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Radial glia – from boring cables to stem cell stars

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Summary

The discovery in the year 2000 that radial glial cells act as neural stem and progenitor cells in development has led to a change in the concept of neural stem cells in the adult brain. Not only are adult stem cells in the neurogenic niches glial in nature, but also glial cells outside these niches display greater potential when reacting to brain injury. Thus, a concept that emerged from developmental studies may hold the clue for neural repair.

Introduction

A fundamental issue in developmental biology is to understand when and how cell diversity is brought about during development. In the nervous system, this means understanding when and how the diverse types of neurons and glial cells are generated. One way to generate distinct types of progeny is to set aside, at some developmental stage, distinct sets of progenitor cells; for example, neuronal progenitors specified to generate only neurons and glial progenitors giving rise only to glia. Such a model, proposed by Wilhelm His more than 100 years ago, implied the co-existence of two different types of progenitor cells, neuroblasts and spongioblasts, based on the position of their soma (His, 1889). However, at the beginning of the 1960s it became clear that these distinct positions are simply a result of cells in different phases of the cell cycle: neural tube progenitor cells move their soma from apical positions during M phase to a more basal location during S phase (for reviews, see Fujita, 2003; Götz and Huttner, 2005). Despite this evidence, the concept of progenitor diversity was so well accepted that the neuroblast and spongioblast terms lingered on, and was refueled when glial fibrillary acidic protein (GFAP) was discovered as a new 'marker' for astrocytes and was also detected in radial glial cells during development (Levitt and Rakic, 1980). This supported their glial nature and thus implicitly their identity as the glial progenitors, the spongioblasts. However, nobody examined whether all stem and progenitor cells lining the ventricle express GFAP: it was automatically assumed that these GFAP-positive cells were 'of course' only a subtype, as they are the spongioblasts and, hence, a minority during neurogenesis. Other cells in the ventricular zone, where dividing cells reside, must therefore be more numerous, act as neuronal progenitors and generate the neurons that migrate along the radial glial cells (Fig. 1A). The next discovery, that neurons migrate along the radial fibers of radial glial cells (Rakic, 1971; Rakic, 1972), led to the assumption that the other neuronal progenitor cells would not have such long radial processes, as they should not have the supporting functions of glial cells, and they were hence depicted with short processes (Fig. 1A), probably based on histological observations in which many processes were cut short by sectioning. Finally, lineage-tracing studies with lipophilic dye on ferret cortex, showing transitional forms between radial glia and astrocytes at the end of the neurogenesis (Voigt, 1989), were the last brick of the wall protecting the concept of dualism between neuronal and glial lineages, implying that neuroblasts and radial glia were as distinct as neurons and astrocytes in the mature CNS.

An idea emerging from a control experiment

Our entanglement with radial glia started, rather unexpectedly, from a research line focused on Pax6. Mutation of *Pax6* had been described to cause rather prominent malformations in the developing cerebral cortex, the dorsal region of the telencephalon (Schmahl et al., 1993), and we were interested in elucidating its role in more detail. In collaboration with Anastassia Stoykova and Peter Gruss we examined the cellular and molecular basis of this phenotype (Götz et al., 1998). As one simple control experiment, we wanted to know which cells actually expressed Pax6 and so aimed to analyze markers for radial glial cells and proteins indicative of the 'other' neuronal progenitors. The outcome was that Pax6 was expressed in radial glia and in virtually all proliferating progenitors at mid-neurogenesis stages (Götz et al., 1998). This prompted the question of how many of the proliferating cells were actually radial glia. We were very surprised to see that all bona fide radial glial markers [RC2, GLAST (Slc1a3) and BLBP (Fabp7)] labeled the majority of dividing cells, triggering a further question as to whether these markers were not valid radial glia markers or whether the concept of radial glia as a glial-specific subpopulation of progenitors had to be revised. Our results showing that at the peak of neurogenesis virtually all proliferating cells were radial glia (Fig. 1B) prompted the idea that radial glia might actually act also as progenitors for neurons. When I (M.G.) started my laboratory in Munich, I set out to tackle this question and was happy that Paolo Malatesta took on this first central question for our laboratory when joining the lab as a postdoctoral fellow (Malatesta et al., 2000).

A technical challenge – how to isolate radial glia?

How to examine the progeny of these cells directly? Although not well established for neural cells by then, fluorescence-activated cell

Box: A *Development* classic

The year 2012 marks 25 years since the journal *Development* was relaunched from the its predecessor, the *Journal of Embryology and Experimental Morphology* (*JEEM*). In 2008, we fully digitised our *Development* and *JEEM* archives, and made them freely available online. At the same time, we took the opportunity to revisit some of the classic papers published in *JEEM*, in a series of commentaries (see Alfred and Smith, 2008). Now, to mark a quarter century of *Development*, we have been looking through our archives at some of the most influential papers published in *Development*'s pages. In this series of Spotlight articles, we have asked the authors of those articles to tell us the back-story behind their work and how the paper has influenced the development of their field. Look out for more of these Spotlight papers in the next few issues.

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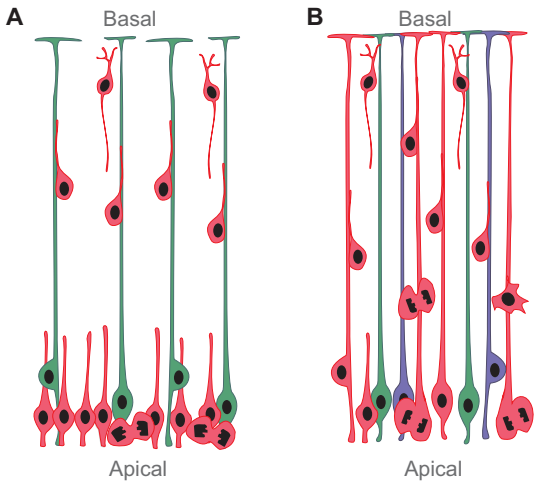

Fig. 1. Comparison between the 'old' and 'new' models of corticogenesis. (**A**) In the traditional view, radial glia (cells with long processes that span the whole apicobasal axis of the neuroepithelium; shown in green), were viewed as cables for guiding migrating neurons (red), and were thought to transform into glial cells at later stages of development. (**B**) The discovery that radial glia were proliferative and gave rise to neurons led to a new concept whereby radial glial cells are the majority of stem and progenitor cells in the ventricular zone, comprising specified neuronal (red) and glial (green) progenitors as well as bi-/multi-potent stem cells (purple).

sorting (FACS) seemed the most straightforward approach to use to isolate cells and then follow their progeny in rather defined conditions. This then required a method for labeling the radial glia. Their most prominent characteristic was their stunning morphology with long extended radial processes, which we could label by applying the lipophilic dye DiI on the surface of the brain. However, what if the neuronal progenitors also had long processes, even though traditionally these were depicted to have shorter ones (Fig. 1A)? Therefore, we aimed at using an independent molecular marker and turned to the glial marker GFAP. A transgenic mouse line had been developed by Albee Messing, in which the fluorescent protein GFP was placed under control of the human GFAP promoter (Zhuo et al., 1997). After having established that the GFP expression almost perfectly colocalized with the radial glial marker GLAST in the embryonic cerebral cortex, we had the ability to isolate radial glial cells by FACS. As our aim was to determine the progeny of a single cell (i.e. to perform analysis of single clones) we plated a small number of individual radial glial cells isolated by FACS together with a bulk of feeder cells isolated from the same brain region at the same stage but from a different species (rat), enabling detection of the sorted mouse cells by mouse-specific antibodies (Malatesta et al., 2000). This procedure allowed us to examine the lineage of single cells in high-density cell cultures, the standard assay in the field for clonal analysis using viral vector labeled cells, without the need of growth factors (Williams and Price, 1995).

As soon as we started to analyze the clones generated from sorted cortical radial glia, it became evident that they were generating a large fraction of pure neuronal clones (Malatesta et al., 2000). Importantly, our clonal analysis showed that radial glia were generating neurons not just occasionally but rather that they behaved just as would be expected for neural progenitors at mid-neurogenesis. Indeed, we observed some heterogeneity among the clones emerging from radial glial cells, with most generating only neurons, some generating glial cells only and a yet smaller subset generating both glial and neuronal progeny, as expected for a neural stem cell (Fig. 2).

However, in the second set of experiments we noted a surprisingly smaller proportion of neuronal clones emerging from the sorted radial glial cells. After some days of puzzling and anxiety, we realized that this difference was due to the threshold chosen for sorting: in the second experiment only cells expressing very high levels of GFP were selected. Further analysis confirmed that these were generating many more glial clones. Interestingly, when we followed this up at later stages, we discovered that the radial glial cells with higher GFAP-promoter activity were also biased to generate neurons via intermediate progenitors undergoing mitosis at an abventricular position (Pinto et al., 2008), opening a rather fruitful avenue into the molecular regulators of these lineages (Pinto et al., 2009) (M.G., unpublished observations). These quantitative issues aside, we were excited to have discovered the first evidence that cells of the glial lineage can generate neurons, a truly unexpected concept at the time. We were further encouraged in our eccentric idea when Arturo Alvarez-Buylla and his colleagues showed that astrocyte-like cells in the subventricular zone of adult mice act as adult neural stem cells (Doetsch et al., 1999). It was then tantalizing to imagine that the apparent exception in the adult brain could actually represent the rule in the developing brain.

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Although based on a well-established marker, we now turned back to the characteristic morphology of these cells and used the approach described above by DiI application onto the pial surface of brains to label the cells with endfeet that reached the pia. The labeling efficiency and, by consequence, the recovery after sorting was pretty low but was more than sufficient for our clonal analyses. The results we obtained were overlapping with those from the GFP-based labeling. This confirmed that cells with an extended radial process and expression of GFAP genuinely comprised a large population of neuronal progenitors as well as bi-lineage progenitors/stem cells and glial progenitors (Fig. 1B). [For three-dimensional reconstruction of ventricular zone cells confirming that most ventricular zone cells have such a long radial process, see Hartfuss et al. (Hartfuss et al., 2003).]

Catch 22 – how to fight a dogma

We learned soon that the hardest thing about unconventional findings is not so much collecting the data but disrupting an established dogma. Although some scientists were truly interested and welcomed our data, others simply could not accept that glia generate neurons; if radial glia were to generate neurons, then they could not be glia but must be 'misnamed'. For other critics, radial glial cells could not generate neurons because their long process would not allow cell division, despite previous evidence based on cell cycle marker expression (Misson et al., 1988; Kamei et al., 1998) and further support from our own work. The most frustrating criticism was that we were not sorting 'radial glia' cells but neural progenitor cells that displayed radial morphology and glial markers. Considering that morphology and marker expression were

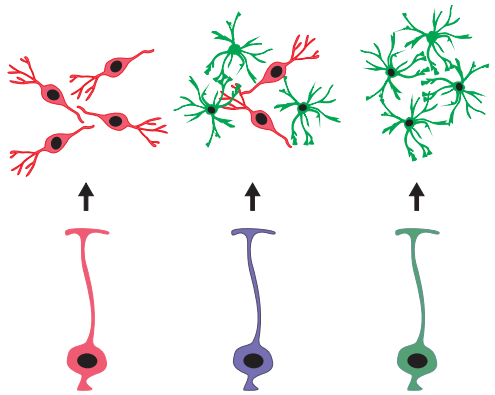

Fig. 2. Schematic summarizing the results of clonal analysis of single radial glial cells isolated by FACS as described in our *Development* paper in 2000 (Malatesta et al., 2000). Clones generated by a single cell were either composed of only neurons (red), of only glial cells (green) or of both glia and neurons. These results suggested for the first time that radial glial cells are a heterogeneous population comprising neuronal progenitors (red), glial progenitors (green) and neural stem cells (purple).

the only way to ultimately define radial glia, this might appear humorous, but it shows how unacceptable the notion of radial glia as neural progenitors was to a large part of the scientific community.

Thus, after presentation of our data at the 1999 Society for Neuroscience Meeting and one year of our manuscript being under review, with editors and reviewers asking for more and more experiments [such as asking us to demonstrate whether or not radial glial cells maintain their radial process during cell division – a key question that was later beautifully answered by live imaging of DiI-labeled cells (Miyata et al., 2001)], we decided to submit to a journal handled by scientists, namely *Development*. This made a huge difference and immediately we were exposed to a rapid and fair reviewing process that allowed our article to be finally published in 2000 (Malatesta et al., 2000). Soon after, we saw a report in *Nature* – where our manuscript had lingered for many months – using live imaging in slice preparation demonstrating radial cells (defined by morphology) dividing and generating neurons (Noctor et al., 2001). Together with the third article also using live imaging to follow radial glial cell division and neuron generation (Miyata et al., 2001), these papers, each taking different experimental approaches, complemented each other very well to successfully convince most scientists that radial glial cells do indeed divide and generate neurons (Götz and Steindler, 2003; Götz and Huttner, 2005). Interestingly, however, this view has still not entered many textbooks, and the notion that these cells may not be ‘true’ glia, but rather ‘neuroepithelial cells’ with some expression of GFAP still lingers. This is despite the fact that a multitude of glial proteins are expressed in these cells (for reviews, see Pinto and Götz, 2007; Robel et al., 2011), showing that it can be difficult to say goodbye to long-held concepts.

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Impact on the field

Despite this initial reluctance, the identification of radial glial cells as neural stem and progenitor cells in many vertebrate species (reviewed by Götz, 2012) and in CNS regions, as well as *in vivo* demonstration by genetic fate mapping (Malatesta et al., 2003), has meant that the concept has caught on and rather now moved to the other extreme – as is often the case. Now, typically, all radial glial cells are described as neural stem cells, even though only a minority of radial glial cells have this activity in the developing brain. Moreover, in some regions, such as the spinal cord, radial glial cells appear only during the latter period of gliogenesis (Barry and McDermott, 2005), implying that neurogenesis actually occurs from the earlier neuroepithelial cells (Götz and Huttner, 2005). Thus, although simple concepts are tempting, cellular and regional heterogeneity should not be overlooked. Most importantly, however, this concept has been extended to the adult brain, where not only adult neural stem cells in various brain regions turn out to be radial glia (Kriegstein and Alvarez-Buylla, 2009), but some glial cells reacting to brain injury regain stem cell hallmarks (Robel et al., 2011), providing an exciting novel source of stem cells right at the injury site. Thus, despite initial problems, this concept has not only caught on for the developmental audience, but has also more broadly penetrated the field of neuroscience even into adulthood and disease contexts.

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