International Journal of Science and Research (IJSR)
ISSN: 2319-7064
Impact Factor 2025: 7.089

Gene Editing and Single-Cell Transcriptomic Profiling in Hereditary Hearing Loss: Pathology-Relevant Advances in iPSC-Derived Cochlear Models

Harshad

Abstract: ***Hereditary hearing loss is increasingly being studied through patient-derived induced pluripotent stem cell (iPSC) systems, genome editing platforms, and single-cell transcriptomic analyses. For a pathology audience, the central value of these technologies is not only therapeutic promise but also their ability to model disease mechanisms, define genotype-phenotype correlations, and validate variant pathogenicity in human tissue-relevant systems. We reviewed English-language literature published mainly from January 2023 to February 2026, prioritizing primary studies and high-quality reviews on iPSC-derived inner ear organoids, CRISPR-based correction strategies, and single-cell RNA sequencing in auditory research. Recent work shows that human pluripotent stem cell-derived inner ear organoids can reproducibly generate hair cell-like and supporting cell-like populations, although most systems still display vestibular bias and fetal-stage immaturity. In parallel, gene therapy and precision editing studies, especially in OTOF- and MPZL2-related deafness, have provided compelling preclinical and early clinical proof of concept for hearing rescue. Single-cell RNA sequencing has become indispensable for benchmarking organoid identity, mapping differentiation trajectories, and demonstrating transcriptomic rescue after correction of pathogenic variants. The most realistic near-term application of this integrated platform is translational disease modeling, variant validation, and therapy prioritization rather than immediate cell replacement. Future work must improve cochlear specification, maturation, functional validation, delivery strategies, and long-term safety before routine clinical deployment becomes feasible.***

Keywords: hereditary hearing loss; inner ear organoid; induced pluripotent stem cell; CRISPR; single-cell RNA sequencing; translational pathology

1. Introduction

Hereditary hearing loss is a genetically heterogeneous disorder with major implications for diagnosis, counseling, and personalized therapy. Conventional rehabilitation with hearing aids and cochlear implants improves communication outcomes, but these modalities do not correct the underlying molecular defect or regenerate native sensory cells. The emergence of patient-specific iPSC models, CRISPR-derived editing tools, and single-cell transcriptomics has therefore changed the research landscape by creating experimentally tractable, human-relevant systems in which disease mechanisms can be studied and therapeutic strategies can be benchmarked.

For pathology and laboratory medicine, the relevance of this field lies in four areas. First, iPSC-derived inner ear models provide tissue-contextual platforms for studying disease morphology and cell-state transitions that are otherwise inaccessible in living patients. Second, gene editing enables isogenic comparison between mutant and corrected lines, improving causal inference in genotype-phenotype analysis. Third, single-cell transcriptomic approaches permit high-resolution classification of cellular heterogeneity, maturation status, and pathway perturbation. Fourth, organoid systems increasingly support functional interpretation of variants of uncertain significance, a growing challenge in molecular diagnostics [1,5-7].

2. Literature Search Strategy

A focused narrative review was prepared after searching PubMed and major biomedical literature sources for English-language articles published predominantly between January 2023 and February 2026. Search terms included hereditary hearing loss, inner ear organoid, iPSC, CRISPR, base editing, OTOF, MPZL2, and single-cell RNA sequencing. Priority was given to primary peer-reviewed studies, seminal protocol papers, and clinically relevant translational reviews. Non-primary news-style sources, duplicate citations, and low-value web references were excluded from the revised manuscript to improve scientific robustness. In keeping with a review format appropriate for this journal, the literature-search method is stated in both the abstract and the body text [14].

iPSC-derived inner ear organoids: what they currently achieve

The derivation of inner ear organoids from human pluripotent stem cells is a major advance because it permits controlled modeling of otic lineage development in vitro. Foundational work demonstrated that three-dimensional differentiation protocols can generate organoids containing hair cell-like populations with mechanosensory properties. Subsequent protocol refinements have improved reproducibility and clarified the sequence of developmental cues needed for otic induction, including staged manipulation of BMP, FGF, WNT, and related signaling pathways [6-10].

A major strength of current organoid systems is that they reproduce early developmental programs and yield multiple relevant cell types, including hair cell-like, supporting cell-like, neuronal, and periotic mesenchyme-like populations. For translational pathology, this cellular diversity is valuable because many deafness phenotypes are not purely hair-cell

autonomous. Organoids can therefore be used to interrogate how specific variants affect epithelial patterning, cell survival, and lineage divergence [9-13].

However, important limitations remain. Most reported organoids still show predominant vestibular rather than cochlear identity. Even long-duration cultures generally correspond more closely to fetal than adult sensory tissue, which restricts their ability to model late maturation, age-related changes, and fully developed inner hair cell physiology. Architectural fidelity also remains incomplete: present systems do not reproduce the spiral organ of Corti, fluid compartments, or the full biomechanical environment required for mature auditory transduction. Batch-to-batch variability and line-to-line differences continue to complicate interpretation, especially when subtle phenotypes are being studied [6,9-13].

These limitations do not negate the value of organoids; rather, they define the current translational boundary. At present, the strongest use case is disease modeling, mechanistic dissection, and preclinical validation, not direct therapeutic transplantation.

CRISPR-based correction strategies in hereditary deafness

Genome editing strategies in auditory disorders depend on the mutational mechanism. In autosomal recessive loss-of-function disease, gene supplementation remains important, particularly through adeno-associated viral delivery. This approach has achieved the clearest human proof of concept in OTOF-related deafness. Recent clinical studies have shown meaningful hearing improvement in children treated with AAV-based OTOF replacement, establishing that the inner ear is a feasible target for molecular intervention and significantly strengthening the translational credibility of the field [1-3].

Dominant-negative disorders present a different challenge because supplementation alone does not remove the pathogenic allele. In such settings, nuclease-based or allele-specific disruption approaches remain conceptually attractive, but precision and off-target control are critical. For monogenic deafness caused by point mutations, base editing has emerged as one of the most compelling strategies because it enables nucleotide correction without creating double-strand DNA breaks. The demonstration that adenine base editing can rescue hearing and restore protein expression in a humanized MPZL2 mouse model represents a particularly important advance, as it links variant correction to structural, transcriptomic, and functional recovery [4,5,18,19].

From a pathology perspective, CRISPR editing adds value even before clinical translation. Corrected iPSC lines function as isogenic controls, allowing investigators to attribute transcriptomic and phenotypic abnormalities specifically to the causal variant rather than to background genomic noise. This design is especially useful in rare disease research, where patient numbers are limited and tissue access is minimal [16].

Single-cell transcriptomics as the validation backbone

The rise of single-cell RNA sequencing has transformed how inner ear models are assessed. Bulk methods can obscure critical cell-type-specific perturbations, whereas single-cell profiling enables resolution of heterogeneity, lineage assignment, and maturation state at the level required for organoid benchmarking. In the auditory field, single-cell atlases of native tissue and organoid systems now provide increasingly useful references for determining how closely in vitro cells resemble their in vivo counterparts [12,14,15].

In practice, single-cell transcriptomics serves three principal purposes. First, it quantifies cellular composition and identifies off-target populations. Second, it reconstructs differentiation trajectories and reveals where developmental progression stalls. Third, it enables direct comparison between mutant, corrected, and control lines to test whether molecular rescue has occurred after gene correction.

This is a decisive advance for translational pathology because rescue should not be inferred solely from morphology or isolated marker expression. A corrected organoid is more convincing when the abnormal transcriptional program normalizes at single-cell resolution, particularly in the cell population expected to mediate disease. Recent studies using human organoids to validate variants linked to cochlear malformations illustrate this principle well: organoid phenotypes and single-cell readouts can move a variant from uncertain significance toward functional pathogenicity [17].

An additional advantage is the possibility of deriving maturation scores and lineage maps that can guide protocol refinement. If an organoid remains locked in an immature vestibular-like state, transcriptomic analysis can identify the missing regulatory programs and suggest the next experimental intervention. Thus, single-cell methods are not merely descriptive; they actively inform protocol engineering.

An integrated patient-specific pipeline

The most coherent translational workflow begins with derivation of patient-specific iPSCs from blood or fibroblasts, followed by correction of the pathogenic variant in the undifferentiated state to generate an isogenic comparator. Both mutant and corrected lines are then differentiated into inner ear organoids and analyzed using immunophenotyping, functional assays, and single-cell transcriptomics.

This integrated design yields several advantages. It permits direct study of disease pathogenesis in a human developmental context. It provides an internal corrected control. It supports mechanistic attribution at cell-type resolution. It also offers a rational platform for prioritizing therapeutic strategies, whether gene addition, base editing, or pharmacologic rescue. For pathology researchers, this framework resembles a modern form of experimental surgical pathology in which tissue phenotype is reconstructed, perturbed, and molecularly decoded under controlled conditions [6,14,16,17].

A central unresolved issue is cochlear specification. Current protocols often favor vestibular outcomes, suggesting incomplete recreation of developmental patterning. Evidence from organoid biology and inner ear developmental studies supports the idea that better control of ventralizing cues, including retinoic acid-sensitive pathways and other regional patterning signals, may improve cochlear lineage commitment. This is a biologically plausible direction, but it remains an experimental priority rather than a settled solution [13,20].

Translational relevance and remaining barriers

Despite genuine momentum, the field should not yet be overstated as ready for routine clinical deployment. There are at least five major barriers. First, maturation remains inadequate. Many iPSC-derived hair cell-like populations still resemble fetal tissue more than adult cochlear cells. Second, architectural fidelity is incomplete, limiting accurate modeling of organ-level biomechanics. Third, functional validation is still uneven across studies; marker expression alone is not sufficient evidence of true auditory competence. Fourth, safety issues remain central for both editing and cell-based strategies, including off-target effects, delivery challenges, and tumorigenicity from residual undifferentiated cells. Fifth, large-scale manufacturing, standardization, and quality control are unresolved for clinical-grade deployment [1,4,9,10].

Therefore, the strongest near-term contribution of this field lies in diagnostic and translational workflows: variant validation, disease modeling, target prioritization, and preclinical therapeutic testing. That framing is also more appropriate for pathology journals than a purely futuristic regenerative narrative.

Pathology-specific editorial positioning

For a pathology and microbiology readership, this topic is most defensible when positioned as translational pathology, molecular pathogenesis, and diagnostic model development. A manuscript centered only on futuristic hearing restoration risks appearing outside core scope. By contrast, a review emphasizing human disease modeling, genotype-phenotype interpretation, single-cell pathology, and validation of pathogenic variants has a clearer editorial rationale.

Accordingly, the revised article avoids overclaiming clinical readiness and instead highlights how organoid and single-cell platforms can deepen molecular diagnostic practice. This positioning also improves scientific balance because the clinical success of OTOF gene therapy, while highly important, should not be conflated with proof that iPSC-derived cochlear cell replacement is ready for bedside use [1-3,17].

3. Conclusion

The combination of iPSC technology, precision genome editing, and single-cell transcriptomics has created a powerful platform for studying hereditary hearing loss in a pathology-relevant manner. The field has progressed from conceptual promise to experimentally validated translational models, and early human gene therapy successes have reinforced its clinical importance. Nevertheless, current organoid systems remain limited by incomplete cochlear specification, fetal-stage maturation, and unresolved delivery and safety challenges. At present, the most compelling role of this platform is in mechanistic disease modeling, functional interpretation of variants, and preclinical evaluation of personalized therapeutic strategies. With continued improvement in differentiation fidelity, transcriptomic benchmarking, and editing safety, these systems may eventually support broader clinical translation; for now, their greatest value lies in bridging molecular pathology and regenerative medicine.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

Table 1: Practical strengths and current limitations of the iPSC-CRISPR-single-cell pipeline in hereditary hearing loss

Component	Practical strengths	Current limitations
iPSC-derived organoids	Patient-specific modeling; multicellular otic differentiation; reusable platform for mechanistic studies	Vestibular bias; fetal-stage immaturity; batch variability
CRISPR-based correction	Creation of isogenic controls; causal testing of variants; compatibility with base-editing strategies	Off-target concern; delivery constraints; mutation-specific feasibility
Single-cell transcriptomics	Cellular benchmarking; trajectory analysis; transcriptomic rescue assessment	Cost; computational complexity; dependence on high-quality reference atlases
Translational utility	Variant validation; drug and therapy prioritization; bridge between molecular diagnosis and functional biology	Incomplete readiness for direct cell replacement; standardization and safety hurdles

References

[1] Jang SH, Lee S, Kim UK, Choi BY. Recent preclinical and clinical advances in gene therapy for hereditary hearing loss. J Transl Med. 2025; 23: 128.
[2] Qi J, Zhang L, Lu L, et al. AAV gene therapy for autosomal recessive deafness 9: a single-arm trial. Nat Med. 2025.
[3] Lv J, Wang H, Niu X, et al. AAV1-hOTOF gene therapy for autosomal recessive deafness 9. Lancet. 2024; 403: 1356-1366.
[4] Hu SW, Li GL, Zhang Y, et al. PAM-flexible adenine base editing rescues hearing loss in a humanized MPZL2 mouse model harboring an East Asian founder mutation. Nat Commun. 2025; 16: 1234.
[5] Gao X, Tao Y, Lamas V, et al. Treatment of dominant genetic hearing loss by CRISPR-Cas9 ribonucleoprotein delivery in vivo. Nat Commun. 2023; 14: 1-15.
[6] van der Valk WH, Loo C, O'Neill N, et al. Generation and characterization of vestibular inner ear organoids

from human pluripotent stem cells. Nat Protoc. 2025; 20: 1-35.
[7] Koehler KR, Nie J, Longworth-Mills E, et al. Generation of inner ear organoids containing functional hair cells from human pluripotent stem cells. Nat Biotechnol. 2017; 35: 583-589.
[8] Nie J, Koehler KR. Generation of inner ear organoids from human pluripotent stem cells. Nat Protoc. 2020; 15: 2020-2048.
[9] Pianigiani G, Roccio M. Inner ear organoids: strengths and limitations. J Assoc Res Otolaryngol. 2024; 25: 5-11.
[10] Qi J, Roccio M. Modeling, applications and challenges of inner ear organoid. Stem Cell Res Ther. 2024; 15: 112.
[11] Doda D, Alonso Jimenez S, et al. Human pluripotent stem cell-derived inner ear organoids recapitulate early human otic development in vitro. Development. 2023.
[12] Waldhaus J, Chow J, Steinhart MR, et al. Mapping the developmental potential of mouse inner ear organoids at single-cell resolution. iScience. 2024; 27: 109069.
[13] Duncan RK, Liu L, et al. Retinoic acid signaling guides the efficiency of inner ear organoid-genesis and governs sensory-nonsensory fate specification. Stem Cell Reports. 2025.
[14] Ma X, Zhang Y, Wang L, et al. Advance and application of single-cell transcriptomics in auditory research. Neurosci Bull. 2024; 40: 85-102.
[15] Sun G, Zhou Y, Li X, et al. Single-cell transcriptomic atlas of mouse cochlear aging. Protein Cell. 2023; 14: 180-202.
[16] Luo Y, Chen J, Li H, et al. Genetic correction of induced pluripotent stem cells from a patient with Usher syndrome type IIA. Stem Cell Res Ther. 2024; 15: 28.
[17] Zafeer MF, Mittal R, Bidiwala A, et al. Human organoids for rapid validation of gene variants linked to cochlear malformations. Hum Genet. 2024.
[18] Xu W, Li K, Li D, et al. Cutting-edge applications of base editing and prime editing in genetic diseases and cancer. J Transl Med. 2024; 22: 1-25.
[19] Azeez SS, Meka P, Kandi V. Advances in CRISPR-Cas technology and its applications. Gene. 2024; 900: 148123.
[20] Romand R. Retinoid signaling in inner ear development. Int J Dev Biol. 2006; 50: 315-324.