Ototoxic Damage Induces Interferon Signaling in Chicken Cochlear Epithelial Stem Cells

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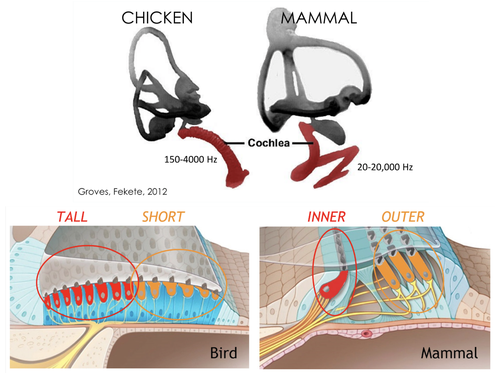
BACKGROUND

The ear is a highly specialized organ. It transduces the mechanical energy of sound waves into electrical impulses that are carried by neurons and interpreted by the brain as the sound we hear. When listening to sounds louder than 20,000 decibels, the mammalian cochlea can be damaged to the point of complete deafness. The mammalian cochlea lacks regenerative capability which is the reason that mammals go deaf. The solution to this problem, that our lab has been interested in, lies in the complex array of stem cells in the avian cochlea, the supporting cells. These supporting cells, which we are just beginning to understand, proliferate into new hair cells, thereby curing deafness in the chicken. The similarities between the avian and mammalian cochlea are evident in the hair cells, which are near-perfect copies; the layout of the hair cells in the cochlea is the main difference between the two models.



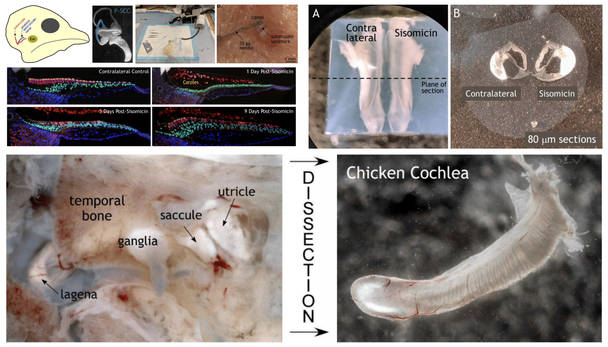
Our lab is interested in characterizing the genes associated with hair cell regrowth in the chicken in order to find the missing link in mammalian hair cell regrowth.

METHODS

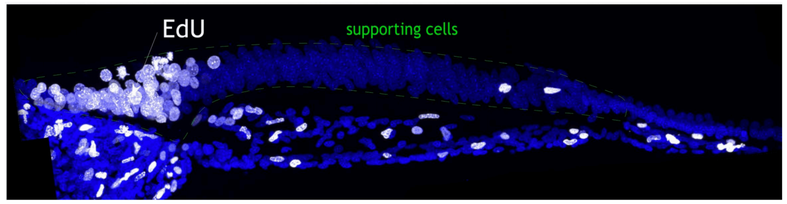
All procedures followed university-approved animal care protocols and guidelines.

Prior to beginning our experiments we analyzed our RNA sequencing data to validate the genes expressed in the day-7 post-hatch chicken cochlea. This protocol helped eliminate genes that were of no importance to us and helped us find genes that we were especially interested in such as those that marked supporting cells, tall, and short hair cells, as well as those which had their own interesting patterns.

We then damaged the chicken cochlea with a single dose of sisomicin, inducing full hair cell death. We then dissected the chicken cochlea and, using *in situ* hybridizations, we characterized the gene expression of the damaged and contralateral chicken cochlea to identify changes in expression between the control and damaged cochlea.

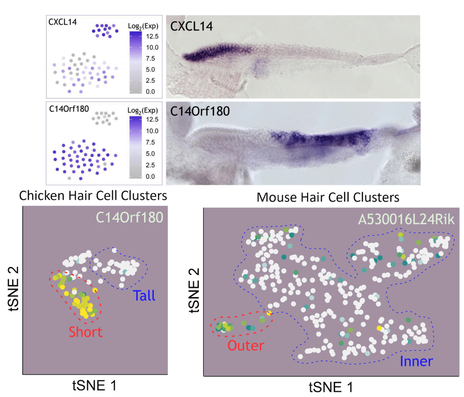


In addition, we stained 40 micron sections of the chicken cochlea with EdU and Sox2 and used virtual reality (VR) analysis to properly characterize neural and abneural hair cells along with verifying supporting cell counts.

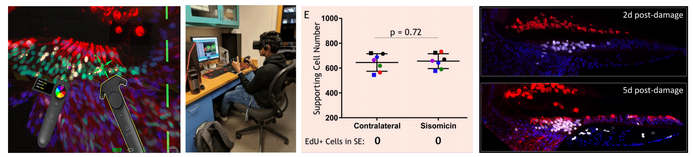


RESULTS

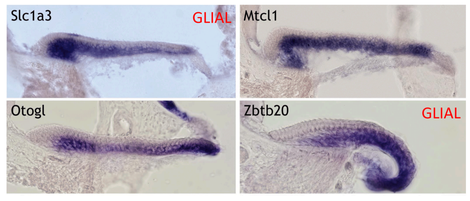
Through our baseline RNA sequencing data, we identified novel hair cell markers such and CXCL14 and C14Orf180 which mark the tall and short hair cells respectively. These two genes also provide the first molecular evidence that avian short hair cells are comparable with mammalian outer hair cells and vice versa.



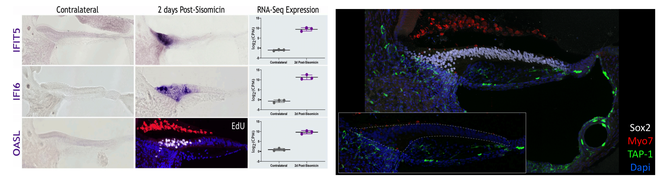
Our VR analysis revealed that the number of supporting cells before and after damage are equivalent and that all neural hair cells mitotically divide while all abneural hair cells undergo phenotypic conversion, demonstrating that only neural supporting cells truly proliferate.



Our In situ hybridizations show supporting cells expressing many glial genes making the supporting cells inherently more stem-like and more likely to proliferate during regeneration.



Lastly, we found that interferon/cytokines genes are regularly unregulated 1-2 days after damage but no immune cells migrate to the cochlea, rather the supporting cells hijack these genes to use for regeneration.



DISCUSSION

Future studies will employ JAK/STAT inhibitors to identify whether interferon-related signaling is essential for regeneration. Ultimately, we aim to compare avian and mouse models in order to identify the missing link in mammals that prevents the cochlea from regenerating the cells that are fundamental for hearing.

REFERENCES

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