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A comparison of cell transplantation and retroviral gene transfection as tools to study lineage and differentiation in the rat spinal cord

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Abstract

Establishing the cell lineage relationships of cells during development allows insight into when and where developmental decisions are made. In the developing spinal cord, the origin and fate of radial glial has yet to be determined. One way in which to address this question is to transplant enriched populations of radial glia into the ventricular zone (VZ) region of host embryos to examine the lineage and differentiation pattern of these cells. An indirect selection procedure using immunomagnetic beads (Dynabeads; Dynal Biotech) was used here to isolate spinal cord radial glia. This negative immunoselection procedure resulted in a high yield of radial glia. A fluorescent cytoplasmic dye (Cell Tracker Green CMFDA) was used to label radial glia before transplantation. The role of radial glia as progenitor cells can also be examined using a green fluorescent protein (GFP)-expressing retroviral vector. The retroviral vector allows dividing cells in the VZ region of the spinal cord to be tracked by labelling them with GFP. Both techniques were utilised here to successfully label and examine embryonic spinal cord radial glia in vivo after a microinjection of either fluorescently labelled radial glia or replication-incompetent GFP-expressing retrovirus in utero.

Keywords: Radial glia; Transplantation; Immunoselection; Retroviral vector; In utero injection; Lineage; Differentiation; Spinal cord

1. Introduction

During development, radial glial cells display a bipolar shape. Their processes span along the full width of the developing CNS. Radial glial cell bodies are mainly situated in the ventricular or subventricular zones. Their short processes anchor the cells in the ventricular zone and their elongated processes reach the pial surface (Rakic, 1995). In contrast to neuroepithelial cells, radial glia exhibit hallmarks of astrocytes, such as the expression of a number of molecules characteristic for astrocytes, for example, the glial fibrillary acidic protein (GFAP) in mammals (Levitt and Rakic, 1980; Choi, 1981). Although rodent and chick radial glia do not express GFAP, they do express brain lipid binding protein (BLBP) and astrocyte-specific glutamate transporter (GLAST), both of which are found in mature astrocytes (Hartfuss et al., 2001). However, radial glia share some molecular characteristics with neuroepithelial cells, such as the neural precursor cell marker nestin (Frederiksen and McKay, 1988). Thus, radial glia express molecules characteristic for both CNS precursors and astrocytes. Radial glia transform into astrocytes in most CNS regions of adult mammals (Chanas-Sacre et al., 2000). The cellular and molecular events that contribute to the transformation of radial glia into astrocytes are not known but the morphological transformation appears to coincide with a change in expression of intermediate filament protein in radial glia, i.e. loss of vimentin (Voigt, 1989) and nestin (Hartfuss et al., 2001; Barry and McDermott, 2005) antigen and acquisition of GFAP immunoreactivity.

Almost all radial glia proliferate throughout neurogenesis (Hartfuss et al., 2001). However, it was Alvarez-Buylla et al. (1990) who first suggested that mitotically active radial glia may give rise to neurons. In recent years, cell lineage analysis has shown that radial glia of the cerebral cortex generate neurons in vitro and in vivo (Malatesta et al., 2000; Miyata et al., 2001; Kriegstein and Gotz, 2003; Kriegstein and Noctor, 2004) and it now appears that these cells may be the sole precursors of the cortical ventricular zone (Noctor et al., 2002; Malatesta et al., 2003). It is unclear whether radial glia also function as progenitors for neurons in other CNS regions. Anthony et al. (2004) showed that the vast majority of neurons in all brain regions derive from radial glia. They showed that radial glia throughout the CNS act as neuronal progenitors and that radial glia within different regions of the CNS pass through their neurogenic stage of development at distinct time points. In the developing spinal

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cord, the lineage of radial glia has yet to be revealed. Barry and McDermott (2005) identified a population of radial cells that resemble radial glia in morphology but do not express radial glial markers. It has been suggested that these are multipotential cells, giving rise to the earliest appearing radial glial cells and also to neurons in the spinal cord.

Cell lineage relations can be revealed by a variety of methods, all of which involve marking progenitor cells and then following their fate. Here, we have used two techniques to study cell lineage of radial glia in the developing spinal cord. Firstly, we have used cell transplantation which is a powerful tool for studying cell fate determination during development. It allows the study of cells of known origin in various kinds of environments. To identify transplanted cells after grafting, individual cells are labelled in suspension with a fluorescent dye. In this paper, we transplanted an enriched population of radial glia, labelled with the fluorescent dye 5-chloromethylfluorescein diacetate (Cell Tracker Green CMFDA), into the developing embryo. This transplantation technique allows transplanted cells to be easily identified in the host microenvironment and allows their fate to be mapped. Here, radial glia are isolated from embryonic spinal cord using a negative immunoselection technique involving the neuronal cell surface marker Mab2F7 (Schubert and Kaprielian, 2001). Before transplantation, the radial glia are labelled with CMFDA, a thiol-reactive cytoplasmic dye that diffuses through the membranes of living cells (Baker et al., 1997). The cytoplasmic dye undergoes several thiol-dependent reactions, including a glutathione *S*-transferase-mediated reaction. The reaction product is membrane-impermeant and persists for several cell divisions.

The second technique we used to examine cell lineage of spinal cord radial glia involves a retroviral vector expressing green fluorescent protein (GFP). Retroviral vectors that are used for cell lineage analysis are modified so that they are replication-incompetent, and thus, cannot spread from one infected cell to another. Noctor et al. (2001) labelled radial clones consisting of radial glia by injecting retroviral vector expressing GFP into the lateral ventricles of E15 rat embryos. After infection, the retrovirus integrates into one daughter cell during the M phase of the cell cycle. We will show in this paper how retroviral vectors expressing GFP can be successfully used to label radial glia in the developing rat spinal cord.

Both of these techniques have proved successful in these experiments and will help unravel the question of what is the origin and fate of radial glia in the developing spinal cord.

2. Materials and methods

2.1. Animals

All experiments were carried out in accordance with national and local ethical committee guidelines. For isolation of an enriched population of radial glia, Wistar rats were obtained from the Biological Services Unit, University College Cork. Embryos at embryonic day 14 (E14) were used with day of conception designated as E0 and birth as postnatal day zero (P0). Injections of retroviral vector were carried out in the laboratory of Dr. Arnold Kriegstein at Columbia University, New York. The injections were carried out on E15 Sprague–Dawley rats (Taconic, NY). These animals were maintained according to protocols approved by the Institutional Animal Care and Use Committee at Columbia University.

2.2. Immunoselection and fluorescent labelling of radial glia

E14 spinal cords were treated with 0.1% trypsin–EDTA (Sigma) and triturated to form a single cell suspension. Cells were incubated with a neuronal cell surface antigen, Mouse IgM Mab2F7, a gift from Dr. Zaven Kapreilian, for 30 min at 4 °C. Cells were washed with buffer and incubated with magnetic dynabeads (Dynal Biotech) coated with rat anti-Mouse IgM for 20 min at 4 °C. After incubation with dynabeads, cells were exposed to a Dynal MPC-S magnet (Dynal Biotech) for 5 min at room temperature. All cells not bound to the dynabeads remained in suspension and were removed by pipetting. Some cells were plated before and after immunoselection to characterise cell phenotype using immunocytochemistry. Cells destined for transplantation were incubated with 20 μM Cell Tracker Green CMFDA (Molecular Probes) for 30 min at 37 °C and washed with DMEM.

2.3. Immunocytochemistry

Two hours after plating cells onto coated glass coverslips, cells were fixed with 4% paraformaldehyde. After blocking non-specific binding with 10 mM phosphate-buffered saline (PBS) containing 3% normal goat serum (Sigma), cells were incubated with the following primary antibodies: the neuronal marker Mouse IgM Mab2F7 (1:20, Dr. Zaven Kapreilian) and the radial glial marker Mouse IgG vimentin (1:100, Sigma) for 1 h. After washing off primary antibodies, cells were incubated with the appropriate secondary antibodies conjugated to fluorescein (1:50, Sigma) for 30 min. All cells were counterstained with bisbenzamide (Sigma) to identify cell nuclei. Five replicates were performed of this immunocytochemical procedure.

2.4. Retrovirus

Moloney murine leukemia virus (MoMuLV) expressing GFP was isolated from a stably transfected packaging cell line (293gp NIT-GFP; Burns et al., 1993; Palmer et al., 1999). This replication-incompetent GFP-expressing retrovirus had a final titre from 5×10^5 to 1×10^6 colony forming units/ml. Retroviral injections were carried out into E15 timed pregnant rats in a sterile biosafety level II hood.

2.5. In utero injections

Pregnant rats were anaesthetised by an intraperitoneal injection of 15 mg/kg ketamine and 3 mg/kg xylazine. A vertical midline incision was made in the lower abdomen and each uterine horn was exposed. A 1 μl injection of CMFDA-labelled radial glia (containing 1×10^5 cells) was injected into each telen-