AMERICAN OTOLOGICAL SOCIETY

Sumana Ghosh, PhD

Instructor, Department of Otolaryngology - Head and Neck Surgery

University of Mississippi Medical Center

"Novel Role of Transcription Factor Six2 in Vestibular Planar Cell Polarity (PCP)"

FELLOWSHIP GRANT 2020

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RESEARCH SUMMARY:

The transcription factor Six2 is known to function in the embryonic development and patterning of many tissues including kidney and heart. Previous studies suggest the highly related Six1 protein plays an important role in the development of the inner ear. However, little is known about the role of Six2 in this context. Here, we investigated the expression pattern and function of Six2 during inner ear development.

Our data suggests that Six2 is expressed in both the pro-sensory and non-sensory cells throughout the development of the otic vesicle. Six2 is expressed broadly, but more ventrally, in the otic vesicle at E10.5. From E12.5 to E14.5, Six2 can be seen in both the otic floor (sensory) and the otic roof (non-sensory). By E17.5 and at postnatal day 0 (P0) Six2 expression in the cochlear floor becomes enriched in cochlear outer hair cells and some modest expression persists in supporting cells. In the vestibular epithelia, Six2 persists in both the type I and type II hair cells and at lower levels in supporting cells. Germline deletion of Six2 led to changes in the morphogenesis of the inner ear organs, with some disruption of the polarity of cells and other regional changes to the organs. For example, the area occupied by the striola was larger in Six2-/- utricles compared to controls, while the number of OCM+ve cells was actually decreased. Reduced numbers of OCM+ cells were also noted in the central region of the lateral cristae. Misorientation of hair cells in Six2-/- animals may not represent disruption of the canonical planar cell polarity pathway as intracellular orientations of VANGL2 and Gαi3 were largely preserved, rather hair cell maturation appears delayed by Six2 deletion leading to disorganization being most prominent in still developing areas of the utricle at E17.5. Consistent with the hypothesis that Six2 may promote hair cell maturation, greater numbers of developing hair cells lack phalloidin and/or espin in the Six2-/- mice vs. controls. However, with overexpression of Six2, the markers of mature type I and type II hair cells, SPP1 and MAPT, were decreased at P3-P4 which would either suggest either dose and/or timing of Six2 may be critical for proper timing of various aspects of vestibular hair cell development and maturation.

OUTCOMES:

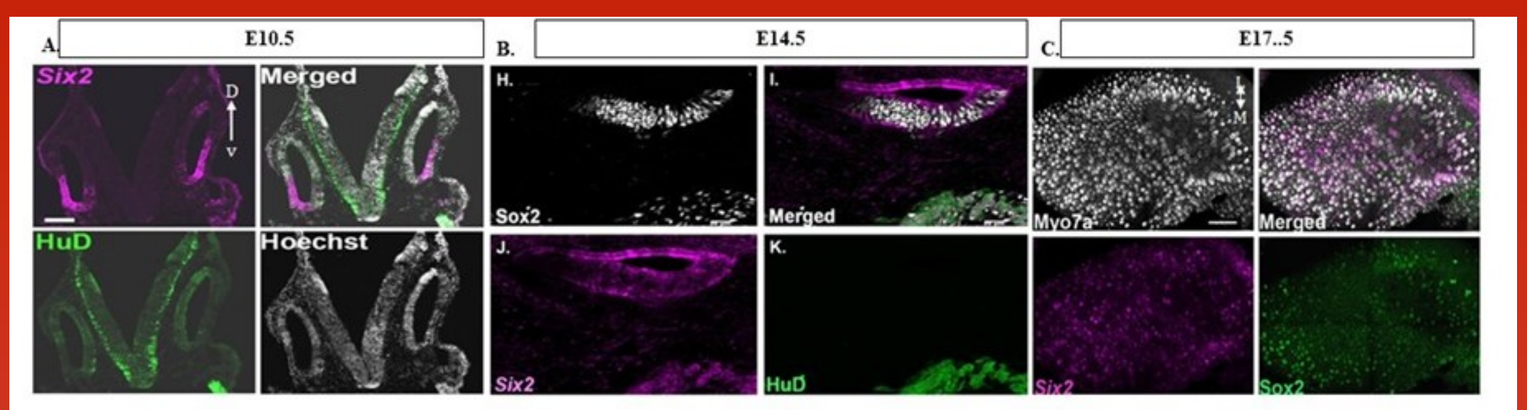

Figure 1: *Six2* is expressed during embryonic development of the inner ear. RNA Expression of *Six2* (magenta) was examined at E10.5, E14.5 and E17.5 followed by immunostaining. **A)** As early as E 10.5 *Six2* is expressed in both sensory and non sensory cells of the otic vesicles. HuD (green) was used to visualize neurons and Hoechst (white) was used to label cell nuclei. **B)** At E 14.5 *Six2* is still present in Sox2 +ve (white) prosensory and non-sensory epithelia and in the ganglion. **C)** *Six2* is expressed at E17.5 in Myo7a (white) positive hair cells and Sox2+ve (green) supporting cells.

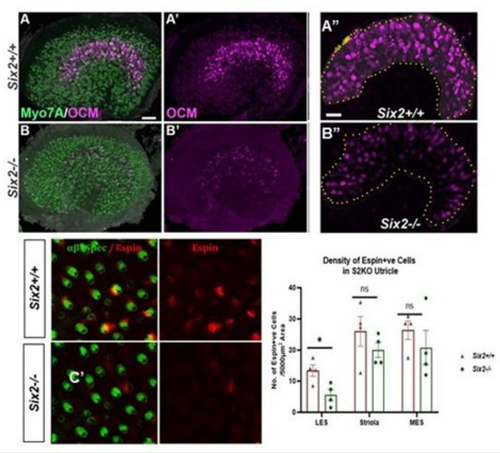

Figure 2: Reduced oncomodulin and Espin expression in response to *Six2* deletion suggests impaired hair cell maturation at E17.5 At embryonic day 17.5 (E17.5) utricles were collected and immunolabelled with OCM and Myo7a (A-B"). Our data suggests that deletion of *Six2* results in reduction of the area and total number of OCM+ve cells. C-E) The utricular hair cells were labelled with αβ-Spectrin II (all hair cells) and espin (mature hair cells). Quantification (E)of our data shows that there is decreased density of the espin+ve cells in the *Six2-/-* mice, suggesting that deletion of *Six2* results in increase of the immature hair cells.

FURTHER FUNDING HAS ENABLED US TO EXPAND OUR RESEARCH TO:

This grant award has enabled us to investigate the role of transcription factor Six2 in the development of the vestibular organ. This work led to unexpected finding that Six2 may play a role in maturation of the sensory hair cells in the utricle. We will further explore the molecular mechanisms how Six2 regulates maturation processing. Our preliminary data suggested that germline deletion of Six2 results in misorientation of hair cells. Therefore, we will further investigate the effect of Six2 deletion in the function of the vestibular system in the conditional Six2 knockout model by performing vestibulo-ocular reflex (VOR), vestibular evoked myogenic potential (VeMP).

LAY SUMMARY OF FINDINGS AND IMPLICATIONS OF THIS RESEARCH:

Vestibular organ located in our inner ear regulates our balance and proprioception. Ototoxic damage such as blast, noise and chemotherapeutic agents and age can result in dysfunctions of the system. This increase fall risk which could be fatal. Despite the importance of the physiological functions of the vestibular system, there is paucity of understanding about the development of this organ. In this current study we investigated the role of a protein (transcription factor) Six2 in the development of the vestibular organs including utricle and cristae. We found that Six2 regulates the development of the sensory cells in the utricle.

During ototoxic damage and natural aging process, the sensory hair cells of the vestibular organ dies. Although some of the hair cells spontaneously regenerate, it fails to fully recapitulate the properties and the function of normal cell population. Therefore, to restore normal vestibular function we need to regenerate the hair cells. Data gleaned from this study will facilitate to understand how the sensory cells develop and this knowledge can be exploite to regenerate sensory cells restoring normal vestibular function.