*. All sgRNAs tested comparably or preferentially cleaved the $Tmc1^{Bth}$ allele, with Tmc1-mut3 exhibiting the greatest selectivity (Extended Data Fig. 1a, b).

We performed lipid-mediated delivery of Cas9–sgRNA RNP complexes into cultured primary fibroblasts derived from wild-type or homozygous $Tmc1^{Bth/Bth}$ mice to evaluate the allele specificity of genomic DNA modification in mouse cells. We delivered Cas9 complexed with each of the four sgRNAs using Lipofectamine 2000 into both wild-type and $Tmc1^{Bth/Bth}$ mutant fibroblasts. RNP delivery into these primary fibroblasts was twofold to fourfold less efficient than with HEK293T cells (Extended Data Fig. 1c). The highest rate of targeted insertions and deletions (indels) in mutant $Tmc1^{Bth/Bth}$ fibroblasts (10%) was observed with Cas9–Tmc1-mut3 RNPs, while lower indel frequencies (0.74–4.1%) were observed using the other sgRNAs (Fig. 1b).

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