Transcriptome profiling and novel isoform of the cochlear *Slc26a4*-expressing cells

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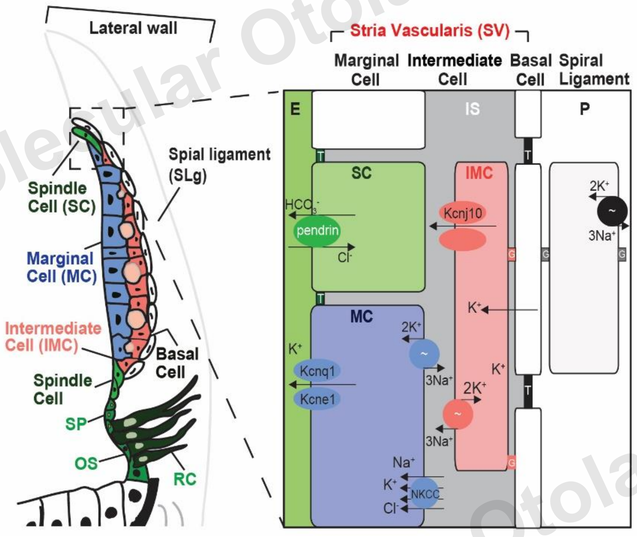
Introduction

- About 90% of Pendred syndrome (PDS) patients and 50-90% of Non-syndromic enlarged vestibular aqueduct (NSEVA) patients are attributed to the gene of *SLC26A4*. *SLC26A4* encodes protein called pendrin.
- In the cochlea, pendrin is expressed in the apical membrane of spindle cells, outer sulcus (OS), and root (R) cells of the spiral ligament (SLg) central regions, and spiral prominence (SP) in the lateral wall.
- The Stria Vascularis (SV) is known to be composed of three cell types based on anatomical features: (i) the marginal cells; (ii) intermediate cells; and (iii) the basal cells.



Methods

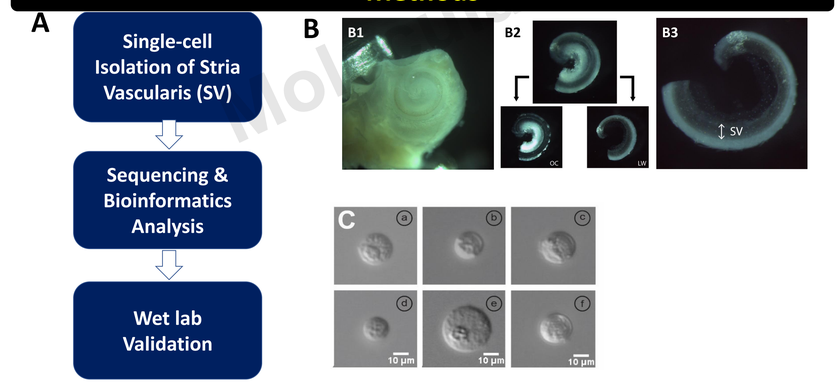


Figure 1. Overall procedures. **(A)** Schematic diagram of single-cell RNA-seq procedures. **(B)** Stria Vascularis (SV) dissection. **(B1)** The temporal bone is removed and opened. **(B2-B3)** The cochlea is dissected and the lateral wall (LW) isolated. **(C)** Representative isolated single cells from SV. **(D)** Illumina Short-read Single-cell RNA-seq. **(E)** Oxford Nanopore Long-read Single-cell RNA-seq. **(F)** Bioinformatics analysis.

Results

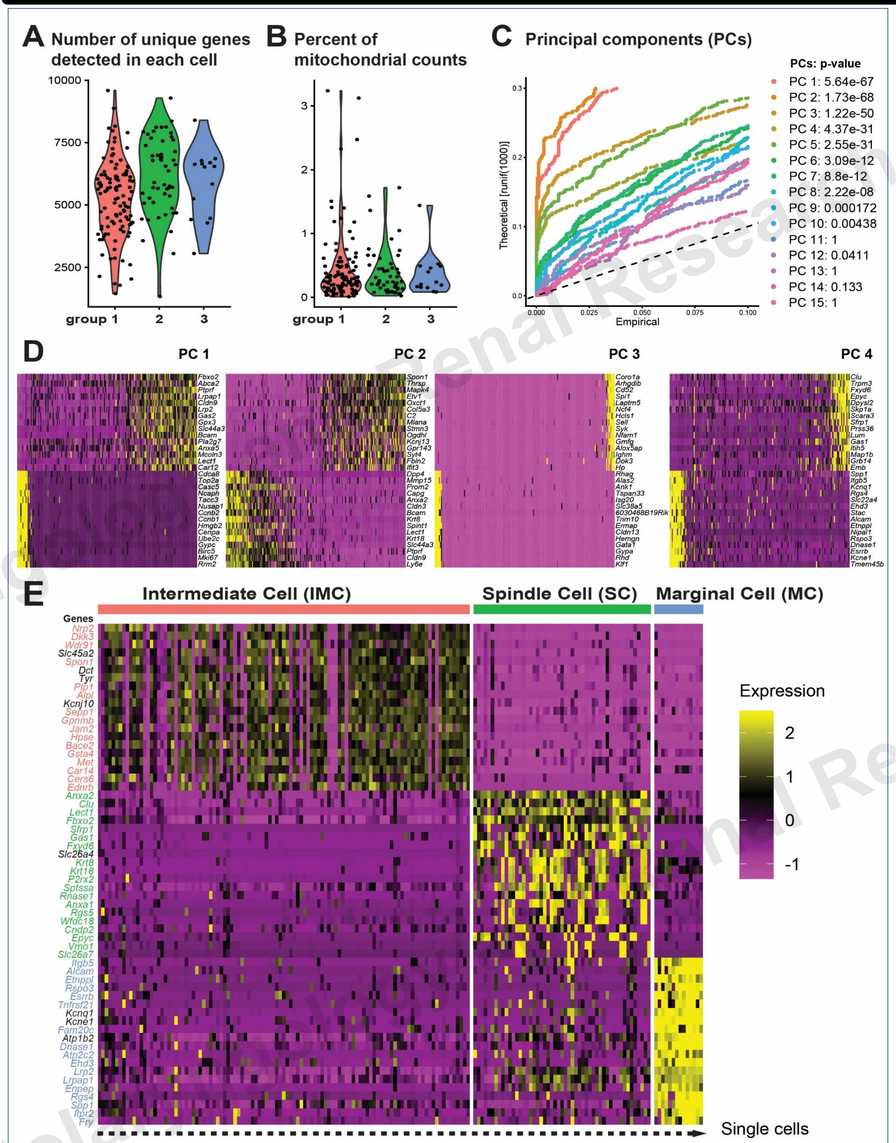


Figure 2. Principal components (PCs) and Cluster-defining Genes of the cochlear stria vascularis (SV). (A) The number of unique genes detected in each cell from the three different groups. Each black dot represents individual cells (n= 172). **(B)** The percent of mitochondrial counts from three different groups. Each black dot is individual cells. **(C)** There is a sharp drop-off in p-values after the first 8 PCs. dashed line is a uniform distribution. **(D)** Heatmap shows the primary sources of heterogeneity in the dataset. PC 1-4 was used for further downstream analyses (The x-axis is 172 cells and the y-axis are genes are ordered according to their PCA scores). **(E)** Heatmap showing cluster-defining genes of intermediate cells (IMCs), spindle cells (SC), and marginal cells (MCs) based on the area under the ROC curve (AUC) classifier.

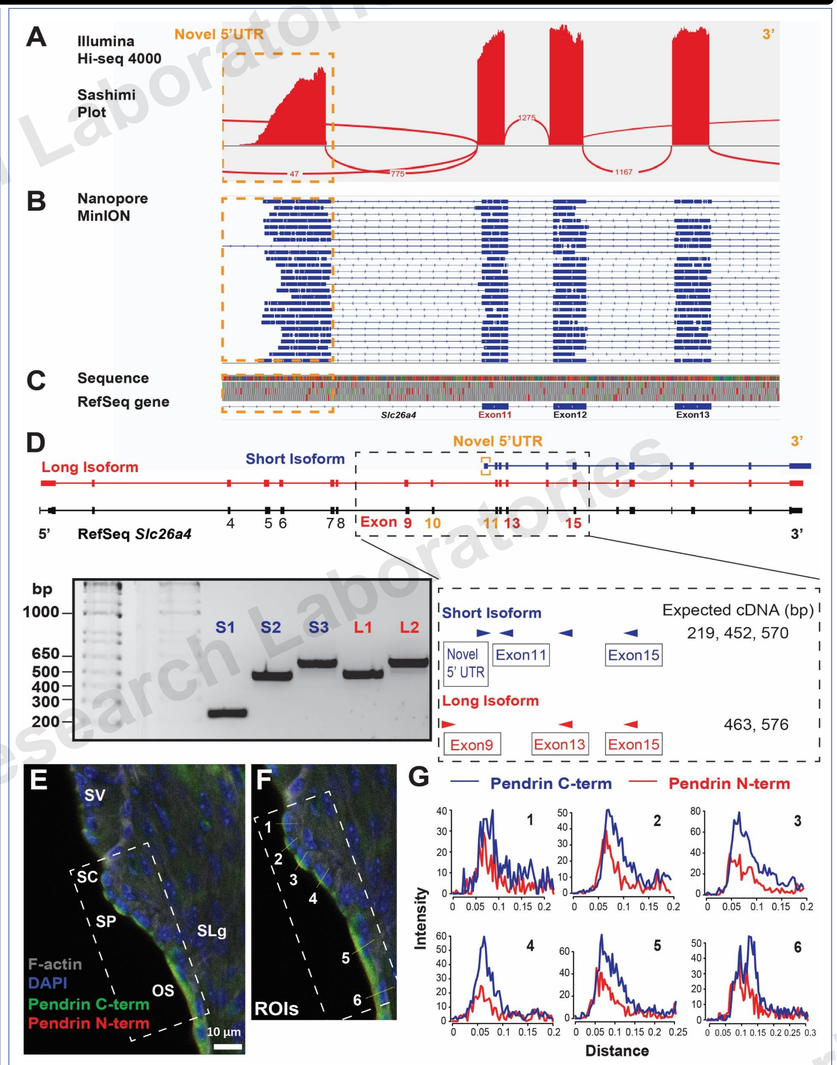


Figure 3. Novel transcription start site (TSS) and isoform diversity of *Slc26a4* and validation of the novel short isoform. (A) A novel 5'UTR preceding exon 11 is shown and is highlighted in the yellow rectangles. **(B)** Long-read scRNA-seq confirms the presence of the novel exon. **(C)** A 5'-UTR sequence without the presence of a methionine start codon is shown in the dashed rectangle region. This exon is unannotated in RefSeq. **(D)** Validation of mRNA expression using the cochlear SV (30 ng) by RT-PCR. The electrophoresis gel image shows expression of the short isoform using a forward primer based on the novel UTR and three different reverse primers. Expression of the long isoform uses a forward primer in exon 9 and two different reverse primers. The novel 5'UTR for the short isoform is found between exons 10 and 11. **(E-G)** Immunostaining to confirm protein expression level at the cellular level. **(E)** Immunostaining of the cochlea using anti-pendrin C-terminal (green) and N-terminal (red) antibodies. F-actin stained with phalloidin (dark grey) and nucleus with DAPI (blue). SV: stria vascularis, SC: spindle cell, SP: spiral prominence, OS: outer sulcus, SLg: spiral ligament. **(F)** Six regions of interest (ROI) for line analysis. **(G)** Line analysis of six different cells. Green represents C-terminal pendrin expression; red represents N-terminal pendrin expression. The y-axis corresponds to fluorescence intensity; the x-axis corresponds to the distance of the ROI.

Conclusions

- **Single-cell RNA-sequencing characterizes intermediate, spindle, and marginal cell of the cochlear stria vascularis by transcriptome profiling.**
- **Nanopore long-read RNA-sequencing identified a novel isoform in the *Slc26a4*-expressing cells.**
- **mRNA expression of the unrecognized transcription start site (TSS) of *Slc26a4* was validated using RT-PCR.**
- **Protein expression of the novel short isoform was confirmed using immunostaining experiments.**

Acknowledgements

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