**ABSTRACT**

Age-related hearing loss (ARHL), one of the most common conditions affecting the elderly, is predominately associated with oxidative stress and sensory cell degeneration. However, cells and tissues are endowed with an antioxidant defense system. Sestrin-2 is a stress inducible protein that has been shown to suppress the production of reactive oxygen species (ROS) in the heart and protect tissues from oxidative stress. Several recent studies demonstrated that Sestrin-2 plays a vital role in preventing cardiovascular diseases by reducing oxidative stress. However, there is a lack of information concerning Sestrin-2 in age-related degeneration in the auditory system. This study investigates the expression of Sestrin-2 in the cochlea and its role in ARHL.

Strong expression of Sestrin-2 has been identified in the cochlea of mice, particularly on the outer hair cells (OHCs). Sestrin-2 knockout mice exhibit early onset of hearing loss compared to the C57BL/6J wild type mice and show significant OHC loss at the basal turn of the cochlea as early as 6 weeks old, much earlier than WT mice. The expression pattern of Sestrin-2 in the peripheral auditory system suggests that Sestrin-2 may play an important role in auditory function and therefore serve as a protective molecule against ARHL.

**SPECIFIC AIMS**

**AIM 1:** To investigate the expression and location of Sestrin-2 protein in the auditory system

**AIM 2:** To determine the functional and pathological changes caused by Sestrin-2 protein deficiency and the role of Sestrin-2 protein in hearing protection

**SUBJECTS:** C57BL/6J WT mice (The Jackson Laboratory) and Sestrin-2 KO mice (C57BL/6J background)

**HEARING EVALUATION:** Auditory brainstem responses were performed under Ketamine/ Xylozone anesthesia at 4, 8, 16, 32 and 48 kHz to assess auditory function

**HAIR CELL COUNT:** To quantify OHCs on the basilar membrane

**IMMUNOCYTOCHEMISTRY:** To determine the location of Sestrin-2 protein in the organ of Corti

**WESTERN BLOTTING:** To quantify Sestrin-2 protein in the cochlea

**METHODS**

**RESULTS**

**AUDITORY BRAINSTEM RESPONSE**

| A: WT mice display average threshold shifts of 20±14 and 18±16 in 3 months, and 18±24 and 15±10 in 5 months at the high frequencies (32 and 48 kHz), suggesting a high frequency dominated hearing loss. B: The KO mice also display threshold shifts, but the threshold increase involves all the tested frequencies (4, 8, 16, 32 and 48 kHz). |

**WESTERN BLOTTING**

<table>
<thead>
<tr>
<th>WT</th>
<th>KO</th>
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<tbody>
<tr>
<td>63 kDa</td>
<td>Sestrin-2</td>
</tr>
<tr>
<td>36 kDa</td>
<td>GAPDH</td>
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</tbody>
</table>

**IMMUNOCYTOCHEMISTRY (Sestrin-2 Staining)**

**IMMUNOCYTOCHEMISTRY (CD45 Staining)**

**FUTURE WORKS**

- Sestrin-2 deficiency exacerbates age-related hearing loss by increasing hearing thresholds and accelerated sensory cell degeneration in KO mice.
- Sestrin-2 expression is detected on cochlear macrophages.
- Age-related decrease of Sestrin-2 expression may contribute to presbycusis.

**CONCLUSIONS**

- Sestrin-2 is identified on cochlear macrophages, however the role of Sestrin-2 in cochlear immune cells is currently unknown.
- Pilot data shows an increase of macrophages with aging and KO mice have more macrophages than WT mice of the same age.
- It is hypothesized that Sestrin-2 may act as an activator of immune cells during stress.
- Further studies will reveal the influence of Sestrin-2 on the role of macrophage in ARHL.

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