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Radial Glial Cells: Defined and Major Intermediates between Embryonic Stem Cells and CNS Neurons

Minireview

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Radial glial cells have been identified as a major source of neurons during development. Here, we review the evidence for the distinct "glial" nature of radial glial cells and contrast these cells with their progenitors, the neuroepithelial cells. Recent results also suggest that not only during neurogenesis in vivo, but also during the differentiation of cultured embryonic stem cells toward neurons, progenitors with clear glial antigenic characteristics act as cellular intermediates.

Radial Glia—Definition

Nomen est omen—radial glial cells were, and are still, defined as cells with a radial morphology and glial characteristics. Like neuroepithelial cells, they stretch from the apical surface—the ventricular lumen—to the basement membrane at the pial surface. As both cell types are then "radial" (Figure 1), the defining and discriminating criterion between these two cell types is the "glial" adjective. Indeed, radial glial cells have cellular and molecular characteristics of astroglia, one of the two major macroglial cell types in the adult brain. Radial glial cells and astroglia contain glycogen granules and other ultrastructural characteristics of astrocytes, in contrast to neuroepithelial cells (for recent reviews and references to the corresponding original publications, see Mori et al., 2005). Furthermore, radial glial cells also express the astrocyte-specific glutamate transporter GLAST, S100β, glutamine synthase (GS), vimentin, and tenascin-C (TN-C), and, in certain species, GFAP (Mori et al., 2005). These molecules are all absent in neuroepithelial cells, but present in mature (GLAST, S100β, GS) or reactive astrocytes (S100β, GS, GFAP, vimentin, TN-C). Importantly, the so-called type B cells, which are astrocytes acting as neural stem cells in the adult subependymal zone, also express the same set of molecules (GLAST, S100β, GS, GFAP, vimentin, TN-C; Alvarez-Buylla et al., 2001; Mori et al., 2005). As a matter of fact, no molecule has been identified so far that would discriminate across species between astrocytes and radial glial cells. Thus, these cells are truly distinct from neuroepithelial cells and are as "astrocytic" as can be assessed by molecular and ultrastructural criteria.

Neuroepithelial and radial glial cells not only both span the entire epithelium, but also are both characterized by interkinetic nuclear migration, by tight or adherens junction coupling, and by a clear apico-basal polarity (for review, see Götz and Huttner, 2005). Both also express the intermediate filament nestin and its posttranslational modification(s) labeled by the RC1 and RC2 antibodies (Mori et al., 2005). Notably, these molecules are also reexpressed by reactive astrocytes, such that the molecular features shared between neuroepithelial cells and radial glia are also mostly shared with neural stem cells and reactive astrocytes. These observations suggest that these markers would allow one to distinguish astrocytes that act as precursors from temporarily or permanently quiescent astrocytes.

Radial Glia—When Do They Appear?

Early studies used RC1/RC2 immunolabeling as the defining criteria of radial glia and placed the transition in the mouse at around E9/E10 (Misson et al., 1988). This is the time when nestin expression can be first detected, consistent with the notion that RC2 recognizes a nestin-linked epitope (see references in Mori et al., 2005). This is also the stage when coupling through tight junction is altered (see references in Götz and Huttner, 2005) and starts to depend on Notch signaling (Hatakeyama et al., 2004). Thus, the emergence of nestin expression correlates with cell biological alterations, even though no specific "glial" features are yet detectable at this stage. Given the nestin immunoreactivity, but the lack of astroglial features of these cells, these cells should be referred to as "neuroepithelial cells," while cells in the previous stage (lacking nestin expression) can be considered as being more similar to "epithelial cells."

Just exactly when radial glial cells begin to express specific markers is also of importance in view of recent and discrepant results obtained using promoters driving the expression of Cre to perform in vivo cell lineage analysis. In particular, while BLBP only becomes immunodetectable around E12 in the ventral telencephalon (Anthony et al., 2004; Hartfuss et al., 2001), its mRNA can already be detected at E10, at a time when neuroepithelial cells are nestin positive, but no radial glial cells can be identified (see below). This is likely to be the explanation for the results of Anthony et al. (2004) indicating that CNS neurons are labeled throughout the CNS when the BLBP promoter is used to drive the expression of Cre (used to remove a stop cassette preventing the expression of a reporter gene potentially expressed in all cells). This result is similar to that obtained using Cre lines driven by the nestin promoter (Backman et al., 2005), while the use of the human GFAP promoter to drive Cre expression only labels a subset of CNS neurons (see below; Malatesta et al., 2003).

As can be expected from other developmental gradi-

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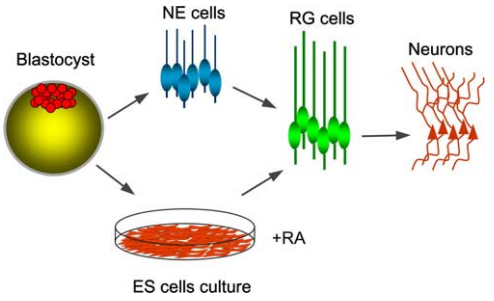

Figure 1. Neurogenesis and the Generation of Radial Glial Cells

ents, the astroglial characteristics of radial glial cells also develop in a ventral to dorsal and lateral to medial gradient, with BLBP being amongst the earliest, followed by GLAST, vimentin, TN-C, and eventually S100β and GS (Mori et al., 2005). Most of these glial features start to appear at E12 in the developing mouse telencephalon, and at E14 the vast majority of ventricular zone cells have glycogen granules, GLAST, TN-C, and vimentin, and subsets have BLBP, S100β, and GS (Malatesta et al., 2000; Malatesta et al., 2003; Hartfuss et al., 2001; Mori et al., 2005). Thus, in most brain regions in the mouse, radial glial cells constitute the majority of progenitors by E13/E14, while virtually no radial glial cells are present at E10/E11. The radial glia is then present until the end of neurogenesis and neuronal migration, when their remainder transforms into astrocytes (Noctor et al., 2004; for review, see Mori et al., 2005). This morphological transformation correlates with molecular changes such as the loss of expression of key neurogenic factors (e.g., Pax6; see below) and remains partially reversible with regard to morphology (Hunter and Hatten, 1995) and cell fate (Heins et al., 2002).

Three Ways of Neurogenesis

In the CNS, neurons are generated from different types of progenitors. Before the appearance of radial glial cells, neurons are generated from *neuroepithelial cells* as well as from the earliest *basal progenitors* (Haubensak et al., 2004; Miyata et al., 2004; Noctor et al., 2004). The latter are defined by the absence of ventricular (apical) contact and their cell division at significant distances from the ventricle at further basal positions. Basal progenitors are molecularly distinct from the apical-located neuroepithelial and radial glial cells, e.g., by the expression of Tbr2 (Englund et al., 2005) and Ngn2 (Miyata et al., 2004) and the lack of GLAST or Pax6 (Malatesta et al., 2003; Haubst et al., 2004). At latter developmental stages (E13 in the ventral and E15 in the dorsal telencephalon of the mouse following the ventro-dorsal and latero-medial gradient), the basal progenitors become frequent enough to form a visible secondary progenitor layer, the so-called subventricular zone (SVZ), located on top of the precursors lining the ventricle arranged in the ventricular zone (for review, see Götz and Huttner, 2005). At these latter stages, basal progenitors also acquire EGF receptor expression that some of them distribute asymmetrically in correlation with the generation of astrocytes or oligodendrocytes, respectively (Sun et al., 2005). Thus, there are important differences between the early embryonic SVZ that is mostly neurogenic and the late embryonic/early postnatal SVZ that is mostly gliogenic. In this regard, it is also important to realize that the adult SVZ is actually a late derivative of radial glial cells (Merkle et al., 2005) rather than of the embryonic SVZ. It may therefore be more appropriate to refer to the adult region containing neural stem cells as the subependymal zone, as the lining of the ventricle at postnatal and adult ages is covered by the ependyma.

Both basal progenitors in the early SVZ and radial glial cells arise from the earlier neuroepithelial cells (Haubensak et al., 2004; Figure 1). At midneurogenesis, *radial glial cells* constitute the majority of ventricular zone progenitors in most CNS regions (Hartfuss et al., 2001; references in Mori et al., 2005). It is now clear that they generate neurons in many, but distinct CNS areas, and especially in those where the population of basal progenitors is small. This is in fact the case throughout the developing CNS of the mouse, with the exception of the ventral telencephalon, where basal progenitors constitute more than half of all dividing cells at midneurogenesis. In the dorsal telencephalon, basal progenitors rise to a sizable proportion only at late stages of development but never exceed 25% of all progenitors (Haubst et al., 2004; Miyata et al., 2004). Thus, by virtue of their dual nature as precursors and radial structures, radial glial cells combine several key functions in development, acting as (neuronal) precursors, boundary and patterning structures, and guides for migrating neurons. The key finding that radial glia do not withdraw their radial process during cell division (Miyata et al., 2001) indicates how precursor and guidance function can be combined if the radial glial cell inherits the radial process (for review, see Fishell and Kriegstein, 2003). Alternatively, the neuronal daughter cell may inherit the radial process and use it for somal translocation (for review, see Fishell and Kriegstein, 2003; Mori et al., 2005).

A key factor in the specification of neurogenic radial glial cells, the transcription factor Pax6, simultaneously suppresses the number of basal progenitors in the developing cerebral cortex (Heins et al., 2002; Haubst et al., 2004). When Pax6 is not functional, as is the case in the *small eye* mutant, radial glial cells in the dorsal telencephalon fail to generate neurons, the remaining neurons being derived from the increased population of basal progenitor cells in the Pax6 mutant cortex or by migration from ventral sources (Heins et al., 2002; Haubst et al., 2004). Thus, in the absence of Pax6 function neurogenesis in the cerebral cortex switches its mode—from a *predominantly radial glia-based neurogenesis* to a reduced but *predominantly basal progenitor-mediated neurogenesis*. Notably, the potent neurogenic role of Pax6 in glial cells is not restricted to the developing cerebral cortex. Pax6 is sufficient to instruct neurogenesis in nonneurogenic postnatal astrocytes (Heins et al., 2002) and drive almost all cells from adult neurospheres toward neurogenesis (Hack et al., 2004). Very recent data demonstrate its important role in adult olfactory bulb neurogenesis in vivo, where Pax6 proved to be necessary and sufficient for adult neurogenesis (Hack et al., 2005). Interestingly, Pax6 is then maintained only in a subset of newly generated neurons

specifying dopaminergic interneurons in the glomerular layer (Hack et al., 2005). Thus, Pax6 is also required in adult neurogenesis in the olfactory bulb both for the progression of multipotent progenitors toward neuronal progenitors as well as for the specification of neuronal subtypes. Thus, the similarity of radial glial cells, adult neural stem cells, and other astrocytes is not restricted to the expression of convenient markers, but it is also evident from the expression and use of common cell fate determinants.

Functional Differences between Radial Glial Cells in Different Brain Regions

While the situation with the ventral telencephalon is unique with regard to its large number of basal progenitors reaching the majority of all progenitors during neurogenesis, it is important to realize that radial glial cells are also localized in this area. In contrast to the dorsal telencephalon, these cells are Pax6 negative (Heins et al., 2002). Most importantly, they are functionally distinct in terms of the lineages to which they give rise. This was perhaps most clearly indicated by in vivo Cre-based fate mapping and in vitro cell sorting experiments (Malatesta et al., 2003). The derivatives of radial glial cells expressing the recombinase Cre under the control of the human GFAP promoter (that drives Cre in the GLAST-immunopositive radial glial cells at E13/E14, i.e., the fully differentiated radial glia stage; Malatesta et al., 2000; Malatesta et al., 2003) differ in the dorsal and the ventral telencephalon. In the dorsal telencephalon, almost all neurons are labeled, but very few are in the ventral telencephalon, while almost all oligodendrocytes are labeled. While the vast majority of Cre-expressing cells in this line have been identified as GLAST-positive radial glia, smaller populations of neuroepithelial cells persisting until later stages, or other minor cell populations, can not be excluded in this fate mapping analysis. In agreement with the in vivo data, radial glial cells isolated at midneurogenesis from the ventral telencephalon (using mice expressing GFP under the human GFAP promoter) hardly generate any neurons, in marked contrast with radial glial cells from the dorsal telencephalon (Malatesta et al., 2003; see also Malatesta et al., 2000). These results were also confirmed by the sorting of radial glial cells from the ventral telencephalon at midneurogenesis on the basis of GFP under the control of the BLBP promoter elements (Anthony et al., 2004) that also generate significantly fewer neurons than those isolated from the dorsal telencephalon at the same stage. Thus, both sets of results indicate that at the peak of neurogenesis (around E14) radial glial cells located in the ventral telencephalon are far less neurogenic compared with radial glial cells of the dorsal telencephalon. These results are further consistent with observations using time-lapse video microscopy, indicating that cortical radial glial cells generate postmitotic neurons directly (Noctor et al., 2004), while radial glial cells in the ventral telencephalon rarely do so (A. Kriegstein, personal communication).

By implication, these results also suggest that most neurons in the basal ganglia are derived from basal progenitors, though it remains to be determined if these cells self-renew their own pool. If this were the case, radial glial cells and basal progenitors should be considered as separate progenitor pools, even though both are derived from neuroepithelial cells. Alternatively or in addition, some early-born radial glial cells may also generate basal progenitors in the ventral telencephalon and thereby indirectly contribute to neuronal sublineages in the ventral telencephalon (Malatesta et al., 2003). In sum then, these in vitro and in vivo results show that, in the developing telencephalon, there are significant functional differences between dorsal and ventral radial glial cells. They also indicate that these cells represent a progenitor pool that is more restricted in its developmental potential than the earlier neuroepithelial cells that do not exhibit these differences in cell fate.

Generation of Specific Radial Glial Cells from Embryonic Stem Cells

The results recapitulated above suggest that the generation of radial glial cells may require a complex series of developmental events, including the generation of a polarized neuroepithelium. In view of this, it is not immediately obvious that a procedure consisting essentially of the addition of retinoic acid to rapidly dividing embryonic stem (ES) cells should lead to the generation of a homogenous population of radial glia cells (Bibel et al., 2004; Figure 1). But such appears to be the case when ES cells are differentiated toward the neuronal lineage (Figure 1), and not only do these cells express the expected radial glial cell markers, but they also all express Pax6, suggesting that they may represent a subtype of neurogenic radial glial cells, as found, for example, in the developing cortex or in the spinal cord. In agreement with in vivo fate map studies, these cells also go on to differentiate into neurons in vitro, and both marker and electrophysiological properties indicate that most of these neurons have characteristics of pyramidal neurons, as found, for example, in the cerebral cortex and the hippocampus (Bibel et al., 2004). That neuronal differentiation of ES cells may occur generally via a radial glial cell state is further suggested by the observation that an ES cell differentiation protocol that differs in major aspects (e.g., no retinoic acid treatment, monolayer differentiation; Ying et al., 2003) from that described by Bibel et al. also leads to the generation of Pax6-positive radial glial cells (S. Pollard and A. Smith, personal communication). Thus, the transition from a pluripotent ES or neuroepithelial cell state toward neurons via an intermediate radial glial cell state may be a common step during the course of neuronal differentiation. Interference with signaling of a major mitogen such as Wnt may be sufficient to induce this shift from a pluripotent (ES cell) to a restricted progenitor (radial glial) state. Indeed, it has been shown that the action of retinoic acid on dividing ES cells can be mimicked by the addition of the frizzled receptor antagonist Sfrp-2, and that this type of ligand is found in the developing cortex (Aubert et al., 2002; Haubst et al., 2004).

The in vitro generation of defined and homogenous CNS progenitors raises the question of their differentiation potential. This was approached by implanting these (marked) ES cell-derived Pax6-positive progenitors in the developing chick embryo (Plachta et al., 2004). Large numbers of (mouse) neurons develop throughout the chick spinal cord. However, when their

identity was assessed by using various markers, only neurons expected to be generated by progenitors expressing Pax6 could be identified, such as, for example, motoneurons in the ventral portion of the spinal cord, as well as interneurons (Plachta et al., 2004). Some of the implanted cells escaped the neural tube, apparently following some of the pathways used by neural crest cells, and colonized adjacent dorsal root ganglia (DRG). However, none of the characteristics expected for sensory neurons, not even axonal elongation, was observed, while undifferentiated control ES cells did display them (Plachta et al., 2004).

It thus appears that ES cell-derived, Pax6-positive radial glial cells are developmentally restricted and cannot readily revert to a more primitive type of progenitor, such as neuroepithelial cells. These cells seem then to behave as other tissue or somatic cells that are characterized by their ability to give rise to a defined progeny. In future experiments, it will be important to test if this conclusion can be extended to other defined neural progenitors. Indeed, the often-discussed possibility to use embryonic or somatic stem cells to repair the damaged CNS critically depends on a better understanding of the developmental potential of defined progenitors.

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