

Sestrin-2 Protein Deficiency Exacerbates Noise Induced Hearing Loss

Student Researcher: Celia Zhang¹ | Faculty Mentors: Dr. Wei Sun¹, Dr. Ji Li², and Dr. Bo Hua Hu¹

¹ Center for Hearing and Deafness, Department of Communicative Disorders and Sciences, College of Arts and Sciences

² Department of Pharmacology and Toxicology, School of Medicine and Biomedical Sciences

ABSTRACT

Noise exposure is the most common cause of hearing loss. It affects approximately 26 million people in the United States, especially those in the manufactory and military industry^[4]. Within the set of cochlear pathologies that occur as a result of noise exposure, the presence of oxidative stress in the cochlear sensory cells and an increased level of reactive oxygen species play a significant role in noise induced hair cell death^[2].

Cells and tissues are endowed with antioxidant defenses to fight against these harmful external stimuli. Sestrins are a family of highly conserved stress responsive proteins that can protect cells from oxidative stress^[1, 3]. Sestrin-2, important for metabolic homeostasis, is a stress inducible protein that has been found in heart tissue and plays a vital role in preventing cardiovascular diseases by reducing oxidative stress^[5]. We hypothesize that Sestrin-2 protein deficiency will cause an increased level of oxidative stress in the cochlea post noise exposure, which will exacerbate hearing loss. Initial Western blot results identified the Sestrin-2 protein in the cochlea, but its role has yet to be studied. This project will explore the novel protein in the auditory system, which may play an important role in hearing protection by suppressing oxidative stress.

SPECIFIC AIMS

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

Aim 2: To determine the degree of hearing loss caused by Sestrin-2 protein deficiency and role of Sestrin-2 protein in hearing protection

METHODS

SUBJECTS: C57BL/6J wild type (WT) mice and Sestrin-2 knock out (KO) mice from The Jackson Laboratory

NOISE EXPOSURE: Bilateral white noise exposure of 110 dB for 3 hours

HEARING EVALUATION: Auditory brainstem responses were performed under Ketamine/Xylazine anesthesia at 4, 8, 16, 32 and 48 kHz pre and 1 week post noise exposure

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

IMMUNOCYTOCHEMISTRY: To determine the location of Sestrin-2 protein expression on the basilar membrane

HAIR CELL COUNT: To quantify hair cells on the basilar membrane pre and post noise exposure

RESULTS

AUDITORY BRAINSTEM RESPONSE

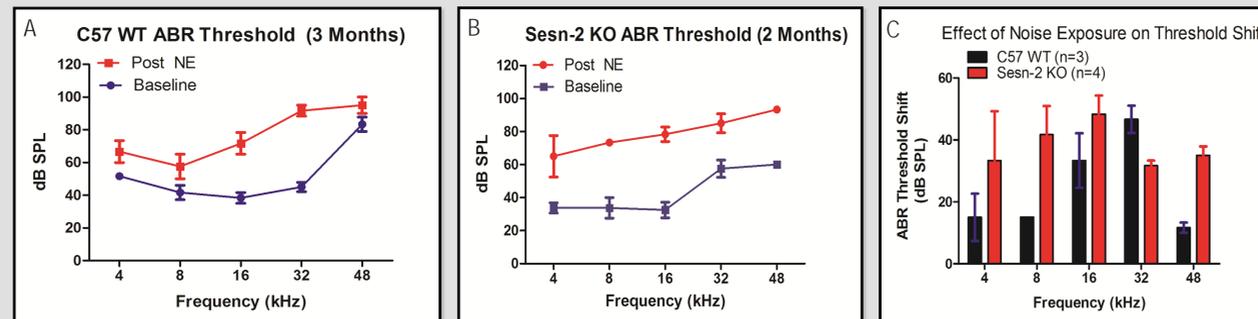


Figure 1: The average ABR threshold in WT and KO mice before and after noise exposure. (A) An increase in hearing threshold after 3 hours of 110 dB white noise exposure in C57 WT mice. (B) An increase in hearing threshold after 3 hours of 110 dB White noise exposure in Sestrin-2 KO mice. (C) The difference in ABR threshold is significantly greater in the Sestrin-2 KO mice in 4 kHz, 8 kHz, 16 kHz and 48 kHz.

IMMUNOCYTOCHEMISTRY

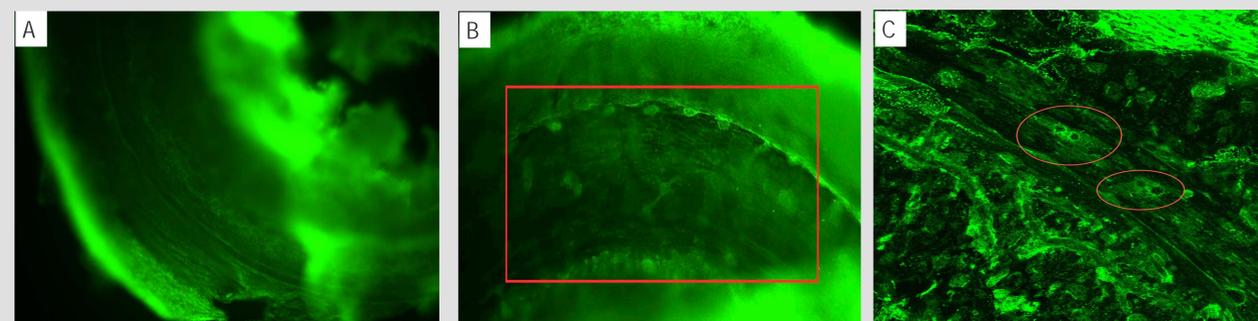
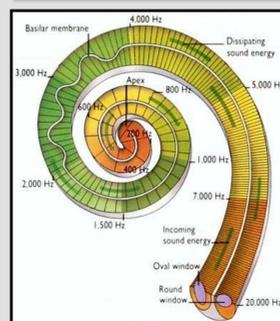


Figure 2: Sestrin-2 protein expression in cochlea of C57 WT mouse (3 months). Positive staining was detected on the macrophages on the basilar membrane of cochlea. The sample was incubated with Sestrin-2 polyclonal antibody over night, then stained with Goat Anti- Rabbit IgG H&L. (A) Apical turn of cochlea: no positive staining was detected. (B) Basal turn of cochlea: positive staining was detected on the macrophages on the basilar membrane. (C) Basal turn of cochlea taken with a confocal microscope: dilation of the structures on a cellular level. Sestrin-2 expression on the macrophages is clearly shown.

COCHLEAR ANATOMY



WESTERN BLOTTING



Figure 3: Expression of Sestrin-2 protein via Western blotting. (A) Sestrin-2 protein is clearly expressed in the cochlea of C57 WT mice. Sestrin-2 has a molecular weight of 63 kD. GAPDH was used as a loading control to normalize the levels of protein. Western blot results show a greater concentration of Sestrin-2 protein in the cochlea compared to the heart. (B) No bands were shown for the KO mice.

HAIR CELL COUNT

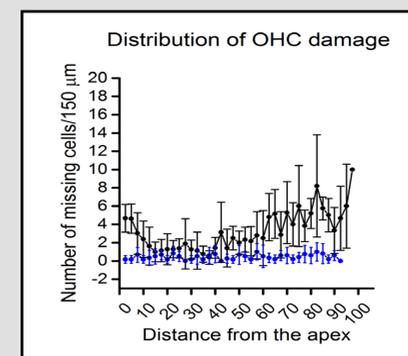


Figure 4: Distribution of outer hair cell damage in C57 WT mice pre (blue) and post (black) noise exposure. C57 WT mice between 6-8 months were noise exposed with narrow band noise of 120 dB for 1 hour. Animals were sacrificed and the cochlea was dissected for hair cell count 2 weeks after noise exposure. (Unpublished data from Dr. Bo Hua Hu's lab)

CONCLUSION AND FUTURE WORKS

- The expression of Sestrin-2 protein has been found in the cochlea of the C57 WT mice via Western blotting. Using immunocytochemistry, we detected Sestrin-2 protein expression on the macrophages on the basilar membrane of the basal turn but not in the apical turn. We hypothesize that the increase in Sestrin-2 protein on the basal turn may be associated with aging related hearing loss.
- Both WT and KO mice showed an increase in hearing threshold after noise exposure in auditory brainstem response, however the threshold shift was larger in the KO mice, implying that Sestrin-2 protein may play a role in hearing protection. Outer hair cell count has been performed and results showed significant hair cell death post noise exposure.
- The role of Sestrin-2 protein in hearing protection will be further investigated. It is expected that post noise exposure, Sestrin-2 KO mice will have drastic increase in their hearing threshold compared to the C57 WT mice due to the lack of protection effect of the Sestrin-2 protein. Western blotting will be used to quantify protein expression pre and post noise exposure and immunocytochemistry will be used to localized Sestrin-2 protein in the cochlea.

REFERENCES

- Budanov, A. V., Lee, J. H., and Karin, M. (2010). Stressin' sestrins take an aging fight. *EMBO Molecular Medicine*, 2, 388-400.
- Henderson, D., Bielefeld, E. C., Harris, K.C., and Hu, B. H. (2006). The role of oxidative stress in noise-induced hearing loss. *Ear & Hearing* (27) 1, 1-19.
- Lao, Y., Feng, L., Yong, Y., Dong, J., Zhang, L., Huang, X., Li, F., and Li, J. (2014). Upregulation of sestrin-2 expression protects against endothelial toxicity of angiotensin II. *Cell Biology and Toxicology*, 30, 147-156.
- National Institute of Deafness and Other Communication Disorders (NIDCD). (2014). *Noise-Induced Hearing Loss*. (NIH Publication No. 14-4233). Retrieved from <http://www.nidcd.nih.gov/health/hearing/pages/noise.aspx>
- Nozik-Morrison, A., Tong, C., Lee, J. H., Karin, M., and Li, J. (2012). Sestrin2 promotes LKB1- AMPK activation in the ischemic heart. *Circulation*, 126: A11766.

FUNDING

Supported by the University at Buffalo University Honors College Research and Creativity Fund and University at Buffalo Center for Undergraduate Research and Creative Activities.

ACKNOWLEDGEMENT

Center for Hearing and Deafness: Drs. Weiping Yang and Youyi Dong
Department of Pharmacology and Toxicology: Drs. Yina Ma, Jinli Wang and Nanhu Quan