

Systematic comparison of IDH wild type GBM and neurodevelopmental trajectories

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Abstract

- To uncover how tumor cells exploit neural developmental pathways and find treatment targets, our study analyzed various single-cell/single-nucleus transcriptome datasets.
- By comparing them with fetal developmental paths, we created a novel GBM classification and aim to identify fine developmental subtypes within GBM using bulk RNA-seq data.
- We'll then correlate these subtypes with clinical data and infer functional connections, potentially shedding light on survival and prognosis implications.

Comparing GBM and Fetal Cell Pathway Activation

After estimating CNAs, we categorized GBCs into two groups: one exhibiting more CNAs, while the other shows no significant CNAs (Figure 1).

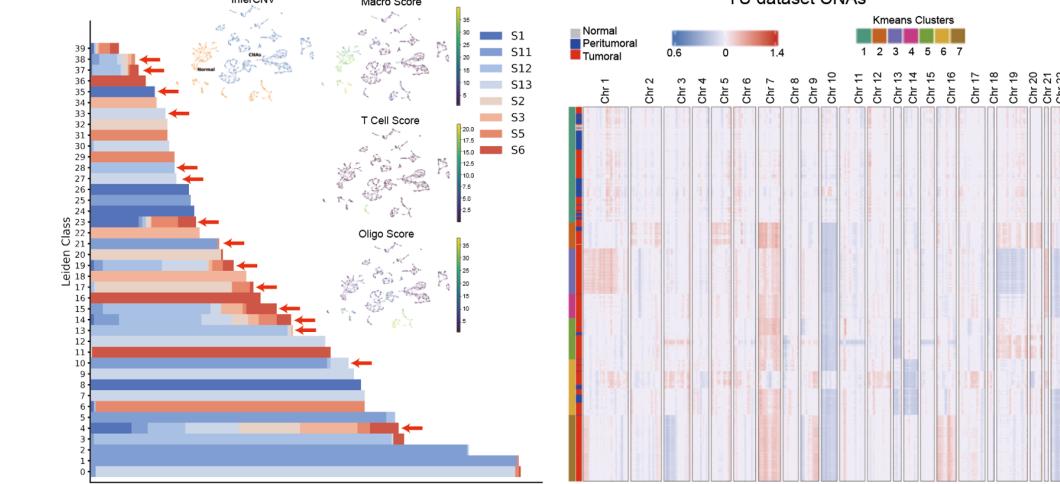


Figure 1: Yu dataset CNAs estimation

- The main activation pathways in developing neurons are partially active in GBM malignant cells but largely inactive in normal cells.
- Neural progenitor cells exhibit partial activation in GBM malignant cells, with limited activation in normal cells.
- tRG cells demonstrate multiple activation pathways associated with cilia, notably evident in fetal data and broadly activated in GBM.

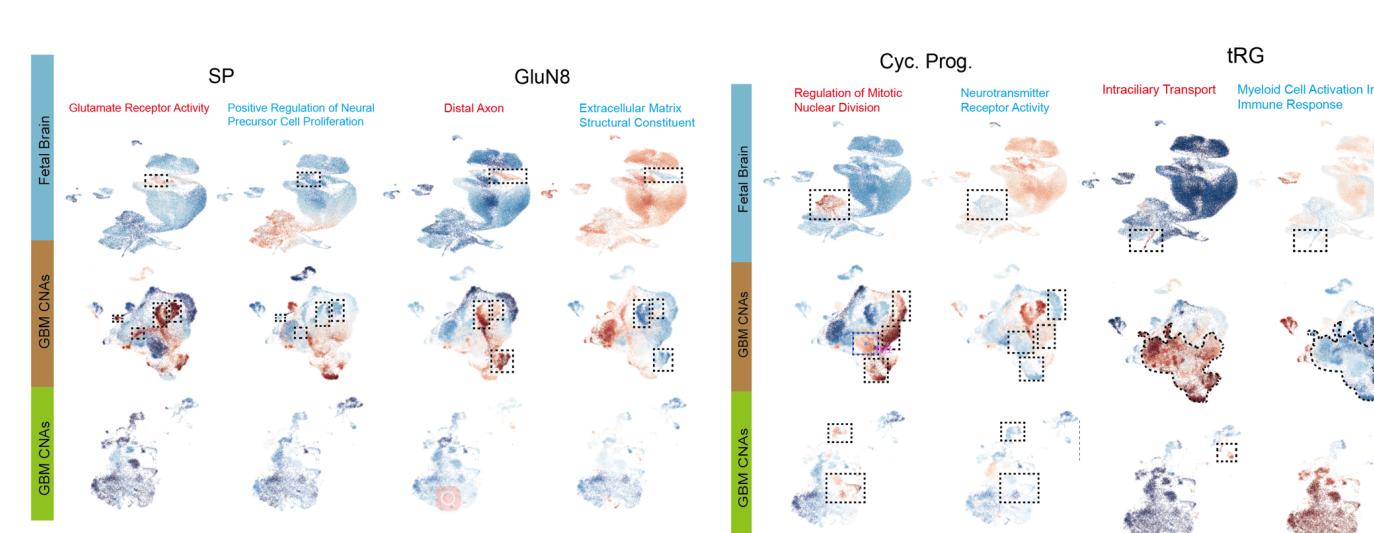


Figure 2: Comparing GBM and Fetal Cell Pathway Activation

My contributions and benefits

This project is my current thesis, which I independently designed, led, and executed. Through it, I honed computational skills like scRNA-seq analysis, dry experiment design, and wrote a successful funding proposal supported by the Postgraduate Research Practice Innovation Program of Harbin Medical University (No. YJSCX2023-120HYD). This experience refined my research abilities and helped me explore potential directions for future doctoral studies.

Filtering GBM populations resembling fetal cells

Calculating Jaccard index between GBM and fetal populations based on pathway activation to filter similarly activated patterns (Figure 3A). Majority of GBCs resemble fetal cells for further investigation (Figure 3B-C).

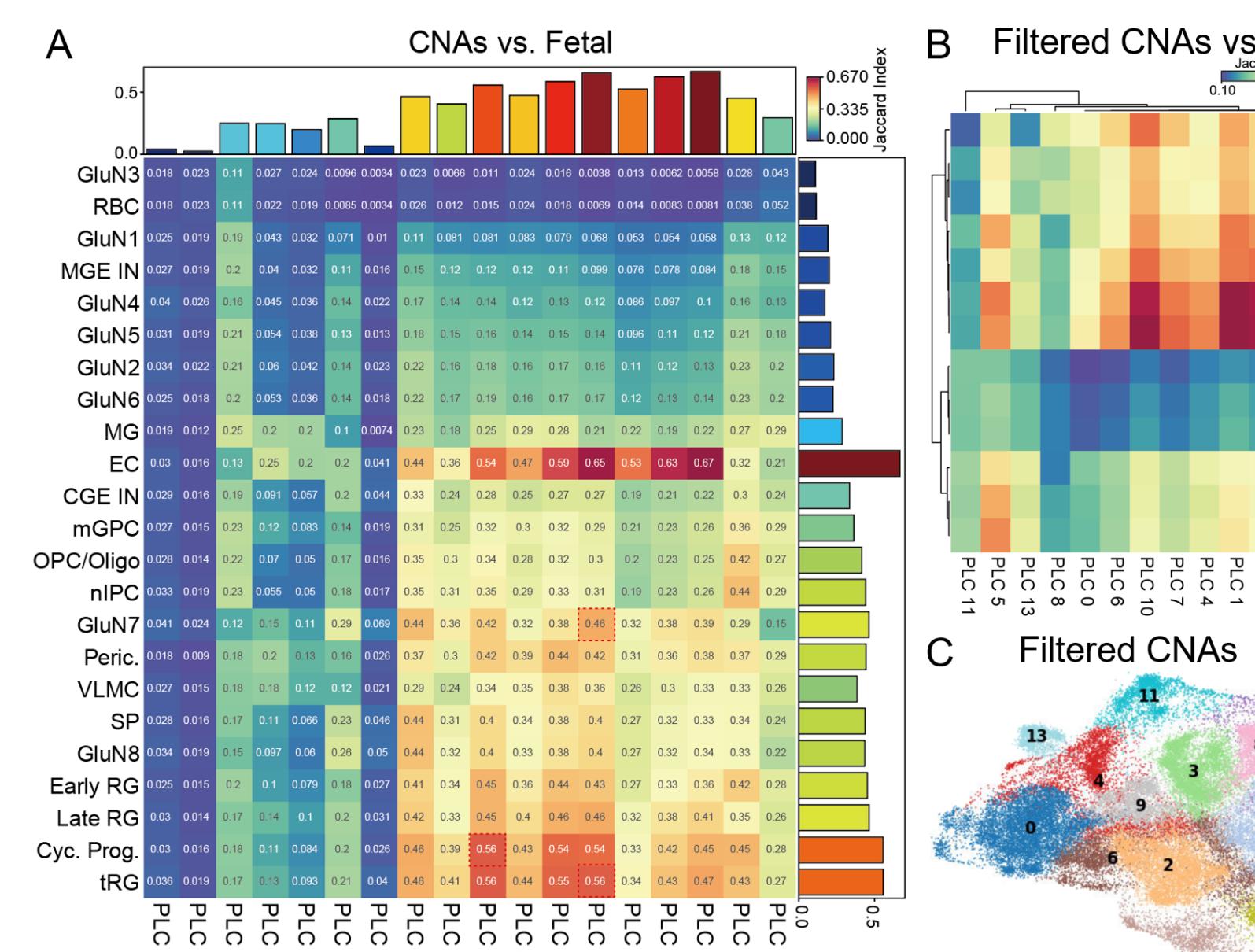


Figure 3: Filtering GBM populations resembling fetal cells

Aligning Fetal and GBM Pseudo-Time Trajectories

To perform pseudotime analysis, ascertain the differentiation trajectory of GBCs, which was then compared to the fetal trajectory (Figure 4A). Remarkably, GBCs exhibited a higher proportion of 'young' cells compared to fetal cells, demonstrating a distinct pattern of differentiation. Fetal development displayed a linear differentiation trajectory, whereas GBCs appeared to form a loop-like structure. This observation suggests a potential enrichment of stem-like cells, influenced by complex forces that affect their differentiation trajectory. Subsequently, we aligned GBCs to fetal cells, from which a 5-branched alignment tree was derived (B1-5), indicating four distinct differentiation directions for GBCs (B2-5) (Figure 4B). Interestingly, while the root consists mainly of cycling progenitors, the GSC is enriched in B3 (Figure 4C). Spatially, the LE region mainly contains B2 and B3 cells, while the PAN area shows a higher presence of B5 cells. Additionally, B4 cells are mainly located in the CT area (Figure 4D). B2 and B3 preferentially align fetal neurons, while B4 and B5 exhibit a higher prevalence of fetal progenitor subtypes, referred to as neural-like and progenitor-like branches, respectively. When compared to different reported classifications, the Neural-like branch corresponds to Richard's developmental state, Nefel's NPC, Wang's Proneural, and Garofano's NEU, while the progenitor-like branch corresponds to Richard's injury response, Wang's Classical, and Garofano's MTC (Figure 4E-F).

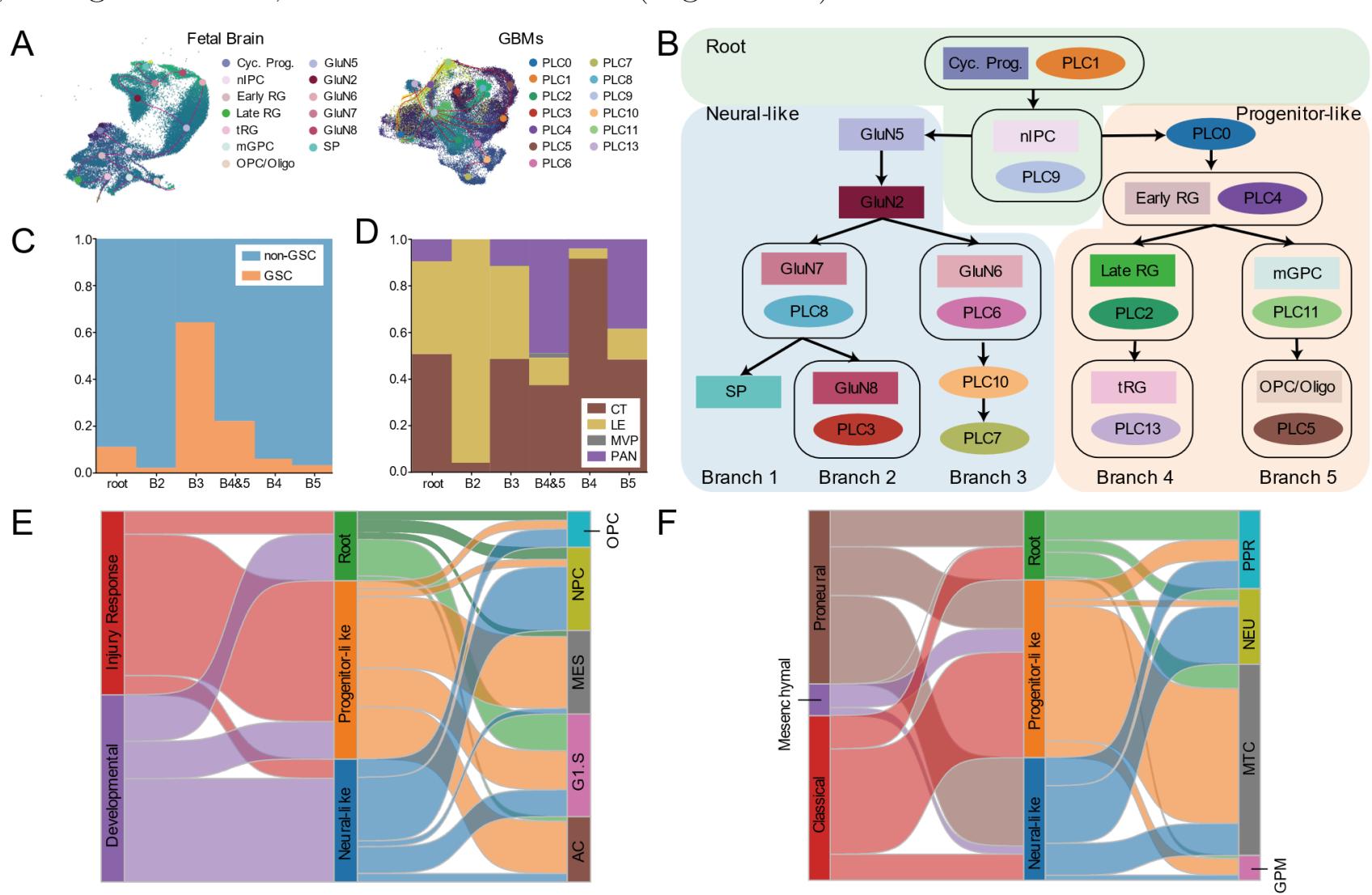


Figure 4: Five braches after alignment of fetal and GBM.
A: Alignment tree of GBM to fetal cells based on pseudo-time trajectory. B: Pseudo-time for fetal and GBM cells respectively. Light green indicates higher time values, while dark blue represents lower time values. C: GSCs distribution among lineages. D: Lineages' generally spacial distribution. E-F: Comparison of main alignment branches with previous classification systems.

GBM tends to cluster two polarities of the timeline

Upon comparison between fetal cells and GBCs across various branches, it was observed that GBCs tend to cluster at two distinct polarities along the temporal axis (Figure 5).

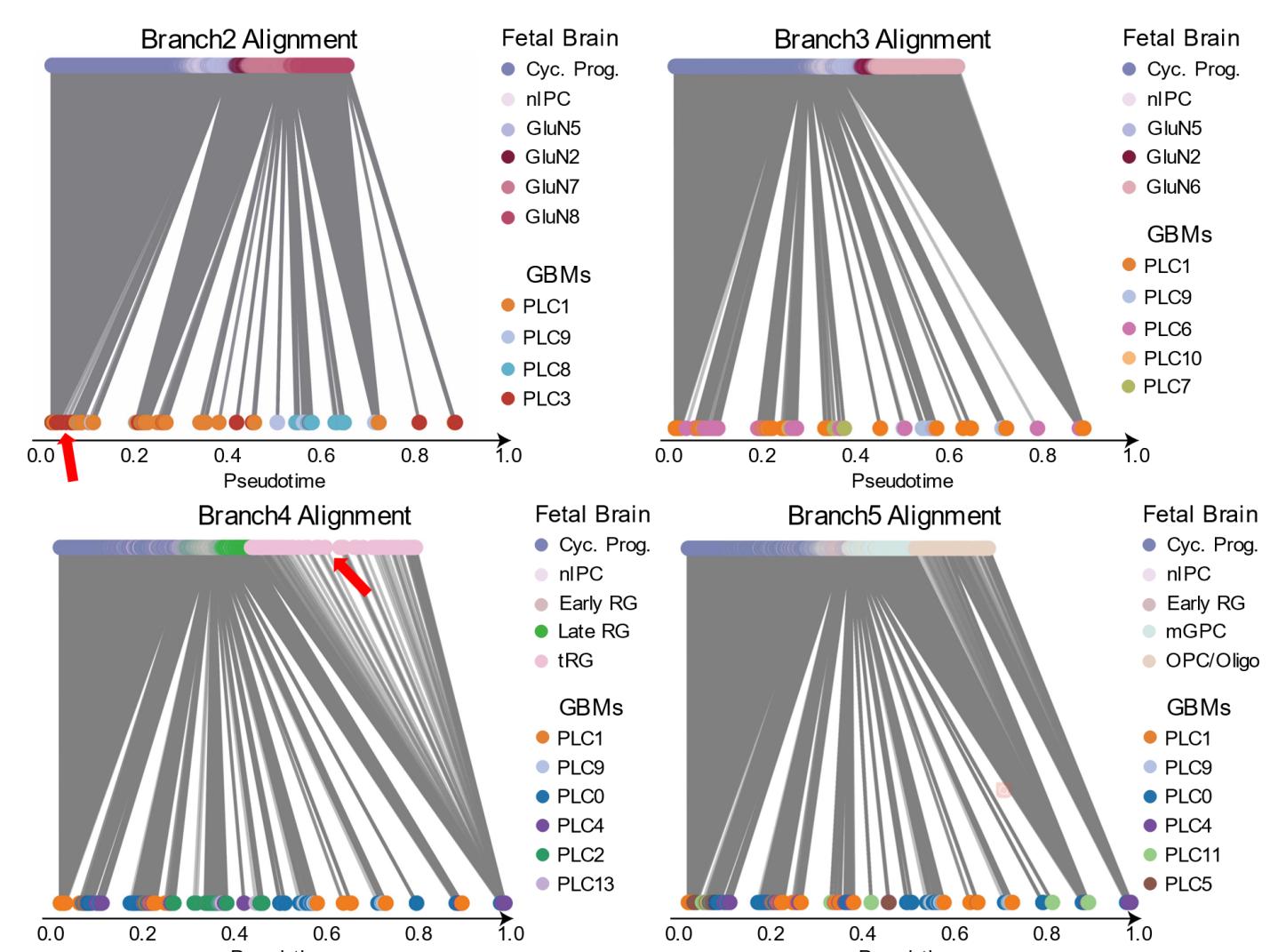


Figure 5: GBM tends to cluster two polarities of the timeline

Specifically, GBCs exhibit an aggregation tendency towards the left termini of branches 2 and 3, indicating a predisposition towards a stem cell phenotype, including some PLC3 aligning with GluN6. Additionally, a minority subset of cells within the PLC1, corresponding to Cyc. Prog, display a comparatively more 'mature' profile aligned with developing neurons along the timeline. Branches 4 and 5 mainly differentiate to neural and glial progenitors, respectively. Notably, certain GBCs are located at the maximum value, suggesting a more mature state despite their alignment with the fetal progenitors, primarily within PLC4 and PLC5. Interestingly, the clustering of GBCs occurs at both ends of the tRG timeline.

Two potential opposing forces

We are examining if two opposing forces influence GBM cells: one pushes towards differentiation into neurons/glial cells, while the other reverses them to a stem cell state, maintaining their stemness. RNA velocity analysis offers some evidence for this (Figure 6).

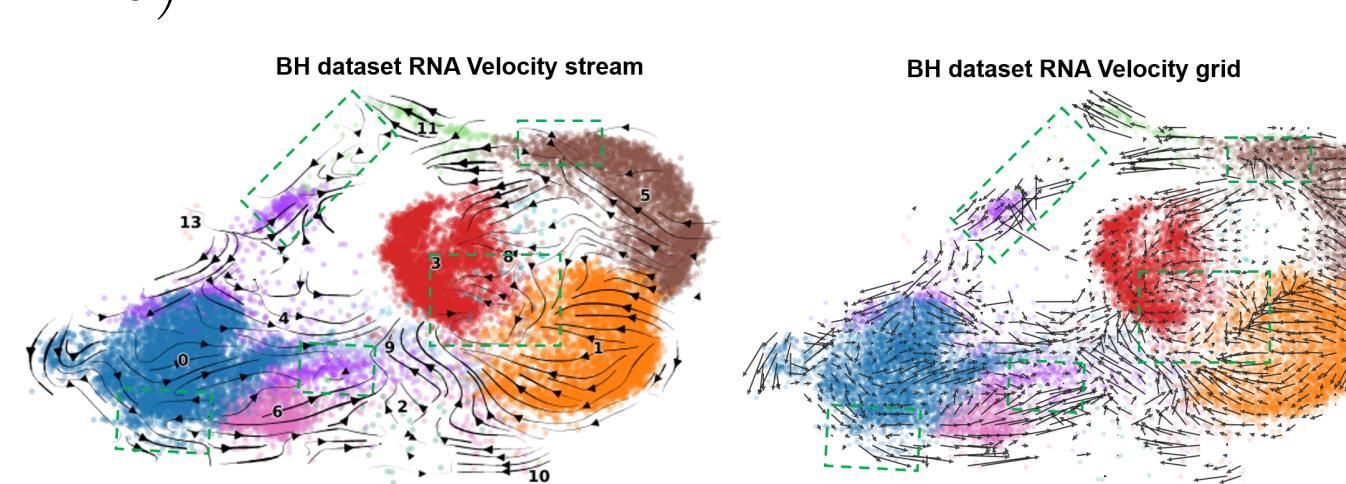


Figure 6: BH dataset RNA velocity analysis

Overall, the majority of cell types exhibit a tendency towards PLC3, indicative of neuronal differentiation. Notably, in specific regions (highlighted by green dashed boxes), particularly in transitions from PLC3 to PLC1, suggesting a potential reversal of neuronal differentiation to a stem cell state, implying the presence of a regulatory mechanism guiding this process. However, it is important to acknowledge that among the datasets examined, only the BH dataset offers raw data suitable for RNA velocity analysis. This limitation leads to fewer cells being assigned to PLC4, 7, and 10, potentially introducing a slight bias into the results. Therefore, further corroborating evidence should be pursued to validate this observation.

Future Work & Other Questions of Interest

In this project, we developed a novel classification system involving five branches of differentiation for GBM. Branches 2-4 are associated with GBC differentiation, with branches 2-3 focusing on neural direction and branches 4-5 on progenitors. Comparison with previous classification systems is required. Additionally, we are going to investigate associations with spatial and phenotypic data, and cell-cell communication. Further validation will be conducted to explore two potential opposing forces underlying GBM progression.

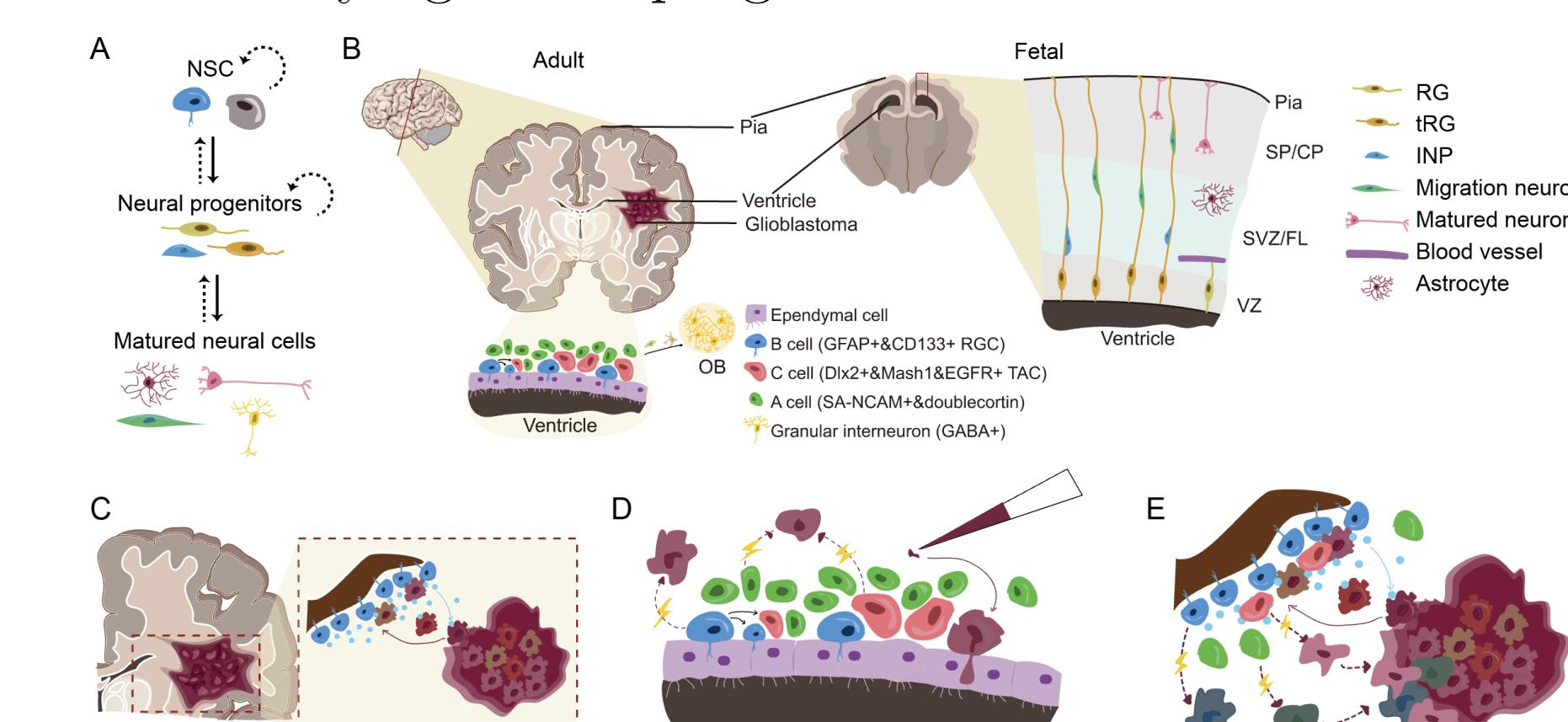


Figure 7: Potential GBCs, Fetal, and Adult Neural Stem Cell Relationships

Other direction I hope to research in the future: GBM stem cells (GSCs) are critical for tumor recurrence, as they share similarities with neural stem cells. Errors occurring at various states of neural cell development are likely to lead to oncogenesis (Figure 7A). Fetal and adult neural stem cells (fNSCs and aNSCs) differ in distribution and activation (Figure 7B) [1]. GBM's origin hypotheses include mature cells acquiring stem-like properties or abnormal differentiation of aNSCs. GBM tends to invade ventricular regions, with cells in VZ inducing migration of GBCs towards them [2, 3] (Figure 7C-D). Some GBCs may relate to aNSCs, but specifics are unknown. Additionally, B cells a kind of aNSC in VZ, expressing same marker with RGCs [1] (Figure 7B), also discovered in GBM [4]. Moreover, tRG is a subtype of RGCs, which have same origin with ependymal cells and shows similar shape with B cells [5]. Our study also found more tRG pathway activation is observed in GBCs, suggesting a potential link between tRG, aNSCs, and GBCs, which could offer new insights into treatment. I'm particularly curious to know whether mutations in mature cells trigger an inflammatory response, activating quiescent aNSCs, leading to abnormal differentiation into tumor cells (Figure 7E). This process could result in a mixture of cells from two different origins in GBM.

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