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CLINICIAN SCIENTIST AWARD 2021-2024

"Cellular Reprogramming of Peripheral Glial Cells to Generate Auditory Neurons"

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AMOUNT AWARDED BY AOS: $205,000

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PUBLICATIONS:

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Claussen AD, Shibata SB, Kaufmann CR, Henslee A, Hansen MR. Comparative Analysis of Robotics-Assisted and Manual Insertions of Cochlear Implant Electrode Arrays. Otology and Neurotology Dec1 2022 PMID:36201552

Iwasa Y, Klimara MJ, Yoshimura H, Walls, WD, Omichi R, West CA, Shibata SB, Ranum PT, and Smith RJH. Mutation-agnostic RNA interference and engineered replacement rescues Tmc1-related hearing loss. Life Sci Alliance 2022 Dec 27 PMID:36574989

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RESEARCH SUMMARY: **The spiral ganglion nerve (SGN) plays a crucial role in hearing by transmitting acoustic signals from the cochlea to the brain. Because the auditory nerve lacks intrinsic regenerative capacity, damage to the nerve leads to permanent deafness. Cochlear implants are ineffective when the SGN is damaged or lost, as observed in auditory neuropathy (AN) cases. Therefore, there is a critical need to develop novel treatments for AN, and regenerative medicine holds enormous potential to restore SGN and treat this condition. Direct neuronal reprogramming converts somatic cells to induced neurons by overexpressing neuronal transcription factors (NTFs), bypassing the pluripotent state. The spiral ganglion glial cells are considered a promising source for cellular reprogramming in the cochlea because of their plasticity, proliferative capacity, survival post-nerve damage, and proximity to the nerve. Recent cellular reprogramming strategies incorporate neuroregulatory microRNAs (miRNAs) to enhance the conversion of fibroblasts into induced neurons in combination with NTFs. The role of these miRNAs in regulating SGN fate is unknown. Our goal is to convert spiral ganglion glial cells into SGNs via gene therapy and to advance cellular reprogramming in the inner ear (Figure 1). The aims of this project are (1) the characterization of age-related glial cell responses to acute SGN apoptosis by creating a neonatal mouse model of AN, (2) optimizing exogenous transgene delivery into cochlear glial cells in neonatal mice, and (3) exploring direct reprogramming of normal or damaged glial cells *in vitro. The expected results of this study will be (1) an improved understanding of age-related changes in reactive gliosis in mice in response to SGN death, (2) improved targeting of cochlear glial cells with a combination of glial cell-specific AAV serotypes and promoters, and (3) providing experimental evidence of the role of NTFs and miRNA in inducing SGNs in normal and post-damaged glial cells.***

OUTCOMES: 1) We have established our AN model in neonatal mice described in aim 2 (Figure 2). We will now focus on the investigation of reactive gliosis in different mouse ages and variations in the transcription of glia, neuron, and apoptosis-associated genes at varying time points before and after the onset of hearing loss using bulk single-cell sequencing of glia following SGN loss. 2) For Aim 2 we have identified several AAV vectors that are candidates for transfecting glial cells. We are targeting glial cells by using glial cell specific promoters. 3) In Aim 3 we are we are exploring ; miR-9/9 and miR-124, that are highly abundant in neuronal tissue and are essential for neural differentiation. Combination treatment of miRNAs with neuronal transcription factors has shown enhanced reprogramming efficiency in the CNS in Dr. Andrew Woo's lab (WashU) who is a collaborator in my project.

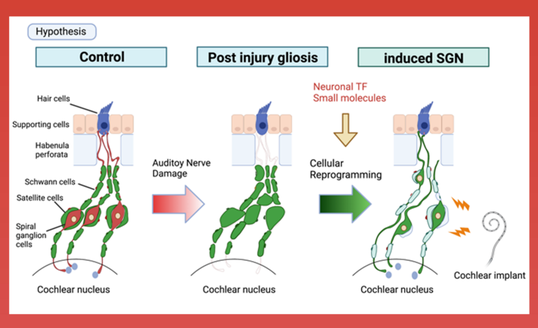
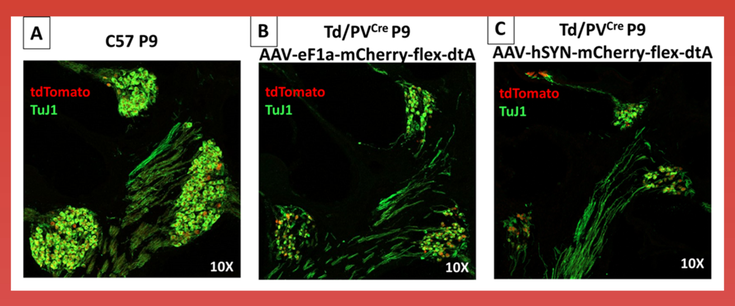
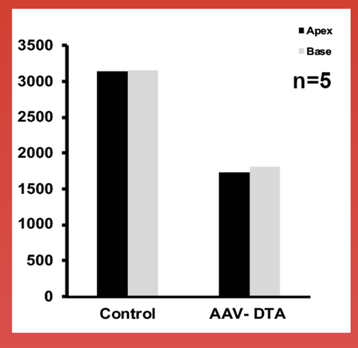


Figure 1. Hypothesis: Direct cell reprogramming of the cochlear glial cells with neuronal transcription factors can generate induced SGN to restore hearing





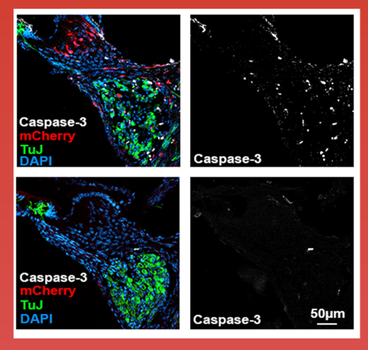


Figure 2. AAV-DTA allows rapid degeneration of SGN cells in Cre-dependent fashion. Control (A) vs AAV-DTA injected mice (B and C), there is notable loss of SGN cells by 1 week using either ubiquitous EF1a promoter or neuronal hSYN promoter to express FLEX-DTA. Quantification of the SGN density shows significant loss of SGN less at one week, this is further decreased to near notal ablation by one month (D). We demonstrate that AAV-DTA injected mice are undergoing apoptosis only in the Rosenthal's canal (E).

FURTHER FUNDING HAS ENABLED US TO EXPAND OUR RESEARCH TO: - Investigate means of direct reprograming of glial cell to neuron. - Develop inner ear glial cell gene therapy.

LAY SUMMARY OF FINDINGS AND IMPLICATIONS OF THIS RESEARCH: Damage to the spiral ganglion nerve leads to permanent deafness, and a cochlear implant is the most effective treatment for severe-profound deafness. However, cochlear implants are ineffective with neuronal degeneration, and regenerative medicine, such as direct neuronal reprogramming to replenish the damaged spiral ganglion neurons, holds enormous potential to cure deafness and improve cochlear implant outcomes. We are exploring approaches to advance cellular reprogramming of cochlear glial cells into induced spiral ganglion neurons using a new mouse model with selective ablation of spiral ganglion neurons, optimizing gene delivery into cochlear glial cells, and enhancing reprogramming efficiency by combining micro RNAs with neuronal transcription factors.