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Finding degrees of separation: Experimental approaches for astroglial and oligodendroglial cell isolation and genetic targeting

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HIGHLIGHTS

- Astrocytes and oligodendrocytes express stage-specific, cell surface markers useful for cell identification and selection.
- Astrocytes and oligodendrocytes can be acutely isolated from CNS tissue, cultured from progenitors or derived from stem cells.
- Genetic models offer a variety of marker genes with which to target stem cells, astrocytes and oligodendrocytes *in vivo*, with overlapping expression across cell types.
- Novel genetic models offer molecular tagging *in vivo* to facilitate gene expression analysis.

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ABSTRACT

The study of CNS glial cell function requires experimental methods to detect, purify, and manipulate each cell population with fidelity and specificity. With the identification and cloning of cell- and stage-specific markers, glial cell analysis techniques have grown beyond physical methods of tissue dissociation and cell culture, and become highly specific with immunoselection of cell cultures *in vitro* and genetic targeting *in vivo*. The unique plasticity of glial cells offers the potential for cell replacement therapies in neurological disease that utilize neural cells derived from transplanted neural stem and progenitor cells. In this mini-review, we outline general physical and genetic approaches for macroglial cell generation. We summarize cell culture methods to obtain astrocytes and oligodendrocytes and their precursors, from developing and adult tissue, as well as approaches to obtain human neural progenitor cells through the establishment of stem cells. We discuss popular targeting rodent strains designed for cell-specific detection, selection and manipulation of neuroglial cell progenitors and their committed progeny. Based on shared markers between astrocytes and stem cells, we discuss genetically modified mouse strains with overlapping expression, and highlight SOX-expressing strains available for targeting of stem and progenitor cell populations. We also include recently established mouse strains for detection, and tag-assisted RNA and miRNA analysis. This discussion aims to provide a brief overview of the rapidly expanding collection of experimental approaches and genetic resources for the isolation and targeting of macroglial cells, their sources, progeny and gene products to facilitate our understanding of their properties and potential application in pathology.

Abbreviations: GalC, galactocerebroside; FACS, fluorescence-activated cell sorting; BAC, bacterial artificial chromosome; PAC, P1 artificial chromosome; EGFP, enhanced green fluorescent protein; Aqp4, aquoporin4; Apoe4, apolipoprotein E4; Blbp, brain lipid binding protein; Cx, Connexin; FGFR, fibroblast growth factor receptor; GFAP, glial fibrillary acidic protein; GLAST, glutamate-aspartate transporter, also known as EAAT1; EAAT, excitatory amino acid transporter; GLT1, glutamate transporter 1, also known as EAAT2; Kir, inwardly-rectifying potassium channel; CNP, 2′,3′-cyclic nucleotide 3′-phosphodiesterase; MBP, myelin basic protein; MOBP, myelin-associated oligodendrocyte basic protein; MAG, myelin-associated glycoprotein; MOG, myelin oligodendrocyte glycoprotein; PLP, proteolipid protein; NG2, neuron-glia antigen 2; PDGFRα, platelet derived growth factor receptor alpha; PDGF, platelet derived growth factor; bFGF, basic fibroblast growth factor.

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1. Introduction

Since their initial discovery, the perception of glia as being mere supportive "glue" for neurons has been transformed into an unexpectedly complex role as valuable partners in cell–cell communication and homeostasis. Diverse glial functions in neuronal conduction, synaptic development and repair have now overshadowed the previous concepts of glial cells as passive structural and, at best, trophic support for neurons. Aside from radial glia, ependymal cells, and cells of the neurovasculature, at least three major subtypes of glial cells: astrocytes, oligodendrocytes and microglia are present in the central nervous system (CNS). A fourth type, NG2 (nerve/glial antigen 2) cells or polydendrocytes, although widely recognized as oligodendrocyte progenitor cells (OPCs) in white matter (WM) structures, show expression patterns and electrical properties that defy conventional glial cell classification. Astrocytes are important regulators of neurotransmitter function, metabolic support, neuronal migration and development, synaptic processing, cell plasticity, excitotoxicity, immune function and blood-brain barrier integrity. Oligodendrocytes are myelin-producing cells of the CNS, and whose layers of compacted cell membranes ensheath axons with lipid-rich insulation that is critical for rapid salutatory conduction by axon fibers. In addition to myelination, oligodendroglia have been found to provide axons with direct metabolic support *via* lactate, a process that prevents axon degeneration and death (Lee et al., 2012). Both astrocytes and oligodendrocytes are described to develop from common progenitor populations. While many brain tumors consist predominantly of precursors of astrocytic nature, each of the individual glial cell types is now recognized as a viable therapeutic candidate in neurological disease, in addition to neurons. The growing importance of glial cells has led to the development of a variety of techniques for their purification and analysis. An important aspect of studying cellular function in the CNS lies in the ability to target individual cell populations, based on identity, developmental stage, and cellular lineage or fate. For their analysis *in vitro* and *in vivo*, specific glial populations may be collected by tissue isolation and culture methods, as well as through the use of genetic animal models. In separate sections of this paper, we will: (A) review culture techniques for the production of astrocytes and oligodendrocyte progenitor cells primarily from rodent brain, and stem cell-based approaches to generate these cells and (B) describe driver mouse strains for glial-specific cell labeling and gene manipulation. In the second section, we will also highlight new reporter and effector mouse strains for driver-assisted gene ablation, cell identification and *in vivo* molecular capture that are now available for gene expression analysis.

2. Cell purification and primary glial cell culture

Glial cells may be acutely isolated from dissociated brain and spinal cord tissue either as a mixed population, or with further purification *via* immunolabeling or reporter fluorescence in recombinant mouse strains. The first step of tissue dissociation consists of subjecting dissected tissue pieces to enzymatic digestion with papain and DNase I, followed by mechanical trituration using a series of needles of decreasing gauge size (*e.g.* 19, 21 then 23 G) (Belachew et al., 2002) and subsequent removal of aggregates by passing the suspension through a cell strainer (Belachew et al., 2002). For mature CNS white matter tissue with high myelin content, an additional purification step prior to cell selection is often beneficial to cell yield (Jiho Sohn, Univ California, Davis, personal communication) (Sohn et al., 2006). This involves layering the dissociated cell suspension onto a pre-formed density gradient of Percoll^{TM}, followed by high speed centrifugation, to separate neural cells from lipid-rich myelin, debris (Avellana-Adalid et al., 1996; Lubetzki et al., 1991) and blood cells. These purified cells, once cleared of Percoll^{TM}, may be maintained in culture (Zhang et al., 2004). Acutely isolated cells may also be selected by immunolabeling before collection by fluorescence-activated cell sorting (FACS) (Nielsen et al., 2006) (Fig. 1). Alternatively, cells from fluorescent reporter mouse strains may be directly collected by single-channel FACS (Belachew et al., 2002) or doubly selected by a combination of immunolabeling and dual-channel FACS collection (Belachew et al., 2003).

Despite the limitation that primary cultured cells in isolation are not morphological and functional duplicates of their *in vivo* counterparts, cell cultures still hold an important and special place in current methodologies. Indeed it was in cultures developed by McCarthy and de Vellis (1980) that astrocytes and oligodendrocyte progenitor cells (OPCs) were prepared from the neonatal rat and characterized in exhaustive detail, forming the foundation of current knowledge of glial cell characteristics, physiology and development. As summarized in Fig. 1 and Table 1, astrocytes and oligodendrocytes are frequently obtained by the establishment of mixed glial cultures from dissociated CNS tissue of neonatal rodents, isolation of their common progenitor by shaking (Levine, 1989; Saneto and de Vellis, 1985) and positive immune-selection – 'panning'-with monoclonal antibody A2B5 against the surface antigen (Stallcup and Beasley, 1987). With the advantages of sensitivity, ease and cost (relative to whole animal models), applications such as high throughput pharmacological analysis (James et al., 2011) rely on cultures for reasons of volume and scalability. Importantly, the establishment of co-cultures between neurons and astrocytes or oligodendrocytes (Jones et al., 2012; Kunze et al., 2013; Pang