Journal Club

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Transplanted Astrocytes Show Functional Flexibility in the Recipient Brain

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Review of Chierzi et al.

Cell type diversity in the brain is an area of intensive study. Although glia are typically categorized simply as astrocytes, microglia, or oligodendrocytes, recent papers have begun to uncover transcriptional differences between glia within these broad categories in different brain regions, a difficult task before the advent of single-cell RNA sequencing (scRNAseq). The extent to which cell-intrinsic versus environmentally supplied mechanisms underpin these transcriptional differences remains largely unknown. Recently, an scRNAseq lineage tracing study proved that cell-intrinsic signaling does not fully determine resulting adult cell type in the mouse brain (Bandler et al., 2022) as the same mother cell could produce daughters of radically different transcriptional states; however, this study did not examine functional differences in daughter cells.

Transplantation paradigms are useful functional experiments to tease apart intrinsic cell development from extrinsic signaling during development. In neuroscience, although the transplantation of neurons into existing circuits is a standard paradigm in developmental research (Frantz and McConnell, 1996), the transplantation of glial cells is a relatively new approach. One notable example is the cross-species transplantation of human primary astrocytes into *Rag2*-null mice (Han et al., 2013) with mice that received human astrocytes displaying improved cognitive performance. Furthermore, several groups have studied microglial functions by completely removing endogenous microglia from the brain and replacing them with transplanted cells (Bennett et al., 2018). Thus, transplantation methods are of great interest to the neuroscience community as tools for assessing cell-autonomous versus environmentally determined functions.

Chierzi et al. (2023) used a new method of astrocyte transplantation to compare the effects of intrinsic and local cues on astrocyte development in two major brain regions. Specifically, the authors cultured immature postnatal day 4–8 (P4–8) mouse astrocytes from neonatal mice, grew the cells in culture for a period of weeks, and subsequently transplanted cultured astrocytes into a new donor mouse brain. To extract and maintain primary mouse astrocytes *in vitro*, the authors purified astrocytes from neonatal mouse cortex or cerebellum expressing inducible *TdTomato* under the astrocytic-specific *Aldh1l1* promoter (Chouchane and Costa, 2018). Cultured astrocytes displayed positive immunostaining for many astrocyte markers, including SOX9, GFAP, and Kir4.1, and expressed *TdTomato* from an astrocytic *Aldh1l1* promoter once induced with tamoxifen.

After 14–34 d *in vitro*, cells were injected into either the cortex or cerebellum of a P3–P8 recipient mouse. The transplanted cells (clearly tagged with *TdTomato*) persisted in the recipient mouse for weeks to years after transplantation, displayed morphology similar to resident astrocytes, and could migrate laterally >1 mm away from the injection site (Chierzi et al., 2023, Fig. 2*D*). The authors profiled the transplanted cells by immunostaining for a variety of astrocyte markers and observed staining suggestive of proper patterning within different layers of the cortex, including the proper establishment of local tiling territories. Furthermore, they quantified the staining for vGLUT1/2 and vGAT to show that transplanted cells seemingly develop proper synaptic interactions with neurons and also display characteristic AQP4+ astrocytic end feet. Importantly, transplanted astrocytes displayed equivalent levels of GFAP immunostaining when compared with resident astrocytes, suggesting that transplanted cells do not maintain a reactive state once integrated into the recipient brain. Beyond immunostaining, the authors also performed preliminary Ca^{2+} imaging and showed that transplanted cells display characteristic astrocytic Ca^{2+} transients that are equivalent to those seen in native astrocytes in the recipient mouse.

The authors next examined donor cells transplanted into the cortices of older mice (P34–P43) and donor cells from different brain regions transplanted into the cerebellum. Notably, donor astrocytes transplanted into older cortices were viable and could still spread laterally from the injection site, although the extent of the spread was

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limited to approximately one-third of that in younger donor-recipient experiments. For their final experiments, the authors attempted to transplant primary cortical astrocytes into the cerebellum of a recipient mouse. Cortical cells transplanted into the cerebellum displayed an intermediate identity, acquiring some characteristics of cerebellar astrocytes, such as characteristic morphology and ability to guide Purkinje cell dendritic growth, but still maintained some properties of cortical astrocytes like expression of EEAT2 instead of EEAT1.

Chierzi et al. (2023) demonstrated that early/immature mouse astrocytes are able to reintegrate into a young brain, even after weeks of *in vitro* culture. This finding suggests that primary astrocytes cultured with animal sera maintain much of their *in vivo* gene expression capacity, although other researchers have shown that serum exposure alters astrocyte gene expression *in vitro* (Foo et al., 2011). Moreover, the cross-region transplantation experiments provide further evidence that regional identity in astrocytes is determined via a mix of intrinsic and local cues, as shown by the intermediate phenotype (correct morphology but incorrect EEAT2 expression) displayed by cortical astrocytes transplanted into the cerebellum.

The study by Chierzi et al. (2023) raises several other compelling questions for future research. First, what are the essential local cues necessary for astrocyte integration into the local CNS environment of both young and old brains? Astrocytes cultured *ex vivo* were able to assimilate better in the young recipient parenchyma compared with old parenchyma, suggesting that some environmental factors in the brain parenchyma that establish astrocytic identity and function change with age. To uncover these environmental factors, one could examine the constitution of the extracellular matrix via microscopy or proteomics; perhaps increased myelin thickness and neuropil density during aging may decrease the ability of transplanted cells to navigate brain parenchyma.

Second, what type of astrocytes are most pliable and able to adapt to a new region? Chierzi et al. (2023) showed that although cortical astrocytes can integrate into the young cerebellum, they have a decreased ability to migrate deeper into the tissue parenchyma. Previous studies report that astrocytes in different brain regions possess unique molecular identities, leading to questions about whether cells from one brain region can ever fully assume the identity of another brain region. Recent work has examined this regional programming at the scRNAseq and the Assay for Transposase-Accessible Chromatin using sequencing level (Lattke et al., 2021), but transplantation is now clearly a viable paradigm to functionally test findings from sequencing experiments.

Beyond regional identity, can transplanted astrocytes maintain the capacity to become reactive and undergo cellular, molecular, and functional transformations in an inflamed or injured brain *in vivo* as observed previously? For example, can they undergo loss of neurosupportive features during injury and gain neurotoxic phenotypes such as the secretion of neurotoxic lipids (Guttenplan et al., 2021)? Although Chierzi et al. (2023) reported normal GFAP levels in transplanted cells, astrocytes may still assume a partially reactive phenotype when transplanted into a previously inflamed or injured brain, which may cause remodeling of the CNS tissue and eventual neuronal dysfunction. Before astrocyte transplantation could be considered as a potential therapeutic, future investigations should examine astrocytic reactive states before and after their transplantation into an aged or diseased brain.

Finally, beyond the astrocytic functions evaluated in this study, other researchers should query whether transplanted astrocytes can perform other critical functions, such as supporting neuronal synapses, phagocytosis of cellular debris and pathologic peptides (Prakash et al., 2021), maintenance of the blood–brain barrier, and secretion of lipoparticles like APOE/J (apolipoprotein E/J) and immunomodulators like TGF-β. Addressing these questions will open new avenues to using transplanted astrocytes as a useful tool to explore and, potentially, modulate CNS states in health, development, and disease.

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