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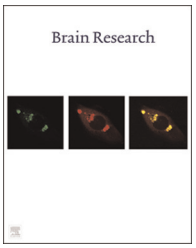

Review

The heterogeneous nature of NG2-glia

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ABSTRACT

In the central nervous system, NG2-glia are the cells responsible for the generation of mature oligodendrocytes during development and adulthood. Some studies could show that NG2-glia can give origin also to astrocytes and neurons, a property that makes them similar to neural stem cells. Beside their important role as progenitors, NG2-glia are believed also to have more functions due to their unique interaction with neurons through synapses. It is however not clear whether these features are common to all NG2-glia or different subpopulations of NG2-glia devoted to different functions exist. Therefore the aim of this review is to highlight the state of the art on NG2-glia heterogeneity from development to adulthood and in different brain areas, and discuss the impact of it on our understanding of the glial neurobiology.

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Contents

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1. Introduction

Since their first identification in the early 80s (Raff et al., 1983), oligodendrocyte progenitor cells (OPCs) have been always described as an abundant glial population widely distributed in the mammalian central nervous system (CNS) at postnatal and adult ages. OPCs have been so far always identified by the common and homogeneous expression of different cellular markers, like the PDGF receptor α and the proteoglycan NG2. However, NG2 expression is also found on pericytes in the brain that are not glial cells and can be easily distinguished from OPCs by their elongated morphology. As OPCs are assumed to have further functions and progeny than only the generation of oligodendrocytes (e.g. during development they also generate astrocytes) (Zhu et al., 2011) we prefer to use the term NG2-glia rather than OPCs. Despite the fact that NG2-glia have been associated to stem cells (Richardson et al., 2011) because of their ability to self-renew (Simon et al., 2011), their long cell cycle length (Psachoulia et al., 2009; Simon et al., 2011) and the generation of more than one cell type (namely astrocytes and neurons, although the generation of the latter one is still under debate), so far their cellular diversity or heterogeneity has never been as deeply discussed (Hill et al., 2013; Tomassy and Fossati, 2014) as for neuronal embryonic and adult stem cells. Along the same line, other glial cells, specifically astrocytes, have been described as a heterogeneous population due to their diverse morphology (fibrous or protoplasmic) and their stem cell-like potential (Bayraktar et al., 2015; Dimou and Gotz, 2014). In contrast, the stellate morphology common to all NG2-glia of the brain has so far probably lead to an underestimation of possible different cellular functions that were however hypothesized by the various electrophysiological properties displayed by these cells (Mangin and Gallo, 2011). In the past years, NG2-glia have been proposed as a good endogenous source for mature and myelinating oligodendrocytes (and maybe also for astrocytes and neurons e.g. after reprogramming) (Guo et al., 2014; Heinrich et al., 2014; Kondo and Raff, 2000; Raff et al., 1983). The advantage of spontaneous or forced differentiation of NG2-glia into different cell types can be of considerable importance to foster repair in case of various diseases or injury of the CNS and not only of demyelinating diseases. To consider such an outcome, we should however understand if all NG2-glia have indeed the same roles and differentiation potential in the developing and adult brain or whether different subtypes are devoted to diverse functions other than the generation of oligodendrocytes. Here we aim to highlight and review the state of the art on NG2-glia heterogeneity from development to adulthood and in different brain areas, to discuss novel technologies to study cellular heterogeneity and the impact that such knowledge could have on our understanding of the mechanisms regulating the behavior of NG2-glia in health and disease.

2. From development to adulthood

The origin of NG2-glia has been mainly characterized in the mouse CNS. In the developing ventral spinal cord NG2-glia appear for the first time at embryonic day 12.5 and are generated from Olig2^{+} motor neuron progenitors (pMN)(Lu et al., 2002; Takebayashi et al., 2002; Zhou and Anderson, 2002) while dorsally they are only generated later, at E15.5. Interestingly, in the ventral spinal cord NG2-glia generation by pMN precursors depends on Sonic-Hedgehog (Shh) activity, whereas no Shh dependency could be observed in the dorsal site (Lu et al., 2002). So far no clear hint is given regarding different potential and/or functions of NG2-glia from these two different origins. In the other area of the CNS, the brain, three waves of NG2-glia generation, spatially and temporally different, have been identified (Kessaris et al., 2006). The first wave overlaps in terms of timing with the first emergence of NG2-glia in the spinal cord (E12.5) and originates from the medial ganglionic eminence, the seconds appears later, at E15.5, in the lateral ganglionic eminence, from where these glial precursors migrate tangentially to populate also the telencephalon. Finally, the third wave arises at P0 from the cerebral cortex (Kessaris et al., 2006). While the cells generated during the first two waves disappear with time, oligodendrocytes generated during the third wave survive and populate the complete brain (Kessaris et al., 2006). Worth noting is that these NG2-glia originate from different neuronal precursors, namely Nkx2.1, Gsh2 and Emx1 precursors for the first, second and third wave, respectively. These precursors generate earlier different classes of neurons, specifically Nkx2.1^{+} and Gsh2^{+} cells give rise to gabaergic interneurons while Emx1^{+} cells generate cortical projection neurons as suggested and discussed by Tomassy and Fossati (2014). The heterogeneousgeneration of neurons during early development could also suggest the generation of different classes of NG2-glia from distinct progenitors. However, the results obtained by Kessaris et al. (2006) argue against this hypothesis, as the direct ablation of NG2-glia generated during one of the waves could be completely compensated by cells originated by the other neural precursors. This strongly suggests that cells originated from different precursors are equivalent/similar or at least plastic enough to replace each other. Moreover, no differences in the progeny of NG2-glia generated in the three different waves have been identified so far in terms of morphology, either in the postnatal or adult brain. Overall, it appears that although developmentally distinct, NG2-glia would represent a rather homogeneous population. However, despite the morphological similarity and developmental compensation, several studies showing generation of astrocytes and neurons by postnatal oligodendrocyte progenitors in vitro and in vivo (Huang et al., 2014; Kondo and Raff, 2000; Zhu et al., 2011) lead again to the questions how homogenous are indeed NG2-glia and whether a reservoir of these cells may retain more stem cell like properties compared to the overall NG2 population. In the same line, postnatal NG2-glia seem more plastic than the adult ones, that under physiological conditions can give

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rise solely to mature oligodendrocytes (Bergles et al., 2000; Clarke et al., 2012; Dimou et al., 2008; Zhu et al., 2011) and to neurons only upon forced reprogramming (Guo et al., 2014; Heinrich et al., 2014). Adult NG2-glia can also proliferate, with a much longer cell cycle compared to postnatal progenitors (Psachoulia et al., 2009; Rivers et al., 2008; Simon et al., 2011; Young et al., 2013). In the same studies, it has also been shown that both postnatal and adult NG2-glia are able to undergo more than one cell cycle (Simon et al., 2011; Zhu et al., 2011); however, it is still unclear if all or only a subpopulation of NG2-glia have this ability. It would indeed be possible that only a particular reservoir of NG2-glia with "more" stem cell-like properties is in charge of keeping the homeostatic network of NG2-glia from development to adulthood, whereas the others may have other functions, among which the best characterized is the generation of mature oligodendrocytes. Along with the temporal differences in proliferation, also differentiation into mature/myelinating oligodendrocytes occurs differentially at diverse ages, with postnatal NG2-glia being faster maturating compared to adult ones (Kang et al., 2010; Rivers et al., 2008; Zhu et al., 2011). This divergence could be explained by the particular extracellular environment of the postnatal mouse cortex compared to the adult one. For example in the first postnatal days OPCs are exposed to an extensive angiogenesis process ongoing in the brain parenchyma that leads to high levels of oxygen and metabolites supply, which in turn support the high level of myelination (Yuen et al., 2014). The contribution of extracellular cues would therefore exclude a major intrinsic variance between postnatal and adult NG2-glia located in the same cortical area. However, it cannot be excluded that intrinsic variance also plays a role, as specific subsets of NG2-glia could show distinct properties in their proliferation and differentiation potential during development. Interestingly, these cells would then specifically expand and survive in the adult brain. An elegant study employing StarTrack analysis could demonstrate that adult NG2-glia are able to generate bigger clones (up to 400 cells per cluster) than during development although they proliferate slower than postnatal ones. The biological significance of this ability is not clear yet but raises questions in regard to the existence of clusters of NG2-glia derived from the same clone/single cell that have a survival advantage compared to other clones, which in turn either get exhausted or terminally differentiated later on (Garcia-Marques et al., 2014). In general terms, the NG2-glia network keeps preserved with aging, and homeostasis is tightly kept even if the mechanisms of self-renewal, proliferation and differentiation get delayed with increased ages (Young et al., 2013). All these processes get strongly accelerated after injury or disease, where adult NG2-glia can resume a certain level of plasticity (Dimou and Gotz, 2014; Dimou and Gallo, 2015; Franklin and Gallo, 2014; Wang and He, 2009). Indeed, also after acute cortical injury, adult NG2-glia show a fast and heterogeneous reaction in terms of proliferation, polarization, hypertrophy and migration (Streitberg and Dimou, unpublished data). It is still unclear to which extent these distinct behaviors are due to intrinsic differences of NG2-glia subpopulations or due to the specific local environment leading to exposure of NG2-glia to different signals, that influence their different behavior. However, as also neighboring NG2-glia show a heterogeneous behavior and hence should receive comparable input of released factors and signaling molecules, the influence of the environment alone is unlikely. This fast reaction, in case of injury or CNS damage, is common to both adult and postnatal cells, equally able to contribute to the more general glia reaction to an insult. Therefore during both, development and adulthood, NG2-glia share many characteristics; however, with the postnatal NG2-glia being more plastic than the adult ones. Understanding the differences among diverse stages of development could help to develop new strategies to force the plasticity of adult NG2-glia, an issue that is very important for repair after injury or disease and that we will discuss later.

2.1. *NG2-glia in gray and white matter*

NG2-glia are homogeneously distributed in all regions of the brain, composing a tight homeostatic network where each cell maintains its spatial domain sensing its microenvironment and the presence of other NG2-glia with its own processes and filopodia (Hughes et al., 2013). Although at macroscopic level no differences are evident among the NG2-glia located in different brain areas in regard to their morphology or expression of different cell markers like NG2 and PDGFRα, a closer analysis of cellular properties revealed that indeed some heterogeneity exists (Fig. 1). Fate mapping analysis using different mouse lines that recombine in NG2-glia (e.g. Olig2^{CreERTM}, PDGFRa-CreER^{T2}, NG2^{CreERT2}) revealed different degrees of differentiation and maturation properties among NG2-glia localized in the white or gray matter of the cerebral and cerebellar cortex of the adult mouse brain, with the former differentiating more extensively than the latter (Dimou et al., 2008; Kang et al., 2010; Rivers et al., 2008; Zhu et al., 2011). To unravel whether these distinct regional differentiation properties are due to intrinsic diversity between cells or due to environmental influence, homotopic and heterotopic transplantations were performed in the adult cerebral cortex. These experiments showed that there is a strong intrinsic determination of white matter NG2-glia towards differentiation compared to gray matter NG2-glia (Vigano et al., 2013). To what extent this determination is a result of developmental origin or to longer exposure to the white matter environment still needs to be resolved. Another interesting trait of white matter NG2-glia is that, besides showing a more pronounced and faster differentiation into oligodendrocytes, they also have a shorter cell cycle length and show a higher proliferative response to PDGFα compared to gray matter cells (Hill et al., 2013; Young et al., 2013). Cells in the white and the gray matter of the cerebral cortex also display different membrane resistance, expression of ion channels and the capacity to elicit immature action potentials (Chittajallu et al., 2004; for review see Dimou and Gallo (2015)). Along this line, Garcia Marquez et al. (2014) showed that some E14 pallial progenitors give rise to a low number of clones that are restricted to the corpus callosum, suggesting a hypothetic intrinsic difference due to their developmental origin compared to cortical gray matter NG2-glia. Other studies showed also that there is a continuous and consistent generation of NG2-glia from postnatal and adult neural stem

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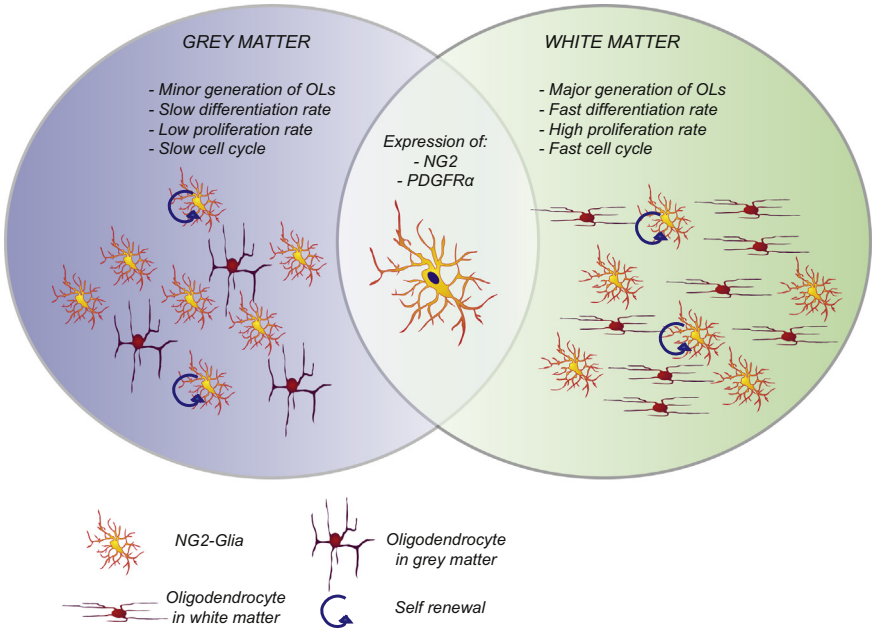

Fig. 1 – Schematic overview of NG2-glia heterogeneity in the cortical gray and white matter: in the adult gray and white matter of the cerebral cortex NG2-glia are commonly expressing NG2 and PDGFRα; however they show some major differences in terms of proliferation and differentiation. While NG2-glia in the gray matter have a long cell cycle length, low proliferation rate and slow differentiation capacity, in the white matter these glial cells are strongly proliferating, have a shorter cell cycle length and faster differentiation rate and generate more mature oligodendrocytes.

cells, and that these newly generated glia migrate from the subventricular zone either to the olfactory bulb or to the corpus callosum (Aguirre and Gallo, 2007; Levison et al., 1993; Levison and Goldman, 1993; Menn et al., 2006; Ortega et al., 2013), further supporting the idea of differential origin of white and gray matter NG2-glia.

Differences in NG2-glia differentiation and proliferation have been additionally observed between cells resident in the brain, spinal cord and optic nerve. Postnatal and adult NG2-glia in all areas are able to generate mature oligodendrocytes, but the rate of differentiation as well as the cell cycle length is prolonged in the spinal cord and in the optic nerve compared to the cortex (Kang et al., 2010; Young et al., 2013). Interestingly, if we specifically concentrate on the white matter of different CNS areas, it is possible to distinguish distinct rates of NG2-glia differentiation into mature oligodendrocytes, showing progressively slower rates in the cerebral cortex, spinal cord and optic nerve (Young et al., 2013). These observations suggest indeed that the environment and the CNS regions, where NG2-glia are located, play an important role in the modulation of their differentiation and proliferation behavior. However, here it is still not clear whether these differences are due to intrinsic determinants or environmental cues. So far, no extensive studies have been conducted on cerebellar NG2-glia (Buffo and Rossi, 2013). It would therefore be interesting to also analyze if the potential of NG2-glia in this area would be more similar to the ones in the cerebral cortex or spinal cord.

The above discussed differentiation properties are concerning the maturation of NG2-glia into mature, myelinating oligodendrocytes. However, fate mapping studies of embryonic NG2-glia in a knock-in NG2CreER^{T2} showed that in the gray matter of the ventral cortex, striatum, thalamus and hypothalamus (but not in the olfactory bulb, cerebellum or brain stem), they could also generate astrocytes at postnatal ages (Huang et al., 2014). Same results were previously obtained with a BAC-transgenic NG2-CreER^{T2} mouse line, showing generation of protoplasmic astrocytes in the ventral forebrain and hippocampus from embryonic but not postnatal or adult NG2-glia (Zhu et al., 2011). Therefore it seems that the astrogenic potential is restricted to a subset of embryonic NG2-glia and that this potential or this subpopulation of cells is lost already at postnatal stages (Huang et al., 2014). The same concept could be translated also to the generation of neurons from NG2-glia in vivo, although the data available in this respect are more controversial (for summary see Table 1). Indeed, some fate mapping analysis showed that NG2-glia in the piriform cortex generate a subset of neurons (Guo et al., 2010; Rivers et al., 2008) while other studies (that were also obtained in one of the laboratories where the former studies were performed) were not able to confirm these results (Clarke et al., 2012; Dimou et al., 2008; Kang et al., 2010; Zhu et al., 2011). Several explanations regarding this discrepancy could be possible like direct expression of CreER by neurons or the transfer of the Cre-recombinase to neurons through exosomes that might be secreted by NG2-glia as it has already been described for oligodendrocytes (Fruhbeis et al., 2013; for review and detailed description of these data see also Dimou and Gallo (2015)). This multi-lineage differentiation potential of NG2-glia should be better investigated and validated in order to

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Table 1 – The table depicts a summary of the data available regarding the generation of neurons by NG2-glia, that were reported using fate mapping studies in transgenic mice. Some of the studies listed (e.g. Dimou et al., 2008; Kang et al., 2010; Zhu et al., 2011; Huang et al., 2014) reported generation of neurons from their fate mapping studies; however this was defined as a result of CreER or CreER$^{T2/TM}$ ectopic expression more than direct generation of neurons from NG2-glia. Cx: cortex; DG: dentate gyrus; OB: olfactory bulb; n.a.: not analyzed.

Cre mouse line	Reporter mouse line	Genetic approach	Postnatal brain	Adult brain	Citation
NG2cre	Z/EG	BAC	NO Cx, forebrain, hippocampus, OB, SVZ, OB	n.a.	Zhu et al., 2008
NG2creER^{TM}	Z/EG gtROSA26R	BAC	NO	NO neocortex	Zhu et al., 2011
NG2CreER^{T2}	Rosa26-YFP Rosa26-tdTomato	Knock in	NO	NO DG, SVZ, OB, Cx, hippocampus	Huang et al., 2014
Olig2-CreER^{TM}	Z/EG; CAG-CAT-GFP	Knock in	n.a.	NO cerebral Cx, DG, OB	Dimou et al., 2008
Plp-CreER^{T2}	Rosa26-YFP	Transgene	+ Dorsal and ventral forebrain, cerebral Cx, and hypothalamus, hippocampus	+ Piriform Cx	Guo et al., 2009; 2010
PDGFRα-CreER^{T2}	Rosa26-YFP	BAC	n.a.	+ Piriform Cx	Rivers et al., 2008
PDGFaR-CreER	Z/EG ROSA26-EYFP ROSA26-mGFP	BAC	NO cerebral Cx, DG, OB, hypothalamus	n.a.	Kang et al., 2010
PDGFaR-CreER^{T2}	Rosa26-YFP	BAC	NO, Cx	NO, Cx	Clarke et al., 2012

determine whether NG2-glia, more than other glial cell types, retain at least some of the stem cell-like properties.

Interestingly, also other parameters have been observed to support a certain level of heterogeneity not only between different regions but also within the very same region of the brain. Indeed, some studies reported the differential expression of proteins in subsets of NG2-glia located in the same area. For instance, Parras et al. (2007) observed the expression of the transcription factor Ascl1, already well studied as an important factor for neuronal fate determination, in a fraction of NG2-glia all over the brain. According to the region analyzed, the proportion of NG2-glia expressing Ascl1 is variable, from 50% in the cortical gray matter to 100% in the corpus callosum (Parras et al., 2007), highlighting the fact that there may be intrinsic differences between cortical gray and white matter cells as well as between cells in the same region (as only subsets of NG2-glia express Ascl1 in the gray matter). More recently also the expression of a G-protein couple receptor 17 (GPR17), involved in the differentiation process of NG2-glia has been shown to characterize only a subgroup of postnatal and adult NG2-glia (Boda et al., 2011; Chen et al., 2009). Whether its expression is temporally regulated in all NG2-glia or whether GPR17 is present only in a subset of cells, needs to be better clarified. The biological significance of the NG2-glia transcriptome and proteome variety and diversity would be easily explained by differential biological functions, that however, as already mentioned previously, have not yet been fully elucidated.

3. Implications of NG2-glia heterogeneity and new approaches to study them

The idea that NG2-glia, at diverse developmental stages but also regionally distinctly located, present not only common features but also different ones has been emerging more and more in the last years. This notion, along with the fact that also other cell types in the central nervous system display a various degree of heterogeneity, makes clear that we should focus our efforts in better determining and defying NG2-glia heterogeneity. As already mentioned, cellular properties and protein profile would suggest that indeed the NG2-glia population is composed of different cell subsets, either having different origins or being primed by the environment where they are hosted. Some efforts to elucidate this dichotomy have been already made. Transplantation of adult oligodendroglia in different cortical regions and experiments on organotypic slice cultures revealed that mainly intrinsic determination but also to some extent environmental influence is important to affect NG2-glia behavior (Hill et al., 2013; Vigano et al., 2013). Transplantations of optic nerve NG2-glia in the spinal cord showed also that these cells that normally would myelinate small caliber axons gain the capacity to myelinate also larger caliber axons, suggesting in this case not only plasticity of NG2-glia but also fundamental influence of the environment on maturation (Fanarraga et al., 1998). Other hetero- or homotopic transplantation experiments would be helpful to shed light on the interchangeability of NG2-glia deriving from various regions and to further understand whether micro-environmental priming would be more

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or less predominant according to the region of study. Postnatal or adult priming of NG2-glia could be identified also by selective isolation of these cells, e.g. by sorting them from different CNS regions and at different developmental stages. Transcriptome and proteome analysis as well as epigenetic profiling would give indeed a better idea on the possible heterogeneous pattern of DNA regulation and transcription in various subsets of NG2-glia. An initial effort in this regard has been made by Zhang and colleagues (and previously also by Cahoy et al. (2008)), that provided a detailed RNA-seq analysis of different differentiation stages of the oligodendrocyte lineage (Zhang et al., 2014). However, a specific comparative transcriptome analysis between embryonic, postnatal and adult NG2-glia, between different regions or different subsets of them at the same age and region, has never been performed. The identification of candidate genes by transcriptional profiling and proteins differentially expressed in regional distinct NG2-glia will be in any case easier than unveiling the possible heterogeneity between cell subsets in the very same CNS region, as the specific markers needed for this type of analysis are largely missing. This on the other hand could be achieved, for example, by alternative investigation of microarray analysis performed on knock-out mouse models of specific transcription factors, a similar approach indeed brought to study the role of GPR17 in NG2-glia maturation (Chen et al., 2009). Different tools in molecular biology and biochemistry are indeed already available to better investigate the proteomic and epigenomic profile of NG2-glia.

What at the moment is still lacking is a general verification of NG2-glia functions by ablation of all or a subset of these cells at any stage of development. Several attempts towards this aim have been reported employing chemical agents, radiation or specific mouse lines inducing cell death in NG2-glia (Birey and Aguirre, 2015; Chari and Blakemore, 2002; Irvine and Blakemore, 2007; Robins et al., 2013); however none of them was successful to ablate the whole NG2-glia population. Recently, a work from McKenzie et al. (2014) showed that specifically the block of newly generated oligodendrocytes in the adult brain is important for learning new complex motor skills. The differentiation of NG2-glia in the adult brain is therefore functionally fundamental for its contribution to neuronal plasticity. Nevertheless, a more complete and specific ablation of NG2-glia might be necessary to gain new insights in further functions besides the generation of mature and myelinating oligodendrocytes. NG2-glia have been indeed shown to have close contact with other glial cells (i.e. astrocytes and microglia) and with neurons (Butt et al., 2002). Of particular interest is that these glia are the only ones able to receive synaptic inputs from neurons (Bergles et al., 2000). The meaning of such a peculiarity among glia populations is not yet resolved. There have been attempts to correlate neuronal activity on NG2-glia with some features of these cells but the original expectations that neuronal inputs would be fundamental for proliferation and oligodendrogenesis could not be confirmed. A study using a genetic mouse model knocking out the NMDA glutamate receptor specifically in NG2-glia showed normal myelin formation and self-renewal of NG2-glia (although with an up-regulation of the AMPA receptor in these cells), pointing to additional roles of neuronal inputs on NG2-glia (De Biase et al., 2011). Thus, more studies will be needed to correlate neuronal activity and NG2-glia functions. Specifically, analysis at single cell level will be necessary also to better understand why in the same cortical area NG2-glia display diverse electrophysiological properties. In vivo real time 2-photon microscopic analysis of NG2-glia behavior has been already shown by Hughes et al. (2013), who introduced and demonstrated for the first time the importance of the homeostatic equilibrium of the NG2-glia network. In this study, general conclusions regarding NG2-glia cellular regulation of proliferation and differentiation have been made; however the analysis was limited to the upper layers of the cortex, a general disadvantage of the method. Hence, before extending the highlights of this work to the behavior of all NG2-glia, new imaging studies should be performed also in other cortical areas, like for instance the white matter or the cerebellum. Finally, to extrapolate better the ideas on single NG2-glia behavior, clonal analysis in adult mice should be performed as it was already done with the StarTrack system for embryonic brains. In this study indeed constructs expressing 12 fluorescent reporters, under the control of the hGFAP promoter, have been electroporated at E14 in order to clonally label progenitors surrounding the lateral ventricle (Garcia-Marques et al., 2014), therefore limiting the final observations probably to a subset of NG2-glia. Regardless this limitation, it was possible to observe that some of these cells could give rise to big clusters of cells in the adult brain with a variance of 40–400 cells. A possible explanation for this huge difference could be that “stem cell like” NG2-glia also exists in the adult brain parenchyma. In order to identify these cells, a combination of different technologies, like clonal analysis, in vivo imaging and transcriptome analysis should be performed.

4. Use of NG2-glia to promote repair and future perspective

A comprehensive understanding of NG2-glia features and functions in the developing and adult CNS is of great importance not only for increasing our basic knowledge of glial cell biology but also to take advantage of this endogenous source of progenitors to foster repair in demyelinating and neurodegenerative diseases. NG2-glia, such as astrocytes and microglia, indeed react to CNS injury and degeneration contributing to tissue response to these insults but have a limited regenerative/repairing capacity (for an extensive review see Gallo and Deneen (2014)). NG2-glia response in terms of proliferation, hypertrophy and migration has been shown and suggested in different types of neuronal insults both in brain and spinal cord (Dimou and Gotz, 2014; Wang and He, 2009). Inflammatory conditions triggered by peripheral cells in case of blood brain barrier destruction or multiple sclerosis also modulate the response of these cells, that seem able not only to respond to but also to release cytokines (for review see Wang and He (2009) and Moyon et al. (2015); and own unpublished data). Reactive NG2-glia, especially those involved in remyelinating processes, upregulate a panel of transcription factors and proteins (i.e. Shh and FGFR1, see Franklin and Gallo (2014)). These data, although very interesting, do not clarify whether all NG2-glia show the

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same response to an insult or if a subset of cells exist as "surveillance" with a stronger reaction than the rest of the NG2-glia. Indeed, thanks to an in vivo analysis employing two-photon microscopy, we observed that NG2-glia do not respond to acute brain injury at the same extent, but on the contrary, their reaction seems to be pretty heterogeneous (Streitberg and Dimou, unpublished data). Up to now, a specific subpopulation of NG2-glia devoted to repair mechanisms, if existing, has not been identified. General transcription profile of NG2-glia present in injured area, like in demyelinating lesions (Moyon et al., 2015), showed at least that activated/reactive NG2-glia share a gene expression pattern more similar to postnatal OPCs than adult oligodendrocytes, suggesting that under pathological conditions NG2-glia could regain their developmental plasticity. Comparative studies on postnatal and adult reactive NG2-glia will be helpful to identify molecular pathways important to trigger differentiation and/or proliferation of NG2-glia and therefore to develop strategies to favor myelination in all CNS diseases characterized by myelin disruption. In the attempts to promote endogenous remyelination, one should also consider the involvement of the other glial cells and the immune system to the course of pathology. This also makes fundamental to investigate with accuracy the fine cross-talk of NG2-glia with astrocytes, microglia and the peripheral immune cells under physiological and pathological conditions. One of the most promising strategies to address demyelinating diseases is the transplantation of progenitor cells able to differentiate into mature oligodendrocytes. This approach has been successfully pursued in rodent models of demyelination not only by using species-specific cells but also with human embryonic progenitors or iPSC derived oligodendrocyte precursors (Sim et al., 2011; Wang et al., 2013; for reviews see Franklin and Gallo (2014) and Goldman et al. (2012)). More recently, encouraging results have been reported also in four children affected by Pelizaeus–Merzbacher disease where human central nervous system stem cells have been implanted in the frontal lobe and patients were followed for one year (Gupta et al., 2012). For a better development of transplantations approaches more key issues should be resolved: 1) a better characterization of the source of progenitors, to understand if they would resemble for instance fast or slow differentiating/proliferating NG2-glia; 2) their migration ability not only during development but also in the adulthood; 3) the possible development of oligodendroglioma and 4) the effects of unsettling the host NG2-glia network homeostasis. Obviously the disadvantages of such approaches should be counter-balanced by the functional amelioration provided. Overall, the full protective and reparative potential of NG2-glia, although now studied since many years, has not yet been understood and most likely information gained by investigating heterogeneity of NG2-glia at different developmental stages and regions will help the optimization of the repair approaches described above.

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