ELSEVIER

Progress in Neurobiology 83 (2007) 53–67

progress in Neurobiology

www.elsevier.com/locate/pneurobio

Investigating radial glia in vitro

Steven M. Pollard [a,*], Luciano Conti [b,**]

[a] *Wellcome Trust Centre for Stem Cell Research, University of Cambridge,Tennis Court Road, Cambridge CB2 1QR, United Kingdom*
[b] *Department of Pharmacological Sciences and Centre for Stem Cell Research, University of Milano, Via Balzaretti 9, 20133 Milano, Italy*

Received 6 November 2006; received in revised form 25 January 2007; accepted 20 February 2007

Abstract

During mammalian neurogenesis newly born neurons migrate radially along the extended bipolar process of cells termed radial glia. Our views of radial glia as a 'static' support/guide cell have changed over recent years. It is now clear that within the developing cortex, and possibly the entire central nervous system (CNS), radial glia actively divide, producing daughter cells that include both neurons and glia. A subset of forebrain radial glia may serve as the founders of adult forebrain neural stem cells and genetic disruption of normal radial glia function can result in tumorigenesis or congenital neurological disorders. Elucidating the cell intrinsic and environmental cues that regulate radial glia behaviour is therefore essential for a full understanding of mammalian CNS development and physiology.

Here, we review those studies in which radial glia have been investigated in vitro following isolation from foetal tissues or differentiation of embryonic stem (ES) cells. We discuss how these approaches, together with an ability to expand radial glia-like neural stem (NS) cell lines, may offer unique opportunities in basic and applied neurobiology.

Keywords: Radial glia; Neural stem cells; In vitro cell culture; Embryonic stem cells; EGF and FGF-2; Neurogenesis

Contents

Abbreviations: BLBP, brain lipid-binding protein; BMP, bone morphogenetic protein; CNS, central nervous system; DRG, dorsal root ganglia; EB, embryoid body; EC cells, embryonic carcinoma cells; EGF, epidermal growth factor; ES cells, embryonic stem cells; FACS, fluorescence-activated cell sorting; FGF, fibroblast growth factor; GABA, gamma-aminobutyric acid; GFAP, glial fibrillary acid protein; GFP, green fluorescent protein; GLAST, astrocyte-specific glutamate transporter; HD, Huntington's disease; ICM, inner cell mass; LIF, leukemia inhibitor factor; NEP, neuroepithelial progenitor; PDGFRα, platelet derived growth factor receptor α; PNS, peripheral nervous system; RA, retinoic acid; RBP-1, retinol binding protein 1; Shh, sonic hedgehog; SVZ, sub ventricular zone

* Corresponding author. Tel.: +44 1223 760281.
** Corresponding author. Tel.: +39 02 5031 8403; fax: +39 02 5031 8284.
E-mail addresses: smp54@cam.ac.uk (S.M. Pollard), Luciano.Conti@unimi.it (L. Conti).

0301-0082/$ – see front matter
doi:10.1016/j.pneurobio.2007.02.008

1. Overview

Understanding how the mammalian brain is generated is one of the most exciting and daunting challenges facing scientists. Although extraordinarily complex, both in structure and function, scientists have made progress in uncovering some of the cellular changes and underlying biochemical mechanisms responsible for the construction of this organ (Jessell and Sanes, 2000). Studies in developmental biology using various model organisms, as well as recently completed genome sequencing projects, have revealed a remarkable functional and genetic conservation of transcriptional regulators and signalling pathways across the animal kingdom. We are therefore faced with the task of understanding how interactions between these intrinsic and extrinsic signals can co-ordinately regulate cell behaviour within the developing mammalian CNS such that the correct numbers and types of mature cells are generated at the right place and right time.

Radial glia are highly abundant within the developing CNS. They have a dual function as a support for migrating neurons and as a progenitor population. In the following sections, we outline the key properties of radial glia and discuss studies in which they have been cultured in vitro. More detailed accounts of radial glia function in vivo can be found elsewhere, including this issue (Campbell and Gotz, 2002; Chanas-Sacre et al., 2000; Ever and Gaiano, 2005; Gotz, 2003; Hevner, 2006; Morest and Silver, 2003). In later sections, we discuss recent work, including our own, which suggests that cells with similarities to radial glia can be isolated from foetal tissues, or generated from embryonic stem cells, and then subsequently maintained indefinitely as tissue specific neural stem cell lines (Bibel et al., 2004; Conti et al., 2005; Liour and Yu, 2003; Pollard et al., 2006c).

2. Sources of radial glia

In order to isolate radial glia in vitro it is first necessary to identify those tissues from which they could be derived. As mammalian radial glia are a transient foetal cell type, it might seem clear that the developing foetal CNS would be the only tissue from which these cells could be isolated. However, there is also evidence that cells with similarities to radial glia can be generated in adult tissues through activation or reprogramming events that occur following injury and disease or within the cell culture environment. Also, the ability to differentiate embryonic stem cells to neural lineages provides an alternative way to generate radial glia in unlimited numbers in vitro. We discuss each of these potential sources below.

2.1. Foetal central nervous system

At early developmental stages in mammals a population of cells becomes specified to the neural lineage. The molecular basis of this neural induction has been studied extensively in many vertebrate model organisms and seems largely conserved across phyla. Induction of the neuroectoderm is promoted through antagonism of the bone morphogenetic protein (BMP) signalling pathway and in some species pro-neural fibroblast growth factor (FGF) signals (reviewed in: (Munoz-Sanjuan and Brivanlou, 2002; Stern, 2005; Weinstein and Hemmati-Brivanlou, 1999). All neural cells of the mature CNS are descended, either directly or indirectly, from this neuroepithelial cell population. Here, we refer to these cells as progenitors rather than stem cells as continuous self-renewal has not been demonstrated for this population.

Neuroepithelial progenitors (NEPs) undergo interkinetic nuclear migration, a process in which the nucleus oscillates between the apical and basal surfaces co-ordinately with cell cycle progression, leading to the formation of a pseudostratified epithelium (Sauer, 1935). The earliest known molecular marker specifically expressed within this population is the transcription factor Sox1 (Pevny et al., 1998). The related and functionally redundant proteins Sox2 and Sox3 are also expressed in these cells, but have broader roles in the development of non-neural tissues such as the epiblast and extraembryonic ectoderm (Avilion et al., 2003; Wood and Episkopou, 1999). The NEPs comprise the neural plate, which undergoes a striking morphogenetic movement that results in formation of the neural tube. Concurrent with these events, signalling molecules secreted by adjacent tissues, such as somite-derived retinoic acid (RA), BMP signals from overlying ectoderm, and notochord-derived sonic hedgehog (Shh), act on the neuroepithelium. These signals lead to activation of various classes of transcription factors, which together convey a positional 'code' establishing sub-regions with the CNS and specifying cells as distinct neuronal and glial subtypes (Briscoe and Ericson, 2001).

It is at this point, approximately embryonic day 9.5–10.5 in mouse, and concurrent with the onset of neurogenesis, that a second morphologically and antigenically distinct cell type, termed radial glia, arises in the neuroepithelial tissue (Gotz and Huttner, 2005; Misson et al., 1988). Radial glia were originally named epithelial cells, spongioblasts, radial cells, or fetal ependymal cells, by investigators around the late 19th century (reviewed in: (Bentivoglio and Mazzarello, 1999; Rakic, 2003). These cells have a bipolar morphology, with one extension and broad endfoot sited at the luminal surface and a longer process extending in the opposite direction through to the basement membrane adjacent to the pia mater. Similar to NEPs, they also exhibit an ovoid cell body and have a nucleus situated in the ventricular zone, adjacent to the lumen that undergoes interkinetic nuclear migration. Ultrastructural studies performed using electron microscopy revealed that radial glia function as a substrate/guide upon which newly generated immature neurons migrate (Rakic, 1971a,b). Radial glia display