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Radial 'glial' progenitors: neurogenesis and signaling

Leah Ever and Nicholas Gaiano

Cells with radial morphology in the developing brain were first identified more than 100 years ago. These cells, later termed radial glia, have been studied primarily as migratory scaffolds and glial progenitors. However, it has become increasingly clear, on the basis of *in vitro* studies and more recent *in vivo* fate mapping experiments, that radial glia also generate neurons during embryonic development. Now the challenge will be to understand the signaling events that regulate the spatial and temporal heterogeneity of these cells and their developmental potential. Recent work has identified the Notch, ErbB, and fibroblast growth factor signaling pathways as central to the regulation of radial 'glial' progenitors.

Addresses

Institute for Cell Engineering, Department of Neurology, Johns Hopkins University School of Medicine, Baltimore, Maryland 21205, USA

Corresponding author: Gaiano, Nicholas (gaiano@jhmi.edu)

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Introduction

One of the most exciting recent advances in our understanding of neural development has been the realization that radial glia (RG) can be neurogenic progenitors [1–4]. Although previous work had suggested that RG might generate neurons [5–7], this view began to develop momentum only after studies showed that isolated RG could generate neurons *in vitro* [8], and that RG could generate neurons in slice culture [9]. Subsequently, several lines of evidence have suggested that most or all ventricular zone (VZ) progenitors have the characteristics of what have traditionally been termed 'radial glia'.

The initial assignment of 'glial' character to radial glial cells was a consequence of studies in primates, in which it was demonstrated that RG acquire many astroglial characteristics including the expression of glial fibrillary acidic protein (GFAP) and the presence of glycogen granules [10,11]. In rodents, these definitive characteristics are not present until late in neurogenesis, although these cells do express some astroglial markers. In light of this

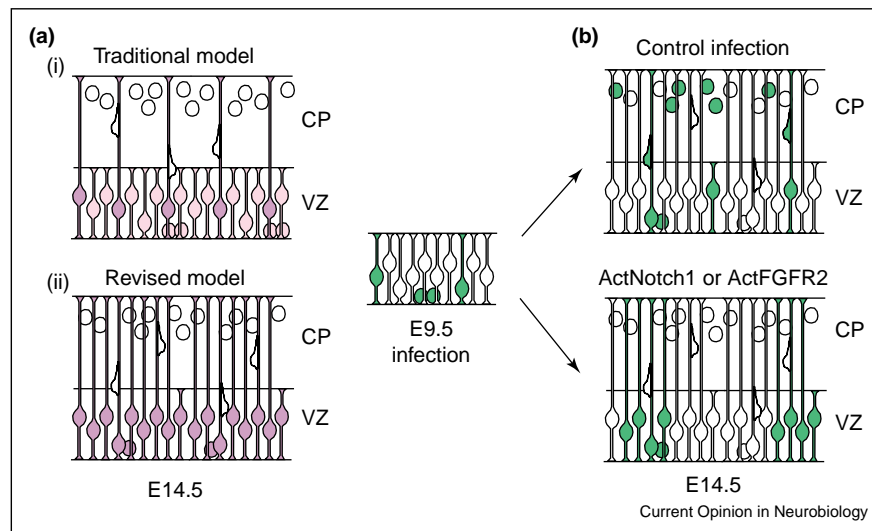
ambiguity, use of the term 'radial glia' should be revisited, as others have also suggested [1,12]. It might be more appropriate for neurogenic and potentially multipotent 'radial glia' to be called 'radial glial progenitors', or even simply 'radial progenitors'. The term 'radial progenitors' reflects the morphology and behavior of these cells, and sets aside the confusing and sometimes semantic question as to whether they should be labeled glia. Regardless of what they are called, the realization that radial glial progenitors are neurogenic has prompted a flurry of new studies to consider the lineage and potential of these cells, in addition to the signaling cascades that regulate them.

Are most ventricular zone cells radial 'glial' progenitors?

Although the neurogenic capacity of radial glial cells is becoming widely accepted, the relationship between these cells and the progenitor pool at large is less clear. The conventional wisdom has been that during neurogenesis in the brain, the VZ contains two primary cell types: neuroepithelial progenitors (NEPs) and RG (Figure 1a). These cell types were thought to differ morphologically, in that NEPs lost contact with the pial surface as development proceeded, whereas RG retained pial contact. Furthermore, NEPs were considered to be the primary source of neurons, whereas RG were considered to be support cells used by newborn neurons for migration out of the VZ. It was generally assumed that NEPs were similar to progenitors that were present before neurogenesis, and that RG were specialized cells that actively retained contact with the pial surface.

In recent years, the traditional view of the VZ pool has been challenged, and several lines of evidence have suggested that most or all VZ progenitors possess RG characteristics (Figure 1a). For example, double labeling experiments have revealed that nearly all neocortical VZ cells express the canonical RG marker RC2 [13]. It should be noted, however, that although RC2 is certainly expressed in RG, its expression in VZ cells that might lack RG morphology (i.e. NEPs) has not been ruled out. Thus, although RC2 expression supports assigning RG identity to a given cell, its expression alone does not definitively demonstrate such identity. More compelling evidence that most VZ cells have radial glial morphology has come from retroviral and lipophilic dye labeling studies [12]. For example, 24 h after infection with a retrovirus expressing green fluorescent protein (GFP), 96% of the infected cells in the VZ had RG morphology. Because murine retroviruses only infect dividing cells, this finding suggests that most proliferatively active cells in the VZ are RG. Furthermore, dye labeling of VZ cells in neocortical explants and slice cultures revealed that most

Figure 1



Radial glia as the predominant ventricular zone (VZ) progenitor cell type. **(ai)** The traditional view was that RG extended processes to the pial surface, whereas neuroepithelial progenitors were confined to the VZ. **(a ii)** Recent data have suggested instead that most cells in the VZ have radial glial morphology. **(b)** Cells infected with activated Notch1 (ActNotch1) or activated FGFR2 (ActFGFR2) maintain radial glial morphology during neurogenesis, whereas control virus-infected cells disperse to the cortical plate (CP). Based upon evidence that most of the VZ is comprised of radial glial progenitors, these data suggest that while promoting radial glial characteristics, Notch and FGF signaling are also maintaining the progenitor pool.

VZ cells contacting the ventricular surface (and thus not likely to be newborn neurons) were of RG morphology.

The emerging view that most VZ progenitors have RG characteristics has important implications with regard to neural development. For example, this view suggests that, at least in certain parts of the developing CNS, most or all neurons are derived from RG progenitors. *In vivo* fate mapping studies have recently addressed this issue, as is discussed below. In addition, if most VZ progenitors possess radial processes that extend to the pial surface, these processes could mediate previously unanticipated signaling interactions between the postmitotic areas and the progenitor pool at large. Furthermore, it will now be important for *in vitro* studies of embryonic neural progenitors to take into account that *in vivo* those cells, as RG, might possess characteristics more complex than those previously ascribed to undifferentiated progenitors.

In vivo fate mapping of radial glial progenitors

As mentioned above, recent *in vitro* studies have provided evidence that radial progenitors can generate neurons. More recently, the neurogenic capacity of radial progenitors has been examined *in vivo* by two studies employing Cre-loxP mediated fate mapping. These studies have used mouse lines expressing the Cre-recombinase driven by either the human glial fibrillary acidic protein (hGFAP) promoter [14••] or the mouse brain lipid binding protein (BLBP) promoter [15••]. Crossing these lines

with the R26R reporter strain results in β -gal expression in Cre-expressing cells and their descendents. Thus, cells that are β -gal+ are presumed to have arisen from a lineage that expressed the Cre-recombinase at some point.

It is crucial in interpreting any Cre-mediated *in vivo* fate mapping to understand when and where Cre is expressed. The BLBP promoter fragment used was found to drive expression in the mouse forebrain as early as embryonic day 10.5 (E10.5) [15••]. This fragment exhibits widespread expression in cells that also express the progenitor marker Nestin, and the glutamate transporter GLAST (glutamate-aspartate transporter). The authors of this study argue that expression from this promoter fragment is "confined to radial glia" and is expressed "in all radial glia" [15••]. However, these conclusions are centered on defining RG as a cell type that differs from NEPs and that can be definitively identified by marker expression, assertions that are somewhat problematic, as mentioned above. Regardless of this, the study does demonstrate that cells expressing *blbp*, a gene expressed in radial progenitors, generate large numbers of neurons during development in both the dorsal and the ventral telencephalon, and at other positions along the neuraxis.

Another study designed to fate map the progeny of RG used the hGFAP promoter [14••]. Although GFAP is not expressed in RG in rodents, it is expressed in RG in primates. Remarkably, the hGFAP promoter drives expression in murine RG during neurogenesis, suggesting

that although *cis*-acting elements have been lost in the mouse GFAP promoter, *trans*-acting signals of glial character are present. Consistent with the study described above using BLBP-Cre, neocortical radial glial progenitors that expressed Cre from the hGFAP promoter gave rise to large numbers of neurons during development [14^{••}]. However, in the ventral telencephalon few neurons were generated. The authors interpreted this result to indicate that there are fundamental differences between dorsal and ventral RG, and that ventral RG are not neurogenic. At first glance, this result appears to be at odds with the BLBP-Cre fate mapping data.

The primary difference between the two studies is that the BLBP-Cre is expressed much earlier in development than the hGFAP-Cre. This is especially true ventrally, where hGFAP does not drive expression before E14.5, a time when much of ventral telencephalic development has already taken place. That said, several conclusions can be drawn. First, from early in telencephalic development cells with some radial glial progenitor characteristics (as marked by BLBP), give rise to neurons both dorsally and ventrally. Whether these cells do so directly or through the generation of secondary progenitor pools is unknown. Second, later in development, those RG in the ventral telencephalon that express Cre from the hGFAP promoter do not generate neurons. It should be noted, however, that a neurogenic subset of RG might not express Cre from the hGFAP promoter, and would have been overlooked by this approach. Nevertheless, this *in vivo* fate mapping work clearly indicates that cells with radial glial characteristics generate neurons *in vivo*. Furthermore, these studies suggest that maturation of radial glial progenitors is accompanied by changes in developmental potential.

Signaling in radial progenitors

Although much progress has been made towards understanding the role of radial glial progenitors during development, we currently have a limited understanding of the signaling cascades that regulate these cells. Below, the roles of the Notch, ErbB and fibroblast growth factor (FGF) signaling pathways in radial glial progenitors are discussed.

Notch signaling

In the developing nervous system, the Notch signaling pathway has traditionally been thought to maintain a progenitor state. Interestingly, recent *in vivo* data have shown that during embryogenesis Notch1 activation promotes radial glial morphology and upregulates the RG marker BLBP [16]. Considered together with the emerging view that BLBP-expressing RG can be neurogenic progenitors (see above), this finding suggests that Notch maintains neural progenitors in the form of RG (Figure 1b; [17]). Direct evidence in support of this notion has come from recent work demonstrating that

Notch1 activation not only promotes RG characteristics *in vivo* but also promotes progenitor characteristics as assayed *in vitro* [18^{••}].

So how is Notch activated to maintain radial glial progenitor characteristics *in vivo*? It has been suggested that newly generated postmitotic neurons expressing increased levels of Notch ligands migrate along the radial processes, thereby activating Notch and maintaining the RG state [16,19^{••}]. Indeed, gene expression analysis in the mouse telencephalon has demonstrated that cells expressing the Notch ligand Delta1 do not express progenitor markers and are in close contact with radial glial processes [20]. The activation of Notch by newly generated neurons would ensure that the radial glial scaffold remains intact for ongoing neuronal migration. Concomitantly, the activation of Notch in this manner would maintain the radial progenitor pool for subsequent waves of neuron production.

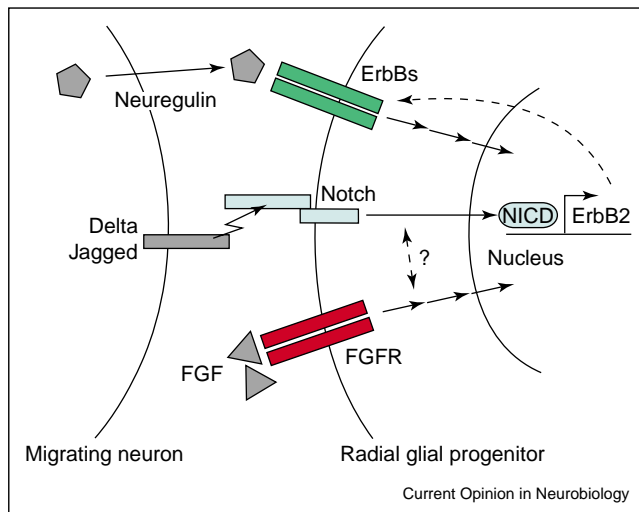
It should be noted that depending upon the developmental context, the promotion of RG characteristics by Notch might not be coupled to progenitor identity. For example, in the cerebellum, Bergman radial glia (BRG) express Notch1, whereas the granule cells that migrate along the BRG fibers express the ligand Jagged1 [19^{••},21]. Unlike the radial glial progenitors present during the bulk of neurogenesis in the telencephalon, the RG present in the developing cerebellum during granule cell migration do not appear to be progenitors, and instead are thought to provide primarily a supportive 'glial' role.

Currently not much is known about the intracellular mechanisms by which Notch promotes radial glial characteristics. It will be interesting to determine the role of the conventional Notch signaling cascade, mediated by C-promoter binding factor 1 (CBF1) and the *HES* genes, and the role of non-conventional targets. With respect to non-conventional targets, two recent studies have suggested that at least part of the mechanism by which Notch regulates radial glial cells is by upregulating the receptor tyrosine kinase (RTK) ErbB2 [19^{••},22^{••}]. In addition, as discussed below, Notch might interact with signaling through another family of RTKs, the FGF receptors (FGFRs), although the molecular nature of that interaction is unclear.

Neuregulin-ErbB signaling

The first evidence that ErbB signaling promoted RG identity suggested that migrating neurons expressing the ErbB ligand Neuregulin (NRG) signal through ErbB receptors to maintain RG character [23]. Subsequent work has examined the effects of NRG deletion in cortical explants and found reduced RG marker (RC2) expression, which could be rescued by addition of exogenous NRG [22^{••}]. Furthermore, using slice cultures this study showed that a dominant negative (DN) form of ErbB2 could promote the premature transformation of

Figure 2



Complexity of signaling in radial glial progenitors. The Notch, ErbB, and FGF receptors all play a part in the maintenance of radial glial progenitor characteristics. Activation of the Notch and ErbB pathways might be achieved by expression of ligands such as Delta and Neuregulin on the migratory neurons that come into contact with RG processes. The identity of the downstream signaling components that mediate the effects of these pathways and how they interact remain to be elucidated.

RG cells into astrocytes. Interestingly, this study went on to suggest that ErbB2 is a direct transcriptional target of the Notch–CBF1 signaling cascade.

A similar recent study examined the role of ErbB2 signaling in cerebellar RG [19**]. This work also showed that a DN form of an ErbB receptor (ErbB4) could antagonize RG characteristics, and that ErbB2 could be upregulated by Notch signaling. Interestingly, in contrast to the neocortical study, this work suggested that ErbB2 was a CBF1-independent Notch target. This apparent discrepancy suggests that regional differences might exist in the mechanism by which Notch activates ErbB2. Because telencephalic RG are progenitors, whereas cerebellar RG are probably not, understanding this difference could provide insight into how the radial glial and progenitor states are coupled. In any event, on the basis of these studies it is becoming apparent that migratory neurons provide multiple cooperative cues to maintain the radial progenitor pool (Figure 2).

Fibroblast growth factor signaling

Currently, evidence is emerging suggesting that in addition to the ErbB signaling pathway the FGFRs, another family of RTKs, play a part in maintaining radial glial progenitors. For example, a recent study used the hGFAP–Cre line described above to delete FGFR1 from radial glial progenitors *in vivo* [24*]. This study focused on the hippocampus and found that lack of FGFR1 led to a

reduction in the number of RG. Interestingly, the study went on to demonstrate that deletion of FGFR1 from RG resulted in a nearly complete loss of cells with the potential to form neurospheres in media containing FGF2 *in vitro*. This finding supports the notion that hippocampal RG are progenitors and suggests that these cells comprise the majority of the neurosphere-forming population present *in vivo*.

Recent gain-of-function studies have further supported a role for FGF signaling in the promotion of RG characteristics [18**]. FGFR2 is expressed in many telencephalic RG and an activated form of FGFR2 promotes RG morphology *in vivo*. Interestingly, however, in contrast to findings with activated Notch1, cells infected with activated FGFR2 showed little to no increase in neurosphere forming potential. This finding supports the view, mentioned above, that the promotion of RG characteristics is not absolutely linked to the promotion of progenitor characteristics.

The fact that two different families of RTKs, the ErbB and FGF receptors, can promote the development of RG characteristics raises the possibility that they are doing so through similar intracellular signaling cascades. Although this might be true, it is clear that the activation of different RTKs can have different effects depending upon the experimental paradigm. For example, in contrast to the data discussed above regarding activated FGFR2, expression of an activated form of ErbB2 *in vivo* does not promote the development of RG characteristics [18**].

In addition, others have found that epidermal growth factor receptor (EGFR, also called ErbB1) signaling, but not FGFR signaling, can promote the acquisition of RG characteristics in embryonic forebrain cells *in vitro* [25*]. Because signaling downstream of RTKs is extremely complex, it is likely that cellular context and signaling levels will determine the effect of activating a given pathway in radial glial progenitors. Further study is needed to generate a comprehensive understanding of the role of RTK signaling in radial glial progenitors, in addition to how that signaling interacts with other pathways.

Conclusions

Our understanding of the neural progenitor pool during development has been permanently altered by the realization that many RG are dividing progenitors capable of generating neurons. Recent work has shown that the Notch, ErbB and FGF signaling pathways have a role in the maintenance of radial glial progenitor characteristics. In addition, this work has provided insight into how neuronal cues, such as Notch and ErbB ligands, mediate regulatory interactions between neurons and RG. Numerous challenges lie ahead as we seek to understand both the developmental potential and the heterogeneity of

radial glial progenitors. In light of the complex signaling pathways involved, and discrepancies in the current literature, it is clear that significant progress will require the close coordination of *in vivo* and *in vitro* approaches.

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This study and that by Malatesta *et al.* [14**] used Cre-loxP mediated recombination to fate-map radial glial progenitors *in vivo*. Anthony *et al.* [15**] used BLBP-Cre, whereas Malatesta *et al.* [14**] used hGFAP-Cre to mark RG. These studies provide important *in vivo* evidence that RG are neurogenic, although they reach different conclusions about the neurogenic potential of RG in the ventral telencephalon. This discrepancy is probably a function of differences in the timing of Cre-mediated recombination.
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