Cell Type Specific Markers in Regenerated Mouse Cochlea

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Abstract

Hair cells and supporting cells are required for hearing function, and their irreversible loss causes permanent hearing loss in humans. The aim of this project is to gain better understanding of ectopic hair cells induced by overexpressing the transcription factor Atoh1 in mouse cochlea.

I used R to cluster single cell RNA sequencing data, identified cell subtype of each cluster based on established markers, and identified novel markers for different types of supporting cells as well as inner and outer hair cells.

Introduction

Hearing loss caused by the loss of hair cells affects a large portion of population. A promising approach to regenerating hair cells is to overexpress the transcription factor Atoh1. Previous studies and work done in our lab have shown that Atoh1 overexpression generates ectopic hair cells that express known hair cell markers in the greater epithelial ridge (GER) region. Some ectopic hair cells also show markers of hair cell subtypes. To gain a better understanding of the phenotypes of regenerated cells, we want to characterize them with subtype specific hair cell and supporting cell markers found in endogenous cells.

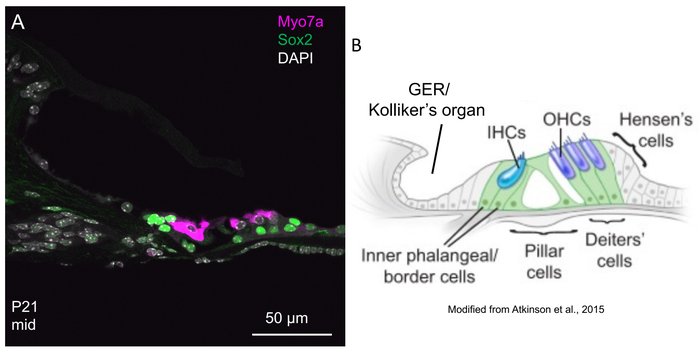

Figure 1. Different cell types in the cochlea

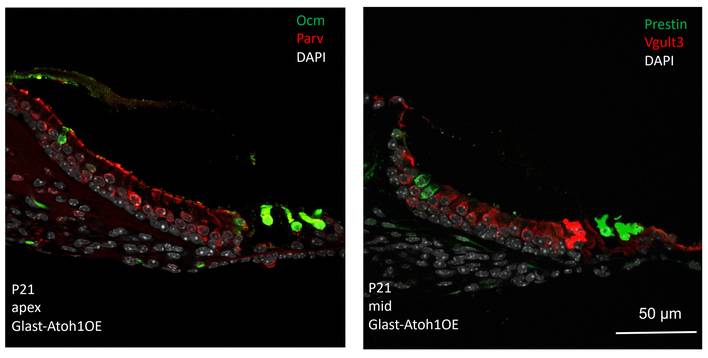

Figure 2. Ectopic cells expressing hair cell markers

Methods

- **Datasets:** Postnatal 1-day-old (P1) and P7 scRNA sequencing data using CD1 mice from Kelley et al. and P20 scRNA sequencing data using C57 mice from Xue et al. Both datasets are available on gEAR website.
- **Clustering:** Done in R Studio using CellTrails package
- **Marker identification:** Summed rankings of gene expression subtraction and fold differences between clusters

Results

Identified markers that are present in P1 only, P20 only, or in both ages.

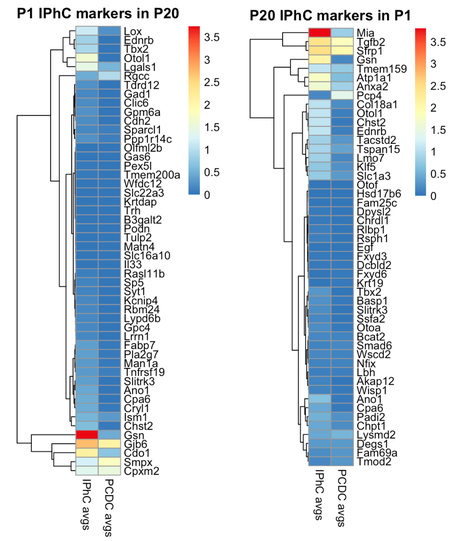

Figure 3. 80% of top 50 inner phalangeal cell markers in P1 are also higher expressed in P20. 78% of top 50 inner phalangeal cell markers in P20 are also higher expressed in P1.

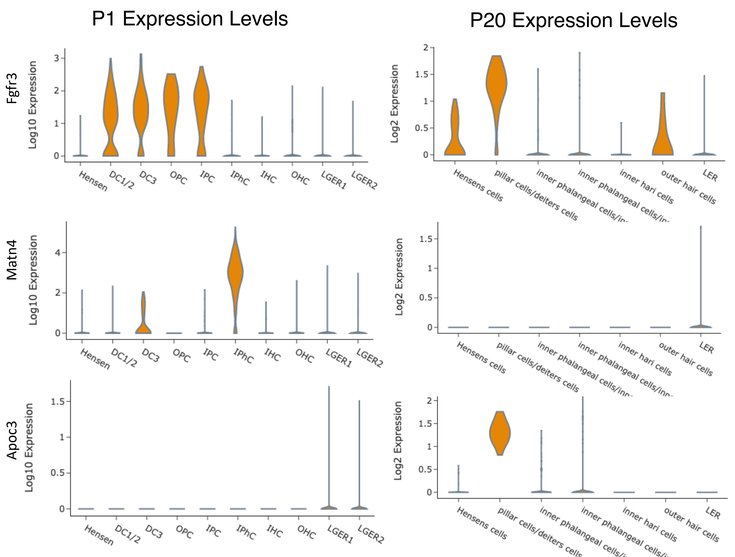

Figure 4. Fgfr3: Pillar/Deiters' cell marker at P1 and P20
Matn4: Inner phalangeal cell marker at P1 only
Apoc3: Pillar/Deiters' cell marker at P20 only

Summary

	Inner Phalangeal Cell	Pillar/Deiters' Cell	Inner Pillar Cell	Outer Pillar Cell	Deiters' Cell 1/2	Deiters' Cell 3
Pan	Ednrb, Otol1	Smagp, Fgfr3, Mansc4, Cep41, Gm13782,		Cd44	Pdzk1ip1	Emilin2, Mgll
Immature	Matn4, Sparcl1, Fabp7	Etv4, Pkd2l1, Dgat2, Dync1i1, Igfbpl1	Npy, Ngfr, Ace			
Mature	Hsd17b6, Fam25c, Fxyd3	Apoc3, Ppp1r17, Crhcr1				

Table 1. Markers identified for supporting cell subtypes

	Inner Hair Cell	Outer Hair Cell
Pan	Atp2a3	4930558C23Rik
Immature	Fgf8, Fam19a3, Ppm1j	Mslnl, Calca, Tac4, Adamts13, Cacng2, Grp, Tmem91, Neurod6, Coro2a, Nppa
Mature	Ephx1, Ano4, Grin2c, Reep6, Slc17a8, Nfasc, Ano3, Arl5a	Myom1, Slc26a5, Ifit1, Prob1, Snhg11, D830039M14Rik, B230217C12Rik

Table 2. Markers identified for hair cell subtypes

Validation

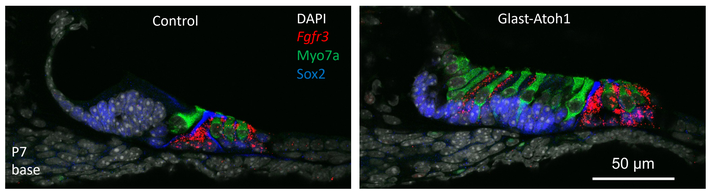

Figure 5. Fgfr3 mRNA is expressed in wildtype pillar/Deiters' cells and medial section of ectopic supporting cells

What's Next

- Evaluate markers identified
- Conduct immunohistochemistry on microdissected sample sections to stain for select markers

Acknowledgements

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References

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