Progenies of NG2 glia: what do we learn from transgenic mouse models?

Abstract

In the mammalian central nervous system, nerve-glial antigen 2 (NG2) glia are considered the fourth glial population in addition to astrocytes, oligodendrocytes and microglia. The fate of NG2 glia in vivo has been carefully studied in several transgenic mouse models using the Cre/loxP strategy. There is a clear agreement that NG2 glia mainly serve as progenitors for oligodendrocytes and a subpopulation of astrocytes mainly in the ventral forebrain, whereas the existence of a neurogenic potential of NG2 glia is lack of adequate evidence. This mini review summarizes the findings from recent studies regarding the fate of NG2 glia during development. We will highlight the age-and-region-dependent heterogeneity of the NG2 glia differentiation potential. We will also discuss putative reasons for inconsistent findings in various transgenic mouse lines of previous studies.

Key Words: astrogliogenesis; cell fate; Cre/loxP system; development; differentiation; embryonic brain; neurogenesis; NG2 glia; oligodendrocyte lineage; oligodendrocyte precursor cells

Introduction

In the mammalian central nervous system (CNS), the nerve-glial antigen 2 (NG2) glycoprotein (also called chondroitin sulfate proteoglycan 4, CSPG4) is immunodetectable only in oligodendrocyte precursor cells (OPCs) and vascular pericytes (Nishiyama et al., 1996; Horner et al., 2002; Stallcup, 2002). OPCs in the adult CNS intensively co-express NG2 and platelet derived growth factor receptor α (PDGFRα), whereas the subcellular expression pattern of those two markers is different during the development (Nishiyama et al., 1996; Diers-Fenger et al., 2001; Dawson et al., 2003; Rivers et al., 2008). Despite their capacity to generate mature oligodendrocytes (OLs) throughout life, NG2-expressing OPCs also possess unique physiological properties such as promoting presynaptic specialization in neurons (Tanaka et al., 2009) and modulating neuroinflammation (Nakano et al., 2017; Zhang et al., 2019; Liu and Aguzzi, 2020). Thereby, to emphasize their status as the fourth glial population in addition to astrocytes, OLs and microglia, OPCs are also termed NG2 glia (Butt et al., 1999; Bergles et al., 2000; Nishiyama, 2001; Greenwood and Butt, 2003; Peters, 2004; Butt and Dinsdale, 2005; Ge et al., 2006; Kukley et al., 2007; Nishiyama et al., 2009; Vélez-Fort et al., 2010; Haberlandt et al., 2011). As the largest proliferative cell population in the adult CNS, NG2 glia are equally distributed over the whole brain (Figure 1Bb and b2) and spinal cord (Nishiyama et al., 2016). Their fate has been carefully studied in several transgenic mouse models using the Cre/loxP strategy in vivo (Doerflinger et al., 2003; Rivers et al., 2008; Zhu et al., 2008a, b, 2011; Guo et al., 2009, 2010; Kang et al., 2010; Hill et al., 2011; Simon et al., 2011; Clarke et al., 2012; Huang et al., 2014, 2018, 2019). Consensus has been achieved for the oligodendrogenic potential of postnatal NG2 glia, though debate still exists regarding the potential differentiation fate of NG2 glia to other cell types like astrocytes and neurons. For this mini review, we searched PubMed for literatures published up to 2020 by using the keyword combination: “NG2” OR “PDGFRα” OR “oligodendrocyte precursor cell” AND “Cre”. We will summarize results of fate-mapping studies of NG2 glia during development by using transgenic mice.

Cre/loxP Mouse Models for Fate-Mapping of NG2 Glia

The Cre recombinase, a 38 kDa protein found in the bacteriophage P1, catalyses site-specific homologous recombination of two particular 34 bp nucleotide sequences (loxP: locus of crossover of the bacteriophage P1, also floxed), allowing specific manipulation of floxed DNA strands (Sternberg and Hamilton, 1981; Abremski et al., 1983; Hoess and Abremski, 1985). To enable a temporal control of Cre activity (CreER), the enzyme was fused to mutated ligand-binding domain of the human estrogen receptor (ER), thereby trapping it by heat shock proteins within the cytosol and preventing its entry into the nucleus (Metzger et al., 1995; Feil et al., 1996; Zhu et al., 2008a, 2008b, 2011; Guo et al., 2009, 2010; Kang et al., 2010; Hill et al., 2011; Simon et al., 2011; Clarke et al., 2012; Huang et al., 2014, 2018, 2019). To map the fate of distinct cell populations, Cre-expressing animals are crossbred to reporter mice, where the expression...
of a transgenic reporter protein is driven by ubiquitously active promoters after Cre-mediated deletion of the floxed STOP cassette (Isl) (Soriano, 1999; Novak et al., 2000; Mao et al., 2001; Srivivas et al., 2001; Madisen et al., 2010). Therefore, Cre-expressing cells and their progeny will irreversibly turn on the reporter gene, allowing the tracing of defined Cre-expressing cell types and their progeny. Hence, fate-mapping studies are largely relying on the specificity of the promoter driving Cre-expression in the target cells.

By and large, Cre-expressing mice can be classified into two groups according to the strategies of transgene insertion: via non-homologous recombination (TgN) by injection of linearized vector DNA strands to oocytes (Gordon et al., 1980) and homologous recombination (TgH, also called knock-in, KI) in embryonic stem cells (Smithies et al., 1985; Thomas et al., 1986). Each strategy has advantages and disadvantages as discussed in previous reviews (Nishiyama et al., 2009; Richardson et al., 2011). Briefly, transgenic mice generated via non-homologous recombination are faster produced, however the transgene is controlled by just a short promoter sequence which may result in ectopic expression of the transgene. Although nowadays the bacterial or phage artificial chromosome (BAC or PAC) approaches greatly improved the specificity of the transgene by using much longer regulatory sequences. Mice generated by non-homologous recombination have still drawbacks due to the random insertion into the genome, which might cause unpredictable transgene expression or unintended gene deletion at the insertion site (Beil et al., 2012). The homologous knock-in strategy introduces the transgene into the endogenous targeted gene locus, ensuring the complete and true control of the transgene expression by all the regulatory elements of the targeted gene. However, it requires more effort and time-cost to generate knock-in mice and usually the knock-in strategy leads to the loss of at least one allele of the targeted gene (in heterozygous mice) unless Internal-Ribosome-Entry-Site strategy is successfully incorporated (Chan et al., 2011).

To achieve lineage tracing of NG2 glia in vivo, a series of transgenic mice has been generated in several research groups via different strategies. In these transgenic mice, the expression of Cre or CreER(T2) is driven by the promoters of either one of the two specific NG2 glia markers, (NG2/PDGFRα), or other genes that are active in the OL lineage such as Olig2, Sox10, or PLP (Table 1). Therefore, the expression pattern of the selected marker gene during development determines the cell populations with the recombined reporter gene. For example, in the developing forebrain at E15.5, Olig2 is expressed in OPCs and a sub-population of interneuronal progenitor cells. When Cre activity was induced at E15.5 in Olig2-CreERT<sup>FLox/FLox</sup> KI mice, both OL lineage cells and interneurons expressing the chosen reporter could be found in the postnatal cortex (Miyoshi et al., 2007). In addition, transgene expression only represents the gene activity of the selected marker rather than the genuine protein expression. For example, the NG2 protein starts to be immunodetectable in OPCs in the rodent brain from about embryonic day 14 (E14), but NG2 gene activity can already be detected in a small portion of PDGFRα OPCs (3%) at E12.5 in NG2-EYFP KI mice (Karram et al., 2008; Huang et al., 2019). Although the proportion of EYFP PDGFRα OPCs increased drastically from E12.5 to birth in NG2-EYFP KI mice, the expression of EYFP could only be found in OPCs or pericytes, indicating that NG2 gene activity is strictly restricted to those two cell populations (Huang et al., 2019).

The sensitivity of the reporter can also lead to different results. The higher sensitivity of the reporter could reveal a broader range of Cre-expressing patterns (Madisen et al., 2010; Van Hove et al., 2020). For instance, compared to the Rosa26-EYFP reporter (Srivivas et al., 2001), a strong and ubiquitous CAG promoter and the WPRE (mRNA stabilizer woodchuck hepatitis virus posttranscriptional regulatory element) cassette were inserted into the construct of Rosa26-tdTomato reporter to improve expression efficiency (Madisen et al., 2010). Therefore, in 4-week old NG2-CreERT2<sup>Huang</sup> KI mice, the Rosa26-tdTomato reporter could label more NG2 glia by at least 20% than the Rosa26-EYFP reporter (Huang et al., 2014). However, reporter with high sensitivity might also generate unexpected recombined cells, which could be due to transient low or even ectopic activity of the selected gene driving Cre expression (Tognatta et al., 2017; Van Hove et al., 2020).

Spatiotemporally Controlled Generation of Oligodendrocytes from NG2 Glia

In the developing brain, OPCs arise in three waves sequentially from ventral to dorsal origin (Kessaris et al., 2006). The first OPCs are derived from Nkx2.1<sup>+</sup> progenitors at E12.5 from the medial ganglionic eminence and anterior entopenduncular area in the ventral brain. By E16.5, the second wave of OPCs from Gsh2<sup>+</sup> progenitors joined from the lateral and caudal ganglionic eminences (LGE and CGE) in the ventral brain. The ventrally derived OPCs quickly migrate to the dorsal cortex, in which those OPCs will be largely eliminated within the first postnatal weeks. The third wave of OPCs is generated from dorsal Emx1<sup>+</sup> progenitors postnatally and contribute to ~80% of the OLs in the dorsal brain (Kessaris et al., 2006; Tripathi et al., 2011). Studies in NG2-EYFP KI mice suggested that at E12.5, the NG2 gene was already active in a small portion of OPCs, which was further confirmed by using NG2-CreERT2<sup>Huang</sup> KI mice (Huang et al., 2019). When NG2-CreERT2<sup>Huang</sup> KI embryos received tamoxifen at E12.5 and were analyzed 2 days later at E14.5 (E12.5:E14.5), only in the ventral brain reporter<sup>+</sup> OPCs were detected. At E12.5:PO (analysis at postnatal day 0), reporter<sup>+</sup> OPCs could also be found in the dorsal cortex, indicating the migration of OPCs from the ventral to dorsal brain. In this similar distribution pattern of reporter<sup>+</sup> OPCs was revealed at E14.5:E16.5 (tamoxifen at E12.5 and analysis 2 days later, Figure 1Aa–a4) and E14.5:PO (Huang et al., 2019). However, at E14.5:PO reporter<sup>+</sup> OPCs (and OLs) migrating to the dorsal brain were greatly eliminated, consistent with previous studies (Huang et al., 2019). When Cre activity was induced embryonically (from E12.5 to E17.5) in NG2-CreERT2<sup>Huang</sup> KI or NG2-CreERT<sup>Huang</sup> BAC mice, some reporter positive cells were found to express mature OL markers and displayed OL morphology after birth which suggest that embryonic NG2 glia give rise to OLs (Zhu et al., 2011; Huang et al., 2019). Quantitative analysis revealed that embryonic NG2 glia have a very low differentiation rate (the percentage of OLs) in the white matter (WM) such as internal capsule than in the gray matter (GM) such as dorsal and ventral cortex (Huang et al., 2019). This region-dependent manner of OL differentiation from NG2 glia was confirmed in postnatal brains of several mouse lines (such as NG2-CreERT2<sup>Huang</sup> KI, NG2-CreERT<sup>Huang</sup> BAC, NG2-Cre<sup>Tsoa</sup> BAC, PDGFRα-CreERT<sup>Tsoa</sup> BAC, PDGFRα-CreERT<sup>Madisen</sup> BAC and Olig2-CreERT<sup>Tsoa</sup> KI). Whenever Cre activity was induced, WM NG2 glia always displayed higher oligodendroglionic potential than their GM counterparts in terms of a greater proportion of reporter<sup>+</sup> OLs within a confined period. In addition, NG2 glia quickly generate OLs within the early postnatal weeks, and such generation of OLs seems to continue throughout life although the rate declines significantly with age in both GM and WM of the brain (Rivers et al., 2008; Zhu et al., 2008a; Kang et al., 2010; Huang et al., 2014; Tsoa et al., 2014). Recent studies further demonstrated that newly generated mature OLs in the adult brain actively participated in myelin modelling (Hill et al., 2018; Hughes et al., 2018).

Results from the constitutive NG2-Cre<sup>+</sup> BAC mice showed that spinal NG2 glia could generate OLs (Zhu et al., 2008a). When Cre activity was induced in neonatal NG2-CreERT2<sup>Huang</sup> KI mice, a great portion of spinal NG2 glia quickly differentiated into OLs although the differentiation rate (the percentage of reporter<sup>+</sup>
mechanisms that regulate the heterogeneous differentiation manner, and it will be important to investigate the precise differentiate into OLs in an age- and region-dependent Taken together, these studies suggest that NG2 glia

...where they contributed to about one third of the total S100B+ astrocytes were mainly found in the GM of the ventral brain, higher oligodendrogliogenic potential than neonatal NG2 glia decreased with age, embryonic spinal NG2 glia did not show the rate of postnatal NG2 glia differentiating into OLs declined (Kang et al., 2010; Huang et al., 2018). In addition, although glia differentiated into OLs with a similar rate in WM and GM activity was induced at P30 or P136 in NG2-CreERT2 Hua... 

NG2 glia (%)

Recombined cell types

OL Lineage Astrocyte Neuron Reference(s)

NG2-Cre<sup>+</sup> BAC Z/EG brain ~86 + + – Zhu et al. (2008a, b)

NG2-CreERT<sup>+</sup>BAC Z/EG brain ~70 + + – Not mentioned

NG2-Cre<sup>+</sup>BAC ROSA26-EYFP brain ~45 + Not mentioned –

NG2-CreERT<sup>+</sup>BAC ROSA26-LacZ brain ~99 + Not mentioned + Tsoa et al. (2014)

NG2-CreERT<sup>+</sup>BAC ROSA26-TdTomato brain ~80 + Not mentioned – +

NG2-CreERT<sup>+</sup>BAC ROSA26-EYFP brain ~95 + + (from Embryonic) – + Huang et al. (2014, 2018, 2019)

NG2-CreERT<sup>+</sup>BAC ROSA26-EYFP brain ~75 + Not mentioned + +

PDGFRα-CreERT<sup>+</sup>BAC PAC ROSA26-EYFP brain ~47 + – – + (in PC) Rivers et al. (2008)

PDGFRα-CreERT<sup>+</sup>BAC ROSA26-tdTomato brain ~40 + – – + Zawadzka et al. (2010)

PDGFRα-CreERT<sup>+</sup>BAC ROSA26-mGFP brain ~86 + – + + Kang et al. (2010)

PDGFRα-CreERT<sup>+</sup>BAC ROSA26-EYFP brain ~87 + – + +

PLP-CreERT<sup>+</sup>TgN TgN ROSA26-EYFP brain ~42 + – + +

Olig2-CreERT<sup>+</sup>TgN TgH ROSA26-EYFP brain ~17 + + + (in PC) Guo et al. (2009, 2010)

Olig2-CreERT<sup>+</sup>TgN TgH ROSA26-EYFP cerebellum ~27 + + +

Olig2-CreERT<sup>+</sup>TgN TgH ROSA26-EYFP brain ~45 + Not mentioned + + –

Olig2-CreERT<sup>+</sup>TgN TgH ROSA26-EYFP brain ~80 + + – +

Olig2-CreERT<sup>+</sup>TgN TgH ROSA26-EYFP cerebellum ~27 + + +

Olig2-CreERT<sup>+</sup>TgN TgH ROSA26-EYFP cerebellum Not mentioned + + –

Olig2-CreERT<sup>+</sup>TgN TgH ROSA26-EYFP brain Not mentioned + + –

Olig2-CreERT<sup>+</sup>TgN TgH ROSA26-EYFP brain Not mentioned + + –

Olig2-CreERT<sup>+</sup>TgN TgH ROSA26-EYFP brain Not mentioned + + –

Olig2-CreERT<sup>+</sup>TgN TgH ROSA26-EYFP brain Not mentioned + + –

Olig2-CreERT<sup>+</sup>TgN TgH ROSA26-EYFP brain Not mentioned + + –

+ Defined cell types were detected; – defined cell types were not detected; BAC bacterial artificial chromosome; NG2 nerve-glia antigen 2; TgH transgene homologous recombination; TgN transgene non-homologous recombination; PAC phage artificial chromosome; PC piriform cortex.

Recombined NG2 glia (percentage) and their distribution in different regions of the brain are shown in Table 1. The first fate-mapping studies of NG2 glia in different transgenic mice

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OLs in all reporter<sup>+</sup> glia in WM was still higher than in GM (e.g., at P1:P13, ~70% and ~90% in the GM and WM, respectively) (Huang et al., 2018). However, the pattern of OL differentiation from NG2 glia in the adult spinal cord is different. When Cre activity was induced at P30 or P136 in NG2-CreERT2<sup>Huang KI</sup> mice, spinal NG2 glia differentiated into OLs with a similar rate in WM and GM (Kang et al., 2010; Huang et al., 2018). In addition, although the rate of postnatal NG2 glia differentiating into OLs declined decreased with age, embryonic spinal NG2 glia did not show higher oligodendrogligenic potential than neonatal NG2 glia (e.g., in the NG2-CreERT2<sup>Huang KI</sup> mice at E17.5:P10, ~50% and ~90% in the GM and WM respectively) (Huang et al., 2018).

Taken together, these studies suggest that NG2 glia differentiate into OLs in an age- and region-dependent manner, and it will be important to investigate the precise mechanisms that regulate the heterogeneous differentiation potential of NG2 glia to generate OLs.

Restricted Astrogliogenic Potential of NG2 Glia

The first in vivo evidence indicating NG2 glia generating astrocytes came from NG2-Cre<sup>+</sup>BAC mice with the Z/EG reporter (Zhu et al., 2008a). In these mice, NG2 glia-derived astrocytes were mainly found in the GM of the ventral brain, where they contributed to about one third of the total S100B<sup>+</sup> astrocytes. In addition, a few NG2 glia-derived astrocytes could be detected in other GM regions such as dorsal cortex and hippocampus, but never in WM areas. In the follow-up study, NG2-CreERT2<sup>BAC</sup> BACxZ/EG mice were treated with tamoxifen either at E16.5 or postnatally to determine if NG2 glia could give rise to astrocytes all the time. However, only mice treated with tamoxifen embryonically displayed reporter<sup>+</sup> astrocytes mostly in the ventral forebrain after birth, indicating that only a subpopulation of embryonic NG2 glia could differentiate into astrocytes (Zhu et al., 2011). These results were further confirmed by studies using NG2-CreERT2<sup>Huang KI</sup> mice crossed to the more sensitive Rosa26-ttdTomato reporter. Only when CreERT2 activity was induced in embryos (at E12.5, E14.5 or E17.5), a significant number of reporter<sup>+</sup> astrocytes could be detected in the postnatal forebrain, and the distribution pattern was similar to NG2-Cre<sup>+</sup>BAC and NG2-CreERT2<sup>+</sup>BAC mice (Zhu et al., 2008a, 2011; Huang et al., 2014, 2018). It was suggested that presumably those reporter<sup>+</sup> astrocytes resulted from the transient activation of NG2 gene in certain radial glial cells (Richardson et al., 2011; Tognatta et al., 2017). However, when NG2-CreERT2<sup>Huang KI</sup> X ROSA26-ttdTomato embryos were analyzed just 2 days post tamoxifen administration, e.g., at E12.5: E14.5 (Huang et al., 2019) or E14.5: E16.5 (Figure 1Aa, and a), all the recombined cells were either PDGFRα<sup>+</sup> OPCs or vascular pericytes, suggesting that reporter<sup>+</sup> astrocytes detected after birth have to be derived from bona fide embryonic NG2 glia rather than radial glial cells. A recent single-cell RNA-Seq study of PDGFRα-CreERT2<sup>Huang KI</sup> BAC mice at E13.5:P3 demonstrated that the data set from reporter<sup>+</sup> cells highly correlated with OLs and astrocytes, indicating again the astrogliogenic potential of embryonic NG2 glia (Marques et al., 2018).

In the spinal cord of NG2-Cre<sup>+</sup>BAC mice, reporter<sup>+</sup> astrocytes were also found in the GM (Zhu et al., 2008b). However, in NG2-CreERT2<sup>Huang KI</sup> KI ROSA26-ttdTomato mice, whenever Cre activity was induced (from E14.5 to adult), reporter<sup>+</sup> cells were always restricted to the Sox10<sup>+</sup> OL lineage cells, suggesting that spinal NG2 glia do not generate astrocytes (Huang et al., 2018). Such discrepancy between those two mouse lines might be attributed to early astrogliogenic progenitors in the spinal cord with transiently...
activated OL genes before E14.5, such as 2',3'-cyclic nucleotide 3' phosphodiesterase (Cnp) (Tognatta et al., 2017).

So far, postnatal fate-mapping studies in healthy mice of several transgenic lines (NG2-CreERT2<sup>Tsoa</sup> KI, PDGFRA-CreERT2<sup>Rob51</sup> PAC, and PDGFRA-CreERT2<sup>Rob51</sup> BAC) showed no incidence for astrogliogenesis from NG2 glia in the CNS after birth. Exceptions were from Olig2-CreERT2<sup>Takahashii</sup> KI and PLP-CreERT2<sup>Duerflinger</sup> TgN mice in the brain and cerebellum (Takebayashi et al., 2002; Doerflinger et al., 2003; Dimou et al., 2008; Guo et al., 2009; Chung et al., 2013). However, the reporter+ astrocytes in postnatal Olig2-CreERT2<sup>Takebayashi</sup> KI mice are mainly due to the direct gene activation of Olig2 in a subpopulation of astrocytes (Cai et al., 2007). In PLP-CreERT2<sup>Duerflinger</sup> TgN mice, recombined astrocytes were detected within three days after tamoxifen administration, suggesting the potential direct/ectopic expression of Cre in astrocytes (Doerflinger et al., 2003; Guo et al., 2009; Tognatta et al., 2017). Thereby, we conclude that only a sub-group of embryonic NG2 glia in the forebrain can generate astrocytes after birth.

Controversial Evidence for a Neurogenic Potential of NG2 Glia

In the early embryonic ventral brain areas (medial ganglionic eminence and anterior entopeduncular area), Nkx2.1+ progenitor cells generate OPCs as well as interneurons, raising the possibility that some embryonic NG2 glia could be neurogenic (Petryniak et al., 2007; Nishiyama et al., 2016). However, in NG2-CreER<sup>z</sup> BACxZ/EG mice, no evidence was found for neurogenesis from NG2 glia (Zhu et al., 2008a). On the contrary, in NG2-Cre<sup>Ad CRE</sup> BAC mice with a more sensitive reporter (Rosa26-LacZ), a significant number of reporter+ interneurons were detected in the postnatal brain, which were assumed as progenies of the NG2/Olig2+ progenitor cells immuno-positive for Cre at E14.5 (Tsoa et al., 2014). In the recent work on NG2-CreERT2<sup>Huang</sup> KI mice, with the sensitive reporter Rosa26-tdTomato, NG2 glia from E12.5 or E14.5 did not generate neurons. Moreover, when Cre activity was induced at later embryonic stages (after E16.5) in NG2-CreERT<sup>Zhu</sup> BAC or NG2-CreERT2<sup>Huang</sup> KI mice, no reporter+ neurons were produced in the postnatal brain (Zhu et al., 2011; Huang et al., 2019). Considering that aberrant transgene activity may also occur in BAC transgenic mice, the reporter+ neurons in NG2-Cre<sup>Ad CRE</sup> BAC mice might be derived from certain neuronal progenitor cells with very low expression level of Cre beneath the detection threshold of immunostaining. Therefore, it is likely that at embryonic stages (at least after E12.5) the NG2 gene is restrictively activated in OPCs and pericytes. Considering NG2 becomes immuno-detectable earliest at age E13.5 (Nishiyama et al., 1996; Diers-Fenger et al., 2001), we conclude that embryonic NG2 glia do not generate neurons.

Also a neurogenic potential of postnatal NG2 glia has been studied intensively. Although reporter+ neurons can be detected in various mouse lines after postnatal Cre activity induction, the existence of neurogenic NG2 glia is still under debate. EdU incorporation experiments demonstrated that virtually all NG2 glia could be labelled by EdU after long-term treatment, indicating that all NG2 glia underwent proliferation (Clarke et al., 2012; Young et al., 2013). Therefore, neurons derived from NG2 glia should have been labelled by any of the thymidine analogues EdU or BrdU. However, the reporter+ neurons detected in NG2-CreERT2<sup>Huang</sup> KI, PDGFRA-CreERT2<sup>Rob51</sup> BACxRosa26-tdTomato, PDGFRA-CreERT2<sup>Rob51</sup> BACxRosa26-EYFP and PDGFRA-CreERT2<sup>z</sup> BACxRosa26-EYFP mice were Edu+ or BrdU negative even after one-month treatment, indicating that those reporter+ neurons were derived from postmitotic cells rather than NG2 glia (Rivers et al., 2008; Kang et al., 2010; Huang et al., 2014). On the contrary, in the hypothalamus of NG2-CreERT2<sup>z</sup> BACxtdTomato mice some reporter+ cells were detected with immunoreactivity to the mature neuronal marker NeuN or neuronal lineage marker HuC/D after adult Cre induction, accumulating to a significant number 60 days later (~4% and ~9% out of all reporter+ cells were NeuN+ and HuC/D+ respectively). In addition, a few HuC/D reporter+ cells demonstrated the incorporation of BrdU (Robins et al., 2013). Given that a small number of neuronal proteins including HuC/D were detected in OL lineage cells (Chittajallu et al., 2004; Clarke et al., 2012), immunostainings for OL lineage markers (such as Sox10 and Olig2) on those BrdU+ or HuC/D+ reporter+ cells would help to better assess their cell types. The number of reporter+ neurons in the brains of NG2-CreERT2<sup>Huang</sup> KI, PDGFRA-CreERT2<sup>Rob51</sup> BACxZ/EG and PDGFRA-CreERT2<sup>Rob51</sup> BACxZ/EG mice did not accumulate over time, further confirming that those neurons were generated from postmitotic cells (Kang et al., 2010; Zhu et al., 2011; Huang et al., 2014). However, the reporter+ neurons in the periformal cortex of PDGFRA-CreERT2<sup>Rosa26</sup> BACxRosa26-EYFP and PLP-CreERT2<sup>Duerflinger</sup> TgN×Rosa26-EYFP mice showed increased numbers of recombined neurons with very low rate (Rivers et al., 2008; Guo et al., 2009, 2010; Clarke et al., 2012). A recent study suggests that these reporter+ neurons might be derived from dormant doublecortin (DCX<sup>+</sup>) progenitors (Rotheinechner et al., 2018). Likewise, very few reporter+ neurons (less than two neurons per cross-section) in the spinal cord of NG2-CreERT2<sup>Huang</sup> KI×Rosa26-tdTomato mice, without incorporation of BrdU, increased in their number after...
If neoglutamic acid 2 glia exist, they should be located in the same brain regions among different mouse lines. However, the distribution pattern of reporter neurons varies in different lines. In adult NG2-CreERT2huang KixRosa26-tdTomato mice, reporter neurons were detected mainly in the cortex where they form clusters in the amygdala, but they are scattered in the dorsal cortex (Figure 1Bb), and could be found rarely in the hypothalamus (Figure 1Bb, -0.5% out of all reporter cells were NeuN), and piriform cortex (Huang et al., 2014). In NG2-CreERT2huang BACxZ/EG mice, reporter neurons appeared sporadically in the neocortex (Zhu et al., 2011). In PDGFRα-CreERT2huang PACxRosa26-EYFP and PLP-CreERT2huang TgNxRosa26-EYFP mice, reporter neurons were mainly located in the piriform cortex (Doerflinger et al., 2003; Rivers et al., 2008; Guo et al., 2009, 2010), while in PDGFRα-CreERT2huang BACxZ/EG mice a few reporter neurons were found in the hypothalamus but never in the piriform cortex (Kang et al., 2010). In Olig2-CreERT2huangKixZ/EG mice, no reporter neurons were discovered (Dimou et al., 2008). It is therefore hard to find a consistent distribution pattern of reporter neurons in common among those mice, suggesting they are derived from different cell populations.

Last, the appearance of morphologically mature reporter neurons happened shortly (within 3 days) post tamoxifen administration without proof of any intermediate transitional stage, suggesting that the reporter expression is due to direct recombination in mature neurons (Kang et al., 2010; Huang et al., 2014). Another explanation for this fast neuronal recombination could be independent from the neurogenic potential of NG2 glia. Recombined neurons could also be resulted from ectopic Cre activity acquired by endocytosis of exosomes potentially released by NG2 glia. In vitro studies have shown that mature OLs release exosomes carrying biomolecules such as myelin proteins (Bakhti et al., 2011; Fitzner et al., 2011; Frühbeis et al., 2019) and might be promoting neuronal long-term maintenance. Cre-carrying exosomes prepared from MOG-iCre mice lead to recombination of loxp sites as indicated by reporter gene activation (Frühbeis et al., 2013). In vivo evidence highlights the uptake of exosomes by neurons (Frühbeis et al., 2013), but the release from OLs in vivo remains to be elusive. Therefore, it is not too tempting to speculate that neurons could be recombined after engulfing Cre (mRNA or/and protein)-carrying exosomes secreted by NG2 glia. Still, whether NG2 glia can release exosomes in vivo remains to be elucidated. Altogether, to date, results from different transgenic mice did not provide adequate evidence for a neurogenic potential of NG2 glia.

Conclusion

In the past decades, the fate of NG2 glia in the CNS has been studied systematically during the development using a variety of transgenic mouse models. It is well established that NG2 glia are the most proliferative cells throughout life and could quickly generate OLs within the early postnatal weeks. The generation of OLs continues throughout life although the rate of OL differentiation declines with age in both GM and WM significantly. The astrogliogenic potential of NG2 glia is regionally restricted to a subgroup of embryonic NG2 glia mainly in the ventral forebrain. Meanwhile, few studies showed a potential neurogenesis from NG2 glia, but the findings were ambiguous. In conclusion, NG2 glia have the potential to generate OLs throughout life and astrocytes in the embryonic brain, but they do not generate neurons.

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