Building a Single-Cell Atlas of the Mouse Cerebellum to Understand the Origins of Group 3 and Group 4 Medulloblastoma

Tanaya Datar[1,2], Divya Singhal[1], Nour Hassan[1], Kathleen Millen[3], Parthiv Haldipur[3], Anders Erickson[4], Shraddha Pai[1,2]

[1] Ontario Institute for Cancer Research, Toronto, Canada [2] University of Toronto, Department of Medical Biophysics, Toronto, Canada [3] Seattle Children's Research Institute, WA, USA
[4] University of Toronto, Laboratory Medicine and Pathobiology, Toronto, Canada

Abstract

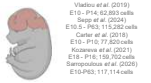
The most prevalent forms of medulloblastoma (MB), Group 3 (G3) and Group 4 (G4) are the least understood. G3 and G4 is believed to arise from the Rhombic lip (RL) to Unipolar brush cells (UBC) developmental lineage. UBCs are a small, overlooked population in the cerebellum and the precise stages of their development are poorly defined. This project aims to build a comprehensive single-cell atlas of the mouse cerebellum to understand this trajectory and identify regulators of UBC lineage commitment. scRNA-seq data from 5 published mouse cerebellar datasets spanning embryonic through adult timepoints were integrated using canonical correlation analysis (CCA). Four transcriptionally distinct postnatal UBC clusters were identified suggesting UBC identity is acquired progressively over postnatal development. These findings lay the groundwork to understand the stage and cell population from which G3 and G4 MB arise, and further to determine if this lineage is conserved between mouse and human.

Introduction

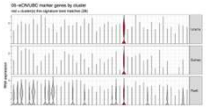
- Medulloblastoma (MB): Most common malignant pediatric brain tumor
- Group 3 (G3) & Group 4 (G4): Most prevalent subgroups (~65% of cases), least understood origins
- Human studies implicate the rhombic lip subventricular zone (RL-SVZ), a unipolar brush cell (UBC) progenitor niche as the shared compartment of origin for G3/G4 MB
- Existing atlases lack resolution to define the RL→ UBC trajectory across species
- **Project Goals:**
  1. Development – Define the UBC developmental lineage(s) in mouse
  2. Evolution – Determine how UBC development differs between mouse and human
  3. Disease relevance – Identify which developmental state(s) G3 and G4 MB most closely resemble
- **Hypothesis:** G3 and G4 MB arise from transcriptionally distinct, sequential stages along the mouse RL→ UBC developmental trajectory, identifiable by stage-specific marker genes.

Methods

Step 1. Integrate five published scRNA-seq mouse datasets of the developing cerebellum
Method: Canonical Correlation Analysis (CCA) (Seurat v5)

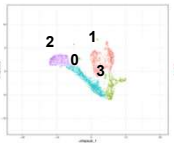


Step 2: Score cluster identity to isolate the UBC population
Method: Signature-based ModuleScore annotation + marker gene DE analysis



Step 3. Gate on Pax6/Eomes to subset UBC-lineage cells
Method: Raw-count marker gating + age filtering

Step 4. Isolate and recluster UBC population (*Pax6+, Eomes+, Lmx1a+, Mki67-*)
Method: SCTransform + reclustering



Step 5
- Cluster-specific / exclusive marker identification (DEG analysis)
- Demographic composition analysis

Results

Integrated mouse cerebellar atlas reveals 19 transcriptionally distinct cell types from embryonic to adult development

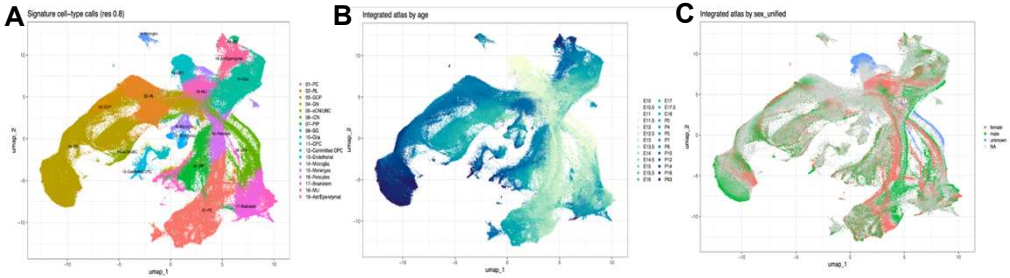


Figure 1. (A) Signature-based cell-type annotation of the CCA-integrated mouse cerebellar atlas (519K cells, 5 datasets, res 0.8), with clusters labeled by best-matching reference cerebellar cell-type signature (ModuleScore, signatures from Aldinger et al. 2021). (B) UMAP colored by developmental age (E10-P63), showing a continuous embryonic-to-postnatal trajectory with minimal discontinuity across timepoints, consistent with successful batch integration. (C) UMAP colored by sex (female, male, unknown, NA), showing no sex-driven clustering, confirming that the observed structure reflects developmental and cell-type identity rather than a technical or demographic artifact.

The mouse cerebellar atlas resolves a single transcriptionally distinct UBC/eCN cluster

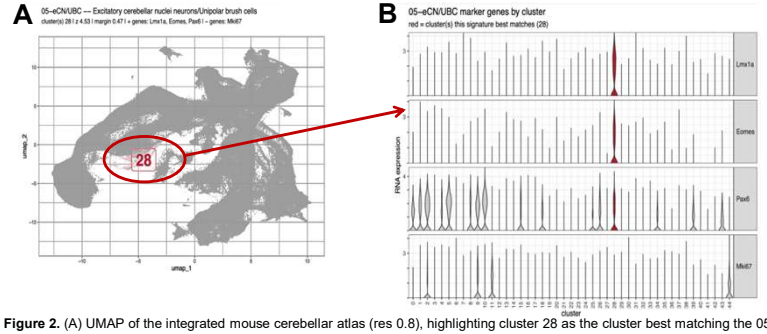
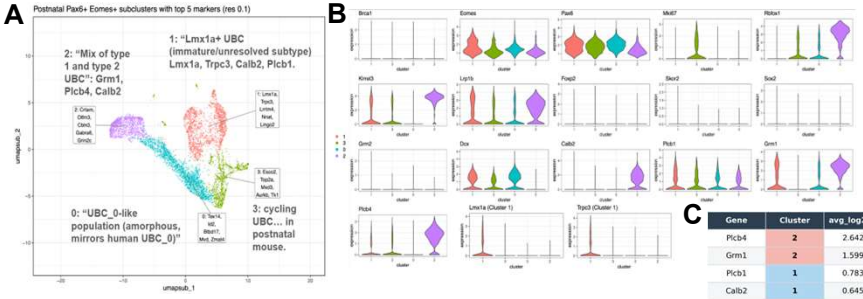


Figure 2. (A) UMAP of the integrated mouse cerebellar atlas (res 0.8), highlighting cluster 28 as the cluster best matching the 05–eCN/UBC reference signature (excitatory cerebellar nuclei neurons/unipolar brush cells; Aldinger et al. 2021), based on ModuleScore signature matching (B) Violin plots of the signature's marker genes (*Lmx1a, Eomes, Pax6, Mki67*) across all 45 clusters, showing that cluster 28 (red) uniquely co-expresses *Lmx1a, Eomes and Pax6*, and is *Mki67*-low/negative — consistent with a postmitotic UBC/eCN identity.

Postnatal *Pax6+/Eomes+* cells resolve into four transcriptionally distinct subclusters



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Gene	Cluster	avg_log2FC	p_val_adj	pct.1	pct.2
Plcb4	2	2.642	0.00e+00	0.863	0.208
Grm1	2	1.599	0.00e+00	0.943	0.358
Plcb1	1	0.783	6.32e-05	0.443	0.402
Calb2	1	0.645	4.83e-09	0.174	0.265
Calb2	2	2.035	0.00e+00	0.695	0.130

Figure 3. Postnatal *Pax6+/Eomes+* cells (N = 6,866) clustered into four subtypes: an amorphous cluster (0) with no canonical UBC markers, an unresolved *Lmx1a+* cluster (1), a mixed Type I/II cluster (2, *Calb2+/Grm1+/Plcb4+*), and a cell-cycling cluster (3, *Top2a+/Aurkb+*). (A) UMAP with top marker genes per cluster. (B) Violin plots of UBC and identity markers across clusters. (C) Representative DEGs (FDR < 0.05) with log2FC and percent expression (pct.1 = cluster, pct.2 = rest).

Discussion

- Four transcriptionally distinct postnatal UBC subclusters were identified, suggesting UBC maturation proceeds through discrete intermediate states rather than a simple Type I/Type II binary.
- Cluster 1 is disproportionately composed of P0 cells and lacks strong Type I/II markers, suggesting an early, immature UBC state that has not yet committed to a mature subtype. Cluster 3's cell-cycle signature (*Top2a, Aurkb*) shows UBC progenitors continue proliferating after birth, not just embryonically.
- **UBC identity is acquired progressively over postnatal development,** a staged model consistent with our hypothesis that G3 and G4 MB may correspond to distinct points along this trajectory.

Conclusion

- These findings advance our understanding of the UBC developmental trajectory and support testing our hypothesis that G3/G4 MB arise from distinct stages along it, informing better developmental models of MB oncogenesis.
- Moving forward, we plan to perform trajectory inference (Monocle 3) to resolve the RL→ UBC lineage at higher resolution and integrate with the human dataset to identify species-specific differences.

References

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Acknowledgements

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