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Lineage, Fate, and Fate Potential of NG2-glia

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Abstract

NG2 cells represent a fourth major glial cell population in the mammalian central nervous system (CNS). They arise from discrete germinal zones in mid-gestation embryos and expand to occupy the entire CNS parenchyma. Genetic fate mapping studies have shown that oligodendrocytes and a subpopulation of ventral protoplasmic astrocytes arise from NG2 cells. This review describes recent findings on the fate and fate potential of NG2 cells under physiological and pathological conditions. We discuss age-dependent changes in the fate and fate potential of NG2 cells and possible mechanisms that could be involved in restricting their oligodendrocyte differentiation or fate plasticity.

1. Introduction

NG2 cells represent a fourth resident glial cell population in the mammalian central nervous system (CNS) that is distinct from astrocytes, mature oligodendrocytes, and microglia. They are defined as non-neuronal, non-vascular glial cells in the CNS parenchyma that express the NG2 antigen and the alpha receptor for platelet-derived growth factor (Pdgfra) (Nishiyama et al., 2009; Hill and Nishiyama, 2014). They are distributed widely throughout both gray and white matter. They generate oligodendrocytes in culture and in vivo and hence are often equated with oligodendrocyte precursor cells (OPCs). Cells with similar properties have been reported in earlier ultrastructural studies as satellite cells apposed to principal neurons in gray matter (Penfield, 1924; Mugnaini and Walberg, 1964), small glioblasts (Vaughn, 1969), oligodendroglioblasts (Skoff, 1976), and β -astrocytes (Reyners, 1982). However, it was not until the 1990s when immunolabeling for NG2 became feasible on routinely processed tissue sections and revealed their multi-processed morphology with their striking coverage of the entire CNS parenchyma that their existence as a resident glial cell population in the CNS was appreciated (Stallcup et al., 1983; Levine et al., 1993; Nishiyama et al., 1996a; Peters, 2004) (Figure 1). The morphology and distribution of these cells were

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not what one expected of immature precursor cells destined to become oligodendrocytes, and the lineage of NG2 cells was intensely debated over the following two decades. The term polydendrocytes has been proposed as an inclusive substitute and synonym for NG2 cells or NG2-glia to avoid using a marker for the name of a cell type and to avoid using the term OPCs when discussing their properties that are not directly related to their ability to generate oligodendrocytes (Nishiyama et al., 2009, Hill and Nishiyama, 2014). Here we summarize our current understanding of the lineage commitment and fate of NG2 cells in normal and pathological states in vivo and discuss some unanswered questions regarding their fate and fate potential.

2. Origin of NG2 cells during development

2.1. Olig2 specifies NG2 cells and the oligodendrocyte lineage

In the mammalian CNS, neurons, astrocytes and oligodendrocytes arise from the neuroepithelium according to a temporally and spatially regulated program. In the rodent spinal cord, oligodendrocyte lineage cells first become specified within a discrete domain in the ventral ventricular zone called pMN domain, which is marked by the expression of the basic helix-loop-helix transcription factor Olig2 (bHLH) induced by the ventral morphogen Sonic hedgehog (Shh). Olig2+ progenitor cells in this domain first give rise to motor neurons at E9-10 and subsequently switch to produce oligodendrocyte lineage cells after E12 (Kessaris et al., 2001). In the forebrain, oligodendrocyte lineage cells are also generated initially ventrally from Olig2+ cells in the medial and lateral ganglionic eminences (MGE and LGE, respectively) that also generate interneurons concomitant with oligodendrocyte lineage cells (Nery et al., 2001; Spassky et al., 2001; Tekki-Kessaris et al., 2001). During neuronal differentiation in both regions, Olig2 expression is downregulated while the expression of neurogenic transcription factors is sustained. Conversely in cells that become committed to the oligodendrocyte lineage and begin to express NG2, Olig2 expression is sustained while neuronal genes are repressed (Novitsch et al., 2001; Petryniak et al., 2007).

2.2. Appearance of NG2 cells shortly after oligodendrocyte lineage specification

Since Olig2 expression is shared by neuronal and oligodendrocyte lineage cells, further specification must occur for the Olig2+ cells in the germinal zone (ventricular zone, VZ) to become committed to NG2 cells. Sox10 is a member of the high-mobility group (HMG) transcription factor family and is expressed throughout the oligodendrocyte lineage. The onset of its expression lags that of Olig2 and precedes that of NG2 cell markers such as NG2 and Pdgfra (Figure 2A), suggesting that Olig2 induces Sox10. Indeed, Olig2 binds to the U2 enhancer sequence in the 5'-flanking region of the mouse Sox10 gene and activates its transcription (Küspert et al., 2011). Development of Pdgfra+ cells is severely compromised in mice lacking both Sox10 and a related transcription factor Sox9, and Sox9 binds to specific sites in the proximal 5'-flanking region of the Pdgfra gene (Stolt et al., 2002; Finzsch et al., 2008). Pdgfra transcription is also reduced in mice lacking Sox10 (Stolt et al., 2002). Since Sox10 expression is restricted to oligodendrocyte lineage cells in the CNS, the induction of Sox10 transcription by Olig2 marks the first step in the commitment of neuroepithelial cells to the oligodendrocyte (NG2 cell) lineage (Figure 2A).

Shortly after the onset of *Pdgfra* transcription in the VZ, cells begin to migrate out into the parenchyma where they undergo extensive dispersion and PDGF-dependent proliferation (Pringle et al., 1992; Calver et al., 1998). NG2 is not expressed in the VZ (Nishiyama et al., 1996a; Zhu et al., 2008; Komitova et al., 2009) but is detected on all the Sox10+ *Pdgfra*+ cells in the parenchyma. Thus the earliest NG2 cells can be defined as Sox10+ cells that have become “committed to the oligodendrocyte lineage” (see below) and exited the VZ. As discussed below and depicted in Figure 2, NG2 cells can be considered as a subset of oligodendrocyte lineage cells that have not yet become terminally differentiated into oligodendrocytes. There is a small subset of NG2 cells that generates protoplasmic astrocytes prenatally. These cells express *Olig2*, *NG2*, and *Pdgfra* and are indistinguishable from OPCs that would eventually become oligodendrocytes (see below), and further analysis is needed to determine whether there are any differences in the transcriptome of NG2 cells with astrocyte and oligodendrocyte fate.

The initial wave of expanding NG2 cells merges with later cohorts of NG2 cells generated from more dorsal domains and together they populate the entire CNS, thus creating a mosaic population with respect to their site of origin (Cai et al., 2005; Vallstedt et al., 2005; Fogarty et al., 2005). NG2 cells reach peak density during the first postnatal week, after which their density declines and their processes become less overlapped but begin to occupy defined tiled domains through the entire parenchyma (Zhou 2014; Hughes et al., 2013).

A property of NG2 cells that distinguishes them from astrocytes is their robust migratory ability. NG2 cells undergo both radial and tangential migration, as do interneurons, whereas astrocytes remain within their radial domains (Tsai et al., 2012; reviewed in Bayraktar et al., 2015). The robust migratory behavior of NG2 cells in the developing spinal cord and optic has been attributed to *Netrin-1*, which is secreted from the floor plate or the third ventricle floor and exerts a chemorepulsive effect on NG2 cells mediated by its receptors *DCC* (deleted in colorectal cancer) and *Unc5H1* on NG2 cells (Sugimoto et al., 2001; Jarjour et al., 2003; Tsai et al., 2003), although a chemoattractive response has also been reported (Spassky et al., 2002). Multiple putative Sox10-binding sites are detected in the mouse *DCC* gene (Jaspar Transcription Factor database; Mathelier et al., 2014), but the number of *DCC*-expressing NG2 cells in the developing spinal cord was unaltered in mice lacking Sox10 (Finzsch et al., 2007), suggesting that Sox10 may need another factor to activate the transcription of *DCC* on NG2 cells. Nevertheless, expression of *DCC* is an early event after NG2 cell commitment and could trigger their exit from the VZ at a time when *Netrin-1* is actively secreted from the floor plate and notochord.

2.3. Lineage commitment from radial glial progenitor cells in the developing neocortex

The neocortex is the last CNS region to be populated by NG2 cells. Here neurons and astrocytes are generated from radial glial progenitor cells according to a temporally regulated program. Radial glial progenitor cells first generate neurons in a defined temporal window during mid-gestation and subsequently generate GFAP+ astrocytes in late embryos. The neurogenesis to gliogenesis switch is highly reproducible, and intrinsic epigenetic changes that occur in the radial glial progenitor cells after completion of neurogenesis

renders them responsive to signals that lead to repression of neurogenic genes and derepression of astrogliogenic genes (Miyata et al., 2010).

The production of NG2 cells from the telencephalic progenitor cells that express the homeodomain transcription factor *Emx1* has been reported to begin around the time of birth (Kessaris et al., 2006). In contrast to the well characterized switch that occurs in the dorsal radial progenitor cells, the timing and the molecular mechanisms that mediate the onset of NG2 cell production from the radial progenitor cells remains unclear. Clonal analysis after injection of lacZ-encoding retrovirus into neonatal rat subventricular zone (SVZ) revealed clones that generated exclusively oligodendrocytes in the corpus callosum, whereas clones in the neocortex consisted of a mixture of oligodendrocytes and astrocytes. When the virus was injected into P14 SVZ, labeled cells were mostly oligodendrocytes, suggesting that NG2 cell production continues after cessation of astrogliogenesis (Levison and Goldman, 1993; Levison et al., 1993). *Olig2* is expressed in the SVZ progenitor cells that generate astrocytes as well as those that generate oligodendrocytes and is required for the development of both lineages (Marshall and Goldman, 2004; Yue et al., 2006; Cai et al., 2007). However, little is known about the spatial and temporal mechanisms that direct an SVZ progenitor cell to generate an astrocyte or oligodendrocyte progeny. In the adult, NG2 cells continue to be generated from the SVZ (Menn et al., 2006). Interestingly clonal analysis combined with real time imaging revealed that oligodendrocyte lineage cells arise more frequently from the dorsal SVZ, while neuronal cells arise from the dorsolateral SVZ, and the oligodendroglial and neurogenic cells appear to arise from distinct stem/progenitor cells (Ortega et al., 2013).

A small number of NG2 cells already appear in the neocortex by birth, mostly in deeper cortical layers (Figure 1A), and these early-born NG2 cells are likely to have been generated and migrated from ventral sources (Kessaris et al., 2006; Tripathi et al., 2011). This is also suggested by the gradient of the density of NG2 cells in P0 telencephalon, with the highest density found in the lateral intermediate zone (Figure 1). The relative contribution of radial glia and SVZ cells to NG2 cell production in the neocortex and corpus callosum is also not well established. It is also not known whether and how the astrogliogenesis program is suppressed when NG2 cells are generated, or whether all progenitor cells across the pallium are equivalent in their ability to generate both astrocytes and NG2 cells after neurogenesis has been completed.

3. The fate of NG2 cells in the normal developing and mature CNS

In culture, glial progenitor cells isolated from perinatal rat optic nerves by immunopanning for the A2B5 polysialoganglioside antigen express NG2 and terminally differentiate into oligodendrocytes in the presence of thyroid hormone, downregulating NG2 and upregulating myelin and oligodendrocyte antigens as they differentiate (Raff et al., 1983; Stallcup and Beasley, 1987). These progenitor cells exhibit some lineage plasticity in culture and can differentiate into astrocytes when exposed to serum or bone morphogenetic protein (BMP). A similar population of A2B5+ cells called GRP cells (glial restricted progenitor cells) were isolated from E13.5 rat spinal cord and shown to generate astrocytes and oligodendrocytes but not neurons (Rao et al., 1998). These are most likely to be the earliest glial committed

cells that arise from the germinal zones described above. However, due to poor preservation and lack of specificity of the A2B5 antigen in fixed tissue, the in vivo correlates of these cells have not been identified, despite widespread use of GRP cells in transplantation studies to promote lesion repair (Cao and Whittemore, 2012). A method for tracing the fate of NG2 cells in vivo had to be developed. In the following sections we summarize the findings from genetic fate mapping studies.

3.1. Oligodendrocyte fate of NG2 cells

Over the past decade, a significant progress has been made in our understanding of the fate of NG2 cells in vivo. The genetic approach using Cre-loxP-mediated recombination has provided a powerful tool to examine the fate of resident NG2 cells in different CNS regions at different developmental stages. This approach involves two transgenic mouse lines. One line must express the Cre recombinase specifically in NG2 cells. This line is crossed to a Cre reporter mouse line in which reporter expression is permanently activated by Cre and thus enables marking of all the progeny of Cre-expressing cells (Nishiyama 2007). In order for the strategy to work, Cre in the Cre driver mouse line must be activated exclusively in NG2 cells, and the promoter driving reporter expression in the reporter mouse line must be active in all cells to allow efficient detection of all lineages besides the cells in which Cre is expressed. There is no perfect combination of Cre driver and reporter mice for NG2 cell fate analysis, and correct interpretation these types of studies requires familiarity with the idiosyncratic pattern of Cre and reporter activation in each mouse line.

Constitutive Cre-expressing cell lines are used to identify the progeny of all NG2 cells from the earliest NG2 cells that appear around E14, whereas inducible Cre lines (CreER^{T/TM/T2}) are used to map the fate of NG2 cells at different developmental stages. So far, the only constitutive Cre line that has been well characterized and used for NG2 cell fate mapping has been the NG2-cre BAC transgenic line generated with the regulatory elements of the NG2 (Cspg4) gene (Zhu et al., 2008). Two additional studies have used NG2-creER^{TM/T2} lines in which Cre was induced embryonically (Zhu et al., 2011; Huang et al., 2014). All the other fate-mapping studies have examined the fate of NG2 cells in the postnatal brain. Of the various inducible Cre driver lines, Cre-mediated recombination occurs almost exclusively in NG2 cells in the CNS parenchyma in NG2-creERTM and Pdgfra-creER^{T2} lines (Zhu et al., 2008; 2011; Rivers et al., 2008; Kang et al., 2010), whereas Olig2-creERTM (Dimou et al., 2008) and PLP-creER^T (Guo et al., 2009) lines target a broader population of cells including neural progenitor cells and oligodendrocytes. Collectively, these studies are in agreement that NG2 cells throughout all stages of development can self-renew and generate oligodendrocytes. They provided direct confirmation that NG2 cells in the postnatal CNS are indeed OPCs (reviewed in Nishiyama et al., 2009; Richardson et al., 2011; Chew et al., 2014), although it remains unknown whether all NG2 cells are equivalent in their potential to give rise to oligodendrocytes.

There are regional differences in the dynamics of their differentiation into mature oligodendrocytes (discussed in more detail in Dimou, this issue). A greater proportion of NG2 cells in the adult white matter become oligodendrocytes than in gray matter over a period of a few months, and an average NG2 cell in white matter differentiates into an

oligodendrocyte faster than that in gray matter (Dimou et al., 2008; Rivers et al., 2008; Zhu et al., 2011; Kang et al., 2010; Young et al., 2013). Cross-transplantation experiments suggest that both cell intrinsic and environmental factors contribute to greater oligodendrocyte differentiation from NG2 cells (Vigano et al., 2013). There are also temporal changes in the rate of oligodendrocyte differentiation from NG2 cells. As detailed below, oligodendrocyte production from NG2 cells occurs at an increasingly lower rate with age. The question of whether all NG2 cells ultimately generate oligodendrocytes during the life of the animal has not been solved. NG2 cells are distributed uniformly throughout different neocortical layers even though oligodendrocyte density is significantly higher in the deep layers. This suggests that many of the NG2 cells in oligodendrocyte-sparse regions remain as NG2 cells and never differentiate into oligodendrocytes (Figure 1; Tomassy et al., 2014). It is unknown whether all NG2 cells have equivalent oligodendroglial fate, and oligodendrocyte differentiation occurs stochastically (Figure 2B1), or whether a subpopulation of NG2 cells loses the ability to become oligodendrocyte at some point during development (Figure 2B2).

Different factors have been shown to positively or negatively affect NG2 cell differentiation into oligodendrocytes (reviewed in Chong and Chan, 2010; Emery, 2010). An area of active investigation is how neuronal activity regulates the fate of NG2 cells. Increasing neuronal activity promotes oligodendrocyte differentiation (Li et al., 2010; Simon et al., 2011; Gibson et al., 2014; reviewed in Hill and Nishiyama, 2014), whereas sensory deprivation reduces the number of oligodendrocytes generated from divided NG2 cells (Hill et al., 2014). The precise mechanism by which neuronal activity translates into fate decision of NG2 cells is currently unknown.

3.2. Astrocyte fate of NG2 cells

When Cre is activated in NG2 cells prenatally before E17.5, more than one-third of the protoplasmic astrocytes in the ventral forebrain are generated from NG2 cells (Zhu et al., 2008, 2011; Huang et al., 2014). NG2 cells in white matter tracts, including optic nerves, do not generate astrocytes, contrary to the findings from the early culture studies (Zhu et al., 2008). When Cre is induced in NG2 cells postnatally, reporter+ astrocytes are not detected in significant numbers (Rivers et al., 2008; Kang et al., 2010; Zhu et al., 2011). A small number of reporter+ astrocytes reported in some studies could have arisen from Cre expression in astrocyte precursor cells rather than in NG2 cells (Dimou et al., 2008; Guo et al., 2009).

NG2 cell-derived astrocytes appear to be converted from parenchymal bona fide NG2 cells in the embryonic forebrain away from the VZ (Figure 2A) and not from radial glia, which do not express NG2, Cre, or Cre reporter (Zhu et al., 2008). There are cells that appear to be in transition from parenchymal NG2 cells to protoplasmic astrocytes in E18.5 ventral forebrain. These cells exhibit weak nuclear Olig2 immunoreactivity, weak NG2 immunoreactivity on tufts of distal processes, and express the astrocyte antigen glial glutamate aspartate transporter (GLAST), suggesting that they are in the process of turning from Olig2+ NG2+ GLAST-negative cells into Olig2- NG2- GLAST+ astrocytes (Figure 3A; Zhu et al., 2008; 2012). At present, NG2 cells that generate astrocytes are

morphologically indistinguishable from those that generate oligodendrocytes and express Olig2, Pdgfra, and NG2. Further RNA- and ChIP-sequencing studies are needed to determine whether they have transcriptional and epigenetic profiles that make them distinct from OPCs.

A similar switch in the fate of NG2 cells in the neocortex is seen when Olig2 is deleted (see below). Analysis of spatially separated astrocyte-containing clusters of the progeny of NG2 cells after a low level of Cre induction at E16.5 failed to reveal clusters that contained both astrocytes and oligodendrocyte lineage cells (Zhu et al., 2011). This suggests that the astrogliogenic subpopulation of NG2 cells undergoes symmetric differentiation into astrocytes in the embryonic brain, thereby exhausting the astrogliogenic NG2 cell population by the time of birth.

3.3. Neuronal fate of NG2 cells

The question of whether NG2 cells generate neurons has been intensely debated and is still not completely resolved. During development NG2 cells arise from discrete ventral domains of the germinal zone that also generate neurons. Despite the shared origin and the initial transcription factor code of domain specification, there is no evidence from the three studies in which Cre was activated prenatally that neurons are generated from NG2 cells during development (Zhu et al., 2008; 2011; Huang et al., 2014), suggesting a tight cross-repression of neurogenic and oligodendroglial programs in cells committed to become NG2 cells or neurons, respectively. Early postnatal to adult induction of Cre in Pdgfra-creER^{T2}:reporter mice also showed no evidence of neurogenesis from NG2 cells (Kang et al., 2010). Similarly, Cre activation in the adult in Olig2-creERTM mice also did not detect neuronal fate of NG2 cells (Dimou et al., 2008).

On the other hand, other NG2 cell fate mapping studies have detected reporter⁺ neurons in the adult brain. In NG2-creERTM mice crossed to a different reporter line, reporter⁺ neurons were observed to gradually increase in number in the hypothalamus after Cre induction in adult (Robins Kokoeva et al., 2013). The NG2 cell-derived neurons exhibited properties of immature neurons characterized by small initial bursts of regenerative action potentials after current injection. In another Pdgfra-creER^{T2}:reporter mouse line, reporter⁺ neurons were detected in the piriform cortex between 28 and 210 days after Cre induction in adult (Rivers et al., 2008). NG2 cell-derived neurons in the piriform cortex did not incorporate BrdU, while those in the hypothalamus did. A follow-up study with the Pdgfra-creER^{T2} mice discusses the validity of their earlier observations, and based on the observation that all NG2 cells are cycling, the conclusion was made that the reporter⁺ neurons that did not incorporate BrdU must not have been generated from NG2 cells (Clarke et al., 2013). In both regions reporter⁺ neurons comprised no more than 5% of the reporter⁺ cells, unlike the case of astrocytes, and the functional implications of the relatively small number of reporter⁺ neurons in the neural circuit remains unclear.

In NG2-cre(creER^{TM/T2}):reporter mice, sporadic reporter⁺ neurons resembling principal and interneurons were detected in the cerebral cortex of adult NG2-cre:reporter mice after P30 but not in younger mice (Huang et al., 2014; Nishiyama et al., 2014). No transitional forms between NG2 cells and neurons were noted, and reporter⁺ mature neurons were

morphologically indistinguishable from reporter-negative mature neurons nearby and did not incorporate BrdU. One possible explanation for the late appearance of reporter+ neurons is that there are spiking bursts of NG2 transcription and hence Cre activation in certain populations of neurons in the mature CNS (discussed in more detail in Nishiyama et al., 2014). Such Cre or NG2 transcriptional activity could occur as a part of a physiological aging or stress-related process.

A recent study describes neuronal gene expression in reporter+ cells in the neocortex of a new NG2-cre mouse line crossed to ROSA-lacZ reporter (Tsoa et al., 2014). The reporter+ “neurons”, which were birth-dated to E14.5, expressed Calretinin but lacked the mature oligodendrocyte marker GST- π (glutathione-S-transferase- π), and they were not tested for the expression of NG2 or the early oligodendrocyte marker CC1. Although further characterization of this mouse line is needed, the most straightforward explanation of these reporter+ cells is that these reporter+ cells are NG2 cells that also express neuronal proteins. Recent transcriptomic profiling studies have revealed that NG2 cells express a number of genes previously considered as neuron-specific genes (Table 1). Notably, Calretinin (Calb2) and Doublecortin (Dcx) used in previous NG2 cell fate and lineage analyses (He et al., 2001; Clarke et al., 2012; Tsoa et al., 2014; Jablonska et al., 2010; Dayer et al., 2005) as well as a number of genes encoding presynaptic terminal proteins are detected at significant levels in NG2 cells in five microarray and one RNA-sequence databases (Nielsen et al., 2006; Cahoy et al., 2008; Lau et al., 2008; Wang et al., 2013; Auvergne et al., 2013; Zhang et al., 2014; Moyon et al., 2015). Other genes that are actively transcribed in telencephalic NG2 cells include the interneuron proteins Gephyrin and Gad1, which may reflect their developmental history. The significance of transcription of neuronal genes in NG2 cells is unclear at present. They could have a function in NG2 cells or their transcriptional activity may simply reflect lineage history. Further analysis of genome-wide transcriptomic and chromatin immunoprecipitation studies would reveal whether it is possible to quantitatively predict lineage plasticity based on the degrees and mechanisms of repression of cell type-specific genes.

4. Fate potential of NG2 cells

Under prolonged culture conditions NG2 cells isolated from the perinatal optic nerve can be induced to differentiate into neurons (Kondo and Raff, 2000). This suggested that it is possible to reprogram NG2 cells to follow other lineages by changing the environmental conditions. Recent studies are uncovering some conditions under which non-oligodendrocyte fate potential of NG2 cells can be revealed.

4.1. Role of Olig2 in restricting the fate of NG2 cells to the oligodendrocyte lineage

A subpopulation of NG2 cells in the ventral forebrain differentiates into astrocytes during normal development, and Olig2 appears to be downregulated during this process (Zhu et al., 2008; 2011, 2012; Huang 2014). In neural stem cells inactivation of Olig2 by cytoplasmic export is necessary for astrocyte differentiation (Setoguchi and Kondo, 2004). These observations suggest that Olig2 functions as the key transcription factor that maintains NG2 cells in the oligodendrocyte lineage. When Olig2 is deleted from NG2 cells, the vast

majority of NG2 cells in the neocortex are converted into protoplasmic astrocytes by the end of the first postnatal week (Zhu et al., 2012). This fate conversion is region-specific, and does not occur in the ventral forebrain where one-third of NG2 cells in wild type mice spontaneously downregulate Olig2 prenatally and become protoplasmic astrocytes. No neurons were generated from Olig2-deleted NG2 cells, suggesting that derepression of neuronal genes requires additional factors besides loss of Olig2. The ability of NG2 cells to become astrocytes upon Olig2 deletion declines with age. When Olig2 is deleted postnatally only 50% of Olig2-deleted NG2 cells are converted into astrocytes, whereas the majority of Olig2-deleted NG2 cells become astrocytes when Olig2 is deleted from the onset of NG2 expression, contributing to 70% of the local astrocyte population (Zhu et al., 2012; Zuo et al., manuscript in preparation).

Olig1, which is closely related to Olig2 and is critical for oligodendrocyte differentiation, is expressed in precursors of interneurons that arise from the ventral telencephalic VZ as well as in oligodendrocyte lineage cells. Deletion of Olig1 increases the number of interneurons at the expense of oligodendrocyte lineage cells (Silbereis et al., 2014). In oligodendrocyte lineage cells, Olig1 represses the homeodomain gene *Dlx1/2*, which is required for interneuron differentiation, and deletion of Olig1 allows *Dlx1/2* expression. Conversely, more NG2 cells are generated at the expense of neurons in mice lacking *Dlx1/2*, suggesting that Olig1/2 and *Dlx1/2* cross-repress each other (Petryniak et al., 2007).

4.2. Fate of NG2 cells under pathological conditions

4.2.1. Fate of NG2 cells after demyelination—After acute experimental demyelination, local NG2 cells quickly proliferate and generate myelinating oligodendrocytes in the corpus callosum and spinal cord (Gensert and Goldman, 1997; Watanabe et al., 2002). More recent cre/loxP fate mapping studies show that NG2 cells in the adult white matter contribute to oligodendrocyte and myelin repair (Sahel et al., 2015). In the spinal cord, when demyelination is accompanied by compromised astrocytes, NG2 cells generate not only oligodendrocytes but also Schwann cells that contribute to myelin repair (Zawadzka et al., 2010). NG2 cells also generate myelinating oligodendrocytes in the inflammatory model of demyelination, experimental autoimmune encephalomyelitis (Tripathi et al., 2010; Guo et al., 2011). While the consensus is that NG2 cells are capable of generating remyelinating oligodendrocytes after acute demyelination, they seem to do so less efficiently after chronic demyelination (Mason et al., 2004; Xing et al., 2014; Beatrice et al., 2015). It remains to be determined whether chronic demyelination exhausts the population of NG2 cells that are capable of generating oligodendrocytes.

4.2.2. Fate of NG2 cells after mechanical or cryoinjury in the brain—It is well established that reactive astrocytes accompany a vast majority of insult to the CNS and that NG2 cell undergo extensive proliferation after injury. Perhaps aided by the historical hypothesis that NG2 cells generate both astrocytes and oligodendrocytes in culture (Raff et al., 1983), the fate of NG2 cells after injury has been investigated by many labs. While earlier studies that examined the fate of proliferated cells were inconclusive, more recent genetic fate mapping studies suggest that NG2 cells undergo proliferation in response to a stab wound and some differentiate into oligodendrocytes but not neurons or astrocytes

(Dimou et al., 2008; Zawadzka et al., 2010; Simon et al., 2011; Komitova et al., 2011). A few reporter+ cells were shown to express GFAP transiently, but these studies did not produce definitive evidence that NG2 cells around a lesion contribute to reactive astrogliosis, even when Olig2 was deleted. By contrast, another study using Olig2-creERTM mice showed that some NG2 cells differentiate into astrocytes following a cryo-injury (Tatsumi et al., 2008). Although appropriate controls are shown, the expression of Olig2 in reactive astrocytes (Chen et al., 2008) confounds the analysis, and confirmatory findings have not been obtained with other cre driver lines. NG2 cells can also generate myelinating Schwann cells after an acute chemically induced demyelinating lesion in the spinal cord (Zawadzka et al., 2010). These observations suggest that NG2 cells that proliferate around different types of injury may initiate reprogramming toward another cell type, but other intrinsic mechanisms could exert a brake to abort this process and restrict their fate (see below).

4.3. Neurogenic reprogramming of NG2 cells in the injured by transcription factors

Two recent studies have suggested that NG2 cells in the adult injured CNS can be reprogrammed into neurons by manipulating transcription factors. The first study showed that infection of proliferating cells around a neocortical stab wound with a retrovirus encoding the neurogenic transcription factor NeuroD1 caused the infected cells to become mostly glutamatergic neurons with a minority of GABAergic neurons (Guo 2014). The glia-derived displayed electrophysiological characteristics of neurons such as action potentials and synaptic currents. However questions remain as to the specific cell type that was initially transduced by the NeuroD1-retrovirus.

In a separate study, transduction of reactive NG2 cells that proliferate around a stab wound with Sox2 or a combination of Sox2 and Ascl1 could convert them into immature neurons expressing DCX (Heinrich et al., 2014). The origin of these cells has been confirmed to be NG2 cells by using a Sox10-dependent lineage reporter. A fraction of these cells expressed the mature neuronal marker NeuN, and a few cells showed sodium channel-mediated inward current and a small number of action potential-like voltage changes upon current injection, although the degree of neuronal maturity was significantly less than that seen after reprogramming of P7 cortical astrocytes in culture (Heinrich et al., 2010).

Interestingly, both of these studies detected glia-derived neurons only after stab wound injury and failed to detect glia-derived neurons after transduction of glial cells in the uninjured brain with the transcription factors. Stab wound injury also caused some local reactive NG2 cells to upregulate GFAP transcription, as described above (Komitova et al., 2011). These observations suggest that intrinsic changes are occurring in NG2 cells in response to injury that lead to derepression of genes normally restricted to other lineages. It will be interesting to learn the nature of the environmental signals that facilitates reprogramming of NG2 cells into other cell types.

5. Age-dependent changes in NG2 cell fate

5.1. Age-dependent decrease in oligodendrocyte differentiation and NG2 cell proliferation

As described above, genetic fate mapping studies have revealed that the rate of oligodendrocyte differentiation from NG2 cells declines with age (Rivers et al., 2008; Zhu et al., 2011; Kang et al., 2011). There appears to be a correlation between the rate of oligodendrocyte differentiation from NG2 cells and NG2 cell proliferation. Although NG2 cells remain proliferative throughout life, their cell cycle time lengthens significantly with age (Psachoulia et al., 2009; Young et al., 2013). However, it is noteworthy that significant clonal expansion of NG2 cells has been detected between P120 and P240 (Garcia-Marques, 2014). Similarly, the rate of oligodendrocyte differentiation and NG2 cell proliferation are greater in white matter than in gray matter (Young et al., 2013; Hill et al., 2013). While these studies showed differences in the average cell cycle times, clonal or cluster analysis *in vivo* indicates that there is some heterogeneity in the degree of expansion of individual NG2 cells (Zhu et al., 2011; Kang et al., 2010; Garcia-Marques et al., 2014). Other approaches must be taken to learn whether there exists a small subpopulation of quiescent NG2 cells that behaves like stem cells and can be recruited after loss of proliferative NG2 cells and whether there is a finite number of divisions that NG2 cells can undergo.

5.2. NG2 cell division and cell fate

Does the apparent correlation between NG2 cell proliferation and differentiation suggest a mechanism during cell division that might influence the fate of the daughter cells? When the fate of divided NG2 cells is examined, there appears to be a finite temporal window during which oligodendrocyte differentiation occurs after division, and the length of this window increases with the age of the animal, from 3–4 days at P8 to 6–10 days at P21 (Hill et al., 2014). In the neocortex, the proportion of the divided cells that become oligodendrocytes during this temporal window after division is similar to the proportion of NG2 cells that become oligodendrocytes over 60 days, suggesting that those that will become oligodendrocytes will do so during this temporal window. Furthermore, myelin and oligodendrocyte damage accelerates oligodendrocyte differentiation from divided NG2 cells within this temporal window. Conversely, loss of whisker sensory input attenuates oligodendrocyte production from divided NG2 cells during this critical window by increasing death of divided NG2 cells that are undergoing terminal differentiation (Hill et al., 2014). In the adult cortex, it has been proposed from *in vivo* imaging study that oligodendrocyte differentiation from NG2 cells does not require cell division (Hughes et al., 2013). However, these types of studies allow analyses of a small number of cells, and it is highly probable that the cells that differentiated into oligodendrocytes during the imaging period had divided immediately prior to the start of the imaging.

The earliest indicator of commitment of a daughter cell to oligodendrocyte differentiation is the onset of appearance of the G-protein coupled receptor GPR17 during telophase of cell division. GPR17 is expressed transiently during oligodendrocyte differentiation (Chen et al., 2009; Fumagalli et al., 2011). Following NG2 cell division the accumulation of GPR17 in a daughter cell is associated with high levels of the cell cycle inhibitor p27/kip1 and terminal differentiation (Boda et al., 2015). The frequency of division events leading to accumulation

of GPR17 in daughter cells is higher in running mice and lower in aged mice, consistent with the findings from the previous population studies on oligodendrocyte differentiation described above.

5.3. Transcriptional and epigenetic changes that occur with age

The observations described above suggest intrinsic changes that occur in NG2 cells as the animal ages. A new transcriptomic profiling study revealed that NG2 cells from adult CNS transcribe more genes expressed in differentiated oligodendrocytes than NG2 cells from perinatal CNS (Moyon et al., 2015). Intriguingly, demyelination changes the oligodendrocyte-like transcriptomic profile of adult NG2 cells to one that more closely resembles perinatal NG2 cell transcriptome, suggesting that the age-dependent changes are reversible under some circumstances.

Oligodendrocyte differentiation requires the activity of histone deacetylases (HDACs) (Marin-Husstege et al., 2002), which repress transcription of oligodendrocyte differentiation inhibitors such as Hes 5 and Id2/4 and Wnt signal (Ye et al., 2009; Swiss et al., 2011). Pharmacological inhibition of HDACs inhibited myelin gene expression when administered during the first postnatal week, but had no effect after the third postnatal week (Shen et al., 2005), suggesting that inhibitors of oligodendrocyte differentiation may become more permanently repressed by other mechanisms beyond the stage of peak oligodendrocyte differentiation.

In addition to histone acetylation, histone methylation also plays an important role in cell fate and differentiation. Transcriptionally active histone methylation at H3K4me3 and the repressive methylation at H3K27me3 are often found on the same genes as stem cells develop into lineage-specific progenitor cells, and one or the other methylation mark is retained as the cells become more firmly committed to the lineage. Such a bivalent histone methylation mark has been detected in the human OLIG2 gene during transition from embryonic stem cells to neural stem cells (Boyer et al., 2006). With further differentiation into oligodendrocytes, neuronal genes become more permanently repressed by additional repressive histone marks such as H3K9me3 (Liu et al., 2015; described in detail in the article by xx in this issue). Taken together, these findings suggest that increasing layers of repressive marks are added to non-oligodendrocyte lineage genes and with age as NG2 cells undergo terminal differentiation into oligodendrocytes. Ultimately, these repressive marks could tether these genes into the periphery of the nucleus for permanent repression (Kohwi et al., 2013). This could also explain why NG2 cells lose fate plasticity with age after deletion of Olig2 (Zhu et al., 2012; Komitova et al., 2011). It will be interesting to learn how environmental changes that occur at sites of injury reverse these repressive mechanisms to facilitate reprogramming of NG2 cells.

6. Concluding remarks

The highly branched morphology of NG2 cells that uniformly cover the CNS parenchyma has posed a challenge in accepting the concept that NG2 cells are “precursor cells” that are destined to generate oligodendrocytes. NG2 cell fate mapping studies have confirmed that oligodendrocytes do indeed arise from NG2 cells. Emerging findings from genomic studies

are consistent with the notion that NG2 cells retain lineage plasticity to some degree by maintaining partial repressive marks on lineage specific genes, and that this plasticity appears to be gradually lost with age. We are still left with the unanswered question of what proportion of NG2 cells ultimately go on to become oligodendrocytes and whether there are NG2 cells that have permanently lost the ability to become oligodendrocytes (Figure 2B). New approaches and techniques are needed to answer these long-standing questions.

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Figure 1. Distribution and morphology of NG2 cells in the neonate and adult

A–B. P0 mouse telencephalon labeled for NG2 (green) and Olig2 (blue). A. Low magnification tiled image of the distribution of NG2 cells. Note the higher density in the nascent white matter and deeper cortical layers and the presence of many Olig2+ NG2- cells in the SVZ. B. Higher magnification of the region boxed in A showing the morphology of individual NG2 cells. NG2 is also highly expressed on the vasculature. Left is medial, top is dorsal. Arrow: a multiprocessed cell. Arrowheads: a cell with asymmetric long processes. svz: subventricular zone.

C–E. P60 telencephalon labeled for NG2 (green) and Olig2 (blue). C. Low magnification view spanning the neocortex and corpus callosum. D. Superficial layer of the cortex. E. Junction of corpus callosum and neocortex.

NG2 cells with similar morphology are similarly distributed in superficial and deep neocortical layers but there are more NG2-negative Olig2+ cells (arrows in E), which are likely to be oligodendrocytes, in deeper layers. ctx: neocortex; cc: corpus callosum

Scale bars, A and C 100 μ m, B, D, and E 20 μ m.

A. Oligodendrocyte lineage commitment and the fate of NG2 cells

B. Oligodendrogligenic potential of NG2 cells

Figure 2. Scheme showing the lineage and fate of NG2 cells

A. Relationship between NG2 cells and the entire oligodendrocyte lineage.

Left: A cartoon showing the appearance of NG2 cells in the parenchyma (blue shade) after specification of the oligodendrocyte lineage in the germinal zone (ventricular zone, gray shade). NG2 cells (blue cells with processes) expand while some generate protoplasmic astrocytes (green cells with bushy morphology).

Right: A diagram depicting the chronology of oligodendrocyte lineage specification and differentiation. Blue shade denotes “committed oligodendrocyte” lineage cells. A subpopulation of these cells with all the molecular signatures of oligodendrocyte lineage cells downregulate and become protoplasmic astrocytes (green).

B. Diagrams highlighting the unanswered question of whether all NG2 cells are capable of differentiating into oligodendrocytes. B1 proposes a scenario in which all NG2 cells are equivalent in their ability to become oligodendrocytes. B2 proposes the alternative possibility of two segregated populations of NG2 cells: one with oligodendrogligenic potential (blue dots) and the other without (red dots).

Table 1

The presence of transcripts encoding “neuronal genes” in NG2 cells

Gene	Gene name	Auvergne, 2013		Cahoy, 2008	Lau, 2008	Moyon, 2015		Wang, 2013	Zhang, 2014
		cortex	white matter			adult	neonatal		
Actb	Actin, beta	13.7	13.9	7.5		7.8	7.4	7.3	FPKM 531.9
Gapdh	Glyceraldehyde-3-phosphate dehydrogenase					9.6	9.0		64.9
Cspg4	Cspg4 (NG2)	7.3	8.7	12.3	9.1	10.1	12.6		115.8
Olig2	Oligodendrocyte transcription factor 2			11.1		9.0	10.7	8.6	206.4
Ascl1	Ascl1 (Mash1)	4.1	4.0	5.7	10.0	8.1	11.0	3.9	9.7
Bsn	Bassoon	3.3	3.3	4.7	4.9	8.2	8.7		3.0
Calb2	Calbindin 2 (Calretinin)	3.5	4.0	8.3	10.1	8.1	9.3		16.0
Dcx	Doublecortin	5.9	5.7	10.3	5.8	9.1	8.8	4.5	1.5
Dlx1	Distal-less homeobox 1			3.8		7.6	8.1		0.2
Dlx2	Distal-less homeobox 2	5.9	7.2	4.5		8.5	9.2		0.2
Gabbr1	GABA B receptor 1	8.6	9.2	3.9	5.9	8.8	8.2		54.9
Gabra1	GABA A receptor subunit alpha 1	3.7	3.3	4.8		6.3	7.5		3.6
Gabra2	GABA A receptor subunit alpha 2	2.5	2.7	4.9		9.8	9.8		5.8
Gad1	Glutamate decarboxylase 1	8.4	8.9	9.4	11.6	8.5	8.9	6.7	11.1
Gphn	Gephyrin	6.5	6.4		10.2	10.9	10.5	6.3	5.6
NeuroD1	NeuroD1	2.5	4.5	3.7	10.7	7.2		5.4	0.1
SLC17A6	Solute carrier family 17, member 6 (VGLUT2)			3.9		10.6	10.3	4.5	0.1
SLC32A1	Solute carrier family 32, member 1 (VGAT)					7.6	8.5	2.9	1.1
Snap25	Synaptosomal-associated protein 25	8.7	8.9	9.2	13.3	10.0	10.3		27.2
Syp	Synaptophysin	6.6	7.6	7.9	10.8	7.7	7.7	5.9	39.5
Syt1	Synaptotagmin I	2.7	3.3	3.8	7.1	8.3	8.5		8.4
Tbr1	T-box brain gene 1	2.5	3.1	3.4		8.9	9.3	5.4	0.9
Tubb3	Tubulin, beta 3 class III			7.1	4.9	10.8	9.8	9.6	13.5

Values for the last stippled column are FPKM values from RNA sequencing. The remaining columns represent results from microarray studies expressed as RMA (robust multi-array average) values. The first two rows represent “house-keeping” genes.

The next two rows are genes known to be highly expressed in NG2 cells.

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Auvergne, et. al., 2013, GSE29796, A2B5+ cells from adult
Cahoy et. al., 2008, GSE9566, P7 mouse OPCs
Lau et. al., 2008, GSE11218, A2B5+ cells from P7 rat whole brain
Moyon et al., 2015, GSE48872
Wang et. al., 2013, GSE40676
Zhang et al., 2014, GSE52564, RNA-sequencing, FPKM values

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