Received: 20 February 2017 | Revised: 23 February 2017 | Accepted: 24 February 2017

DOI: 10.1111/cns.12690

REVIEW ARTICLE

WILEY CNS Neuroscience & Therapeutics

Roles of NG2-glia in ischemic stroke

Fei-Er Song[1] | Jia-Lv Huang[2] | Si-Han Lin[2] | Shuo Wang[2] | Guo-Fen Ma[1] | Xiao-Ping Tong[1]

[1]Discipline of Neuroscience and Department of Anatomy, Histology and Embryology, Shanghai Jiao Tong University School of Medicine, Shanghai, China.

[2]Department of Clinical Medicine, Research-Based Learning training program (RBL2015-29), Shanghai Jiao Tong University School of Medicine, Shanghai, China.

Correspondence
Xiao-Ping Tong, Discipline of Neuroscience and Department of Anatomy, Histology and Embryology, Shanghai Jiao Tong University School of Medicine, Shanghai, China.
Email: xtong@shsmu.edu.cn

Summary

Recent studies have shown that a widely distributed class of glial cells, termed NG2-glia, engages in rapid signaling with surrounding neurons through direct synaptic contacts in the developing and mature central nervous system (CNS). This unique glial cell group has a typical function of proliferating and differentiating into oligodendrocytes during early development of the brain, which is crucial to axon myelin formation. Therefore, NG2-glia are also called oligodendrocyte precursor cells (OPCs). In vitro and in vivo studies reveal that NG2-glia expressing receptors and ion channels demonstrate functional significance for rapid signaling with neuronal synapses and modulation of neuronal activities in both physiological and pathological conditions. Although it is well known that NG2-glia play an important role in demyelinating diseases such as multiple sclerosis, little is known about how NG2-glia or OPCs impact neurons and brain function following ischemic injury. This review summarizes recent progress on the roles of NG2-glia in ischemic stroke and illustrates new approaches for targeting NG2-glia in the brain to treat this disease.

KEYWORDS

ischemia, NG2-glia, oligodendrocyte precursor cells, stroke

1 | INTRODUCTION

NG2-glia, also called oligodendrocyte precursor cells (OPCs), constitute the fifth major cell population in the central nervous system (CNS).[1-3] These cells were first characterized through expression of the chondroitin sulfate proteoglycan (NG2 antigen) in cerebellum since the 1980s.[4-6] To distinguish these cells from the pericytes, which also express NG2 in the CNS,[7] they are now broadly named NG2-glia instead of simply NG2-positive cells.[8,9] NG2-glia can also be found in the literature as polydendrocytes,[1] because of their branched morphology revealed by immunolabeling for NG2 (Figure 1) and the platelet-derived growth factor receptor α (PDGFRα). During early mammalian brain development, NG2-glia play a fundamental role as cell reservoirs for oligodendrocytes, which are crucial for the myelination of axons. Hence, NG2-glia are often equated with oligodendrocyte precursor cells (OPCs). Although NG2-glia are associated with the generation and maintenance of the oligodendrocyte population, De Biase et al. described that both mRNA transcript and glutamate receptors and Nav channel expression in NG2-glia are significantly altered compared with preoligodendrocytes (Pre-OLs) and mature oligodendrocytes (OLs) (Figure 2),[10] further illustrating that NG2-glia are a constitutive distinct cell population in the brain. After recognition that NG2 glial cells are widely distributed in the brain, their functional roles started to draw attention in the brain research field.[11,12] Interestingly, NG2-glia also have direct synaptic contacts with both glutamatergic and GABAergic neurons in adult mammals, suggesting they have as yet to be defined physiological functions by their membrane-expressing ion channels and receptors.[13-15] Furthermore, the morphological, physiological, and biomolecular studies of NG2-glia have shown this cell group is involved in a variety of human CNS pathologies, such as demyelinating multiple sclerosis and ischemia. As NG2-glia demonstrate self-renewal functions as multipotent stem cells and have direct contact with neuronal synapses,[1-3,6,8,10,11,13,15] it raises the possibility that this unique cell group could be a valid therapeutic target for neural disorders.

Stroke is a neural disease clinically manifested by transient or permanent brain dysfunction symptoms. It is caused by various factors

The first two authors contributed equally to this work.

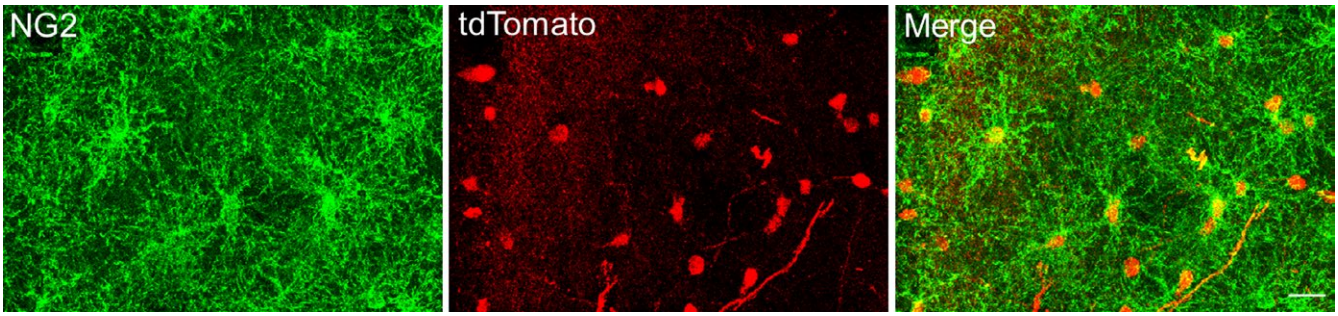

FIGURE 1 Ai9 (RCL-tdT) transgenic mouse strain harbors a targeted mutation of the *Gt(ROSA)26Sor* locus with a *loxP*-flanked STOP cassette preventing transcription of red fluorescent protein variant (tdTomato). NG2-CreBAC transgenic mouse strain expresses Cre recombinase under the control of the mouse chondroitin sulfate proteoglycan 4 (*Cspg4*) promoter/enhancer. When NG2-CreBAC transgenic mice are bred with Ai9 mouse strain, resulting offspring express tdTomato in a pattern dictated by the Cspg4-Cre promoter. As shown in the immunohistochemistry images, NG2-glia with branchy morphology are widely distributed in the mouse brain characterized by NG2 antibody staining (in green) colocalized with tdTomato reporter gene (in red). Scale bar: 20 μm

such as cerebral artery stenosis, occlusion or rupture, and eventually induced acute cerebral blood circulation disorders. As one of the three most common diseases in the world, stroke has a high mortality and disability rates, which severely threaten people's life and health. Ischemic stroke is the most common form, accounting for 87% of strokes. This type of stroke mainly causes impairment of neural cells and ultimately the loss of brain function due to ischemia and hypoxia. However, treatment options to date are very limited. To meet the need for clinical therapeutic approaches, experimental stroke models have been generated. Most researchers use permanent or transient occlusion of the middle cerebral artery (MCAO) in mice or rats to mimic the most common causes of ischemic stroke as seen in patients. Through interruption of blood flow in the entire territory of the artery, the intraluminal MCAO model offers the advantage of inducing reproducible transient or permanent ischemia of the MCAO territory in a relatively noninvasive manner,[16,17] thus giving a convenient experimental ischemia model to study the mechanisms of stroke. This review illustrates the developmental origin of NG2-glia, how NG2-glia change their properties in the pathology of ischemic stroke experimental model, and provides a new insight into the mechanisms of ischemia for clinical therapy.

2 | DEVELOPMENTAL ORIGINS AND HETEROGENEITY OF NG2-GLIA IN THE BRAIN

In the developing brain, NG2-glia emerge in different temporal and regional waves. In vivo fate-mapping analysis demonstrated that the first oligodendrocyte progenitors (OPCs) appear in the cerebral cortex at approximately E16 (embryonic day 16) and migrate from ventral areas of the medial ganglionic eminence. A second wave of NG2-glia arises in the lateral and/or caudal ganglionic eminence to populate the entire cortex by E18 evidenced in a Gsh2-Cre mouse line. Finally, a third wave arises from Emx1-positive cells within the postnatal cortex.[18] Separate studies also showed that the subventicular zone (SVZ), a region derived from the embryonic lateral eminence and lateral cortex, is the major source of NG2-glia and oligodendrocytes in the postnatal brain.[19,20] However, this hypothesis has been challenged recently by Ortega et al., who demonstrated that NG2-glia are generated by distinct stem cells from the dorsal wall of the ventricle by using live imaging and single-cell tracking.[21]

NG2-glia are prone to be intrinsically heterogeneous cell populations, with different developmental and physiological properties, environmental influences and multiple generating regions. Although NG2-glia can divide, gray matter (GM) cells have a longer cell cycle than their white matter (WM) counterparts.[22] For instance, the majority of adult NG2-glia located in the white matter (WM) of the cerebral cortex differentiate into mature, myelinating oligodendrocytes. However, NG2-glia in gray matter (GM) generate fewer mature oligodendrocytes, where the two NG2-glia cell populations have distinct intrinsic properties.[23-26] Moreover, the transcription factor Ascl1 and G-protein-coupled receptor GPR17 are also found expressed by a subset of NG2-glia.[27-30] For example, in the cortical gray matter, only 50% of NG2-glia express the transcription factor achaete-scute homolog 1 or mammalian achaete-scute homolog 1 (Ascl1 or Mash1), an important factor for neuronal fate determination,[28] further supporting the idea of the heterogeneity of NG2-glia in the brain.[27-30]

3 | REACTIVE NG2-GLIA DURING ISCHEMIA

NG2-glia respond to traumatic injuries, including stab wound lesions,[23,31,32] spinal cord injury,[33] and ischemia.[34] It was found that the number of NG2-glia was significantly decreased in infarct core area, whereas they were greatly increased in peri-infarct area, termed penumbra after focal ischemia in rat brain.[35] However, the features and the time course of their responses are strongly dependent upon the nature of the insult and the developmental stage at which this occurs. Ahrendsen et al. have reported age-related changes of NG2-glia in white matter vulnerability to ischemia. They found OPCs (NG2-glia)

(A)

Affymetrix probe ID	Gene symbol	Gene title	OPC vs. Pre-OL		OPC vs. OL	
			Fold change	*P* value	Fold change	*P* value
1438946_at	Pdgfra	Platelet derived growth factor receptor, alpha polypeptide	4.8	.02	62.7	1.40E-08
1423341_at	Cspg4	Chondroitin sulfate proteoglycan 4	5.2	.007	151.6	3.00E-06
1418980_a_at	Cnp	2',3'-cyclic nucleotide 3' phosphodiesterase	−9.7	5.00E-05	−11.9	6.00E-06
1451961_a_at	Mbp	Myelin basic protein	−155.2	2.50E-08	−192.1	5.00E-07
1448768_at	Mog	Myelin oligodendrocyte glycoprotein	−190.9	3.80E-05	−733.6	3.00E-07
1460219_at	Mag	Myelin-associated glycoprotein	−182	2.00E-05	−238.7	1.00E-06
1421010_at	Mobp	Myelin-associated oligodendrocytic basic protein	−487.2	8.00E-06	−703.3	2.00E-06
1450121_at	Scn1a	Sodium channel, voltage-gated, type I, alpha	4.9	.02	18	2.00E-05
1427280_at	Scn2a1	Sodium channel, voltage-gated, type II, alpha 1	3.7	.007	6.8	.001
1439204_at	Scn3a	Sodium channel, voltage-gated, type III, alpha	2.8	.008	19	1.00E-04
1450557_at	Scn4a	Sodium channel, voltage-gated, type IV, alpha	1.1	.5	−1	.9
1422194_at	Scn5a	Sodium channel, voltage-gated, type V, alpha	−1.1	.02	−1.3	.04
1436044_at	Scn7a	Sodium channel, voltage-gated, type VII, alpha	1.1	.01	1	.7
1439889_at	Scn8a	Sodium channel, voltage-gated, type VIII, alpha	1.3	.06	1.9	.02
1421660_at	Scn9a	Sodium channel, voltage-gated, type IX, alpha	−1.3	.04	−1.3	.04
1450266_at	Scn10a	Sodium channel, voltage-gated, type X, alpha	1.1	.09	−1	.8
1420784_at	Scn11a	Sodium channel, voltage-gated, type XI, alpha	1	.8	−1.3	.2
1435239_at	Gria1	Glutamate receptor, ionotropic, AMPA1 (alpha 1)	5.8	.008	22.1	9.00E-05
1453098_at	Gria2	Glutamate receptor, ionotropic, AMPA2 (alpha 2)	1.7	.003	6.1	.0004
1434728_at	Gria3	Glutamate receptor, ionotropic, AMPA3 (alpha 3)	2.2	.03	46.3	5.00E-05
1435722_at	Gria4	Glutamate receptor, ionotropic, AMPA4 (alpha 4)	1.9	.02	3.2	.002
1437968_at	Grin1	Glutamate receptor, ionotropic, NMDA1 (zeta 1)	2.9	.004	4.7	.002
1421616_at	Grin2a	Glutamate receptor, ionotropic, NMDA2A (epsilon 1)	1	.7	−1	.9
1431700_at	Grin2b	Glutamate receptor, ionotropic, NMDA2B (epsilon 2)	−1.1	.08	−1.1	.09
1449245_at	Grin2c	Glutamate receptor, ionotropic, NMDA2C (epsilon 3)	1.1	.8	−1.2	.04
1442328_at	Grin2d	Glutamate receptor, ionotropic, NMDA2D (epsilon 4)	1.7	.1	1.9	.02
1436575_at	Grin3a	Glutamate receptor ionotropic, NMDA3A	5.1	.002	50	1.00E-05

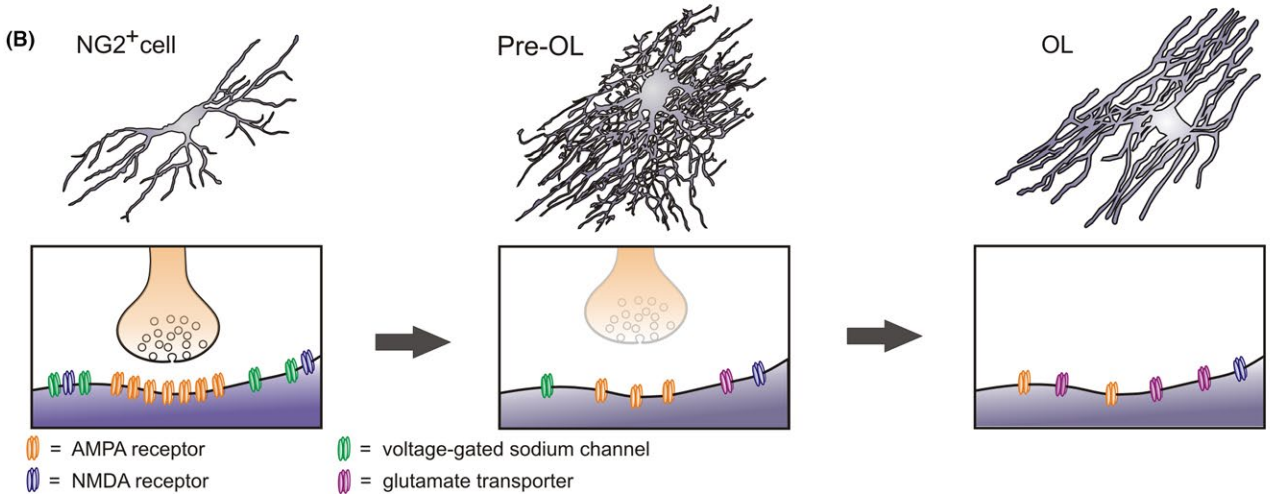

FIGURE 2 Gene expression profiling shows that mRNAs encoding glutamate receptor and NaV channel subunits decrease as NG2-glia differentiate. (A) Table showing mRNA transcripts that are significantly altered in NG2-glia (OPCs) compared with pre-OLs and OLs. Transcripts that should be highly enriched in NG2-glia (eg, PDGFαR and CSPG4/NG2) and mature oligodendrocytes (CNP, MBP, MOG, MAG, MOBP) are shown at the top. For each transcript, the Affymetrix probe set showing the most significant alteration across differentiation is shown. Transcripts significantly enriched in OPCs are highlighted in red. Transcripts significantly downregulated in OPCs are highlighted in blue. Some gene names have been shortened to optimize space, fold changes have been rounded to the nearest decimal, and *P* values have been rounded up.(B) Diagram illustrating the morphological changes (top) that NG2 cells undergo as they differentiate into oligodendrocytes. Bottom, Extent of synaptic signaling and relative abundance of surface glutamate receptors and NaV channels during these distinct stages of oligodendrocyte development

appeared highly resistant to ischemic damage in the juvenile striatum, while the number of PLP-EGFP-positive OPCs was significantly reduced in adult mice as early as 24 hours posttransient middle cerebral artery occlusion (tMCAO) model.[36] Moreover, the OPC numbers were significantly elevated and remained increased in the injured juvenile striatum at 7 days post-tMCAO, which indicates OPCs may respond

to injury and increase their proliferation rate.[9,36] In adults, NG2-glia undergo morphological changes around ischemic lesions. For example, NG2-glia showed enlarged cell bodies with hypertrophied processes after 90 minutes MCAO followed by 2 weeks reperfusion in rat brain.[32,35,37]

In addition to the cell numbers and morphological changes of NG2-glia in ischemic infarction, Boda et al. reported that one type of NG2-glia become reactive and can "sense" brain damage by expressing GPR17 in adult brain.[29] GPR17 is a deorphanized receptor for both uracil nucleotides and cystein leukotrienes, cysLTs (eg, UDP-glucose and LTD4).[38] Both ligands for GPR17 are secreted after brain injury. GPR17-positive NG2-glia in the adult brain increase in density after acute cortical stab wound injury in the gray matter surrounding the lesion and in the white matter underneath the lesion. BrdU-based fate-mapping of GPR17-positive NG2-glia has shown that these cells robustly differentiate into oligodendrocytes to repair the injured tissues after stab wound injury and in the MCAO ischemia model.[29,39]

4 | ALTERATION OF POTASSIUM CHANNELS IN ISCHEMIA

NG2-glia in both gray and white matter exhibit voltage-dependent K^+ currents, which consist of initial rectifying potassium currents (*IA*) and sustained rectifying currents (*IK*).[40,41] Pathological evidence indicates that NG2 cells are particularly susceptible to perinatal hypoxic-ischemia brain damage (HIBD), which results in decreased myelination and infant cerebral palsy. Chen et al. reported that the failed rectification of K^+ channels leads to depolarized membrane potentials of NG2-glia due to changes in K^+ channel permeability, which results in transmembrane cation flow activation and edema. The instability of the NG2-glia perturbs the K^+ concentration gradient in the extracellular space and may be the primary cause of irreversible metabolic disorders in HIBD.[42] Inwardly rectifying K^+ channel, namely the Kir4.1 channel, is well known to be expressed in astrocytes and maintains the resting membrane potentials and plays a fundamental role in the pathology of Huntington disease.[43,44] However, there is a study which found that after 3 days of ischemia in adult rat hippocampus, NG2-glia showed weakly inwardly rectifying K^+ channel current impaired, whereas the cell proliferative activity was largely increased.[45] This sequential correlation indicates that the altered homeostasis of K^+ channel triggered by ischemic injury may induce NG2-glia proliferation. However, it also could be caused by NG2-glia migrating toward the injured core, as previously reported by Tong et al.[12] The Tong group in a recent study found a similar phenomenon in adult mouse brain, that is, the inwardly rectifying potassium channel Kir4.1 in NG2-glia was significantly impaired in tMCAO as evidenced by electrophysiological recordings from acute hippocampal tissue slices (unpublished). Taken together, the altered Kir4.1 channel function in NG2-glia at the early stage of ischemia could be the causative factor of glial and neuronal cell loss.

5 | EXCITOTOXICITY INDUCED NG2-GLIA CELL DEATH IN ISCHEMIA

In both white and gray matter of the brain, neuronal cell death is often caused by a rise of extracellular glutamate concentration under ischemic conditions, which activates N-methyl-D-aspartate receptors and leads to an excessive rise of intracellular Ca^{2+} concentrations. Oka et al. showed that excessive glutamate release can induce NG2-glia death in an in vitro system and found that 24 hours exposure to glutamate caused NG2-glia death by reversing cystine-glutamate exchange and glutathione depletion.[46] However, in the developing cerebral white matter, Deng et al. found that prior exposure of OPCs to sublethal oxygen-glucose deprivation (OGD) resulted in enhanced vulnerability associated with an increased Ca^{2+} influx, which is directly due to aberrantly enhanced activation of Ca^{2+}-permeable AMPA/kainate receptors.[47] In addition to Ca^{2+} overload-induced NG2-glia cell death in stroke, Bcl-2/E1B-19K-interacting protein 3 (BNIP3), a proapoptotic member of the Bcl-2 family proteins, has been known as inducing neuronal death in a caspase-independent manner in stroke.[48] In primary OPC cultures exposed to oxygen-glucose deprivation, BNIP3 was also found upregulated, and the high expression level of BNIP3 was correlated with the death of OPCs. Knockout of BNIP3 significantly reduced death of OPCs in the MCAO mouse model. This study provided further evidence of the molecular pathway underlying NG2-glia cell death in ischemia.[49] Furthermore, when Lee et al. compared the response of glial cell populations to focal ischemia and reperfusion-induced tissue damage, the immunoreactivity to the NG2 protein, but not astrocyte or microglia nor myelinating oligodendrocytes showed degradation during the early postischemic reperfusion following 3 hours MCAO in adult rat brain.[50] Taken together, these findings suggest that NG2-glia are possibly more vulnerable to severe ischemia and might be a crucial factor for axon's demyelination and consequent neuronal loss.

6 | NG2-GLIA FATE CONVERSION IN ISCHEMIC DEMARCATION ZONE

NG2-glia are widely accepted as a distinct glial cell population giving rise to oligodendrocytes by NG2 cell fate-mapping studies[1-6]; however, emerging findings from genomics and epigenetic studies showed reactive NG2-glia can differentiate into GFAP-labeled astrocytes and DCX-expressing immature neurons in a stab wound injury.[51-53] The transcriptional gene changes occurred in NG2-glia in response to injury and environmental signals could lead to depression of conservative genes such as oligo 1/2 and PLP, which are normally restricted to oligodendrocyte lineages and facilitate reprograming of NG2-glia into different cell types intrinsically.[54] On the other hand, NG2 gene can be activated and upregulated by transcriptional factors in other cell type in injured brain. In a transient MCAO model which caused large ischemic lesions in the basal ganglia and adjacent cerebral cortex, Sugimoto et al. reported that the cells termed BINCs (brain Iba1+/NG2+ cells) are largely accumulated in the demarcation zone between

the peri-infarct tissue and lesion core area in rat brain.[55] BINCs which express both NG2 and macrophage markers can be found in pathologic brains, such as 6-OHDA-induced Parkinsonism,[56,57] lipopolysaccharide (LPS)-induced systemic inflammation,[58] or lysolecithin-induced demyelination.[59] In normal conditions, microglial cell somata do not adhere to neurons, but give way to NG2-glia migration capability.[60,61] The upregulated NG2 proteoglycan derived by transforming growth factor-β1(TGF-β1) in activated microglia transforms "NG2-positive microglia" attaching to neurons, suggesting that BINCs actively migrate toward damaged neurons exerting a tissue remodeling process in ischemia.

7 | CONCLUDING REMARKS

Stroke is a leading cause of mortality and morbidity worldwide, with ischemia representing 87% of stroke cases. Researchers have been seeking therapeutic approaches to conquer this disease and attempt to extend the patients' life span. One way of seeking cures for the disease is to first fully understand the pathology of the disease in the brain. In ischemic stroke, we provided evidence that NG2-glia experience morphological changes, altered ion channels and membrane receptors, gene-regulated cells reprograming, as well as excitoxicity-induced cell death. These alterations have direct and/or indirect effects on neurons and other glial cell populations during and after ischemia. By overexpression of Netrin-1 or CXCL12 gene in OPCs, studies showed those factors promote OPCs proliferation, migration, axon remyelination, further facilitating white matter repair and remodeling, which provides a therapeutic perspective for targeting NG2-glia in ischemia.[62,63] These conclusions, however, need to be further confirmed in vivo. A recent study reported that NG2-glia can be reprogramed into both glutamatergic and GABAergic neurons after NeuroD1 expression in the brain, opening a completely new direction in the therapy of neurodegenerative diseases.[64]

In the past two decades, NG2-glia have attained recognition in exerting multiple functions in both normal and pathological conditions in the brain. In addition to the fact that NG2-glia constitute a ubiquitous glial population, which are distinct from astrocytes, oligodendrocytes, and microglia in the CNS, they have multipotent properties of self-renewal and repair in many kinds of brain injuries.[23,31-34] In response to injury, NG2 glial cells are not only capable to proliferate and migrate to the lesions but also differentiate into oligodendrocytes to form new myelin sheaths wrapping around damaged axons and leading to functional recovery.[65,66] It might also be the case when studying NG2-glia function in stroke. In conclusion, given that NG2-glia are actively involved in fast response to neuronal diseases, we can suggest that future studies of NG2-glia will unravel a potential therapeutic target in the treatment of ischemia.

ACKNOWLEDGMENTS

This work is supported by grants from the Natural Science Foundation of China (31571063), the program for Professor of Special Appointment (Eastern Scholar for Dr. X.T.) at Shanghai Institutions for Higher Learning (1510000084), Shanghai Pujiang Program (15PJ1404600). Special thanks to Dr. Dwight E. Bergles for providing Figure 2 image of the review, Dr. Carlos Cepeda and Mr. William R. Bazzarre for critical comments during manuscript writing.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

1. Nishiyama A, Watanabe M, Yang Z, Bu J. Identity, distribution, and development of polydendrocytes: NG2-expressing glial cells. *J Neurocytol*. 2002;31:437-455.
2. Dawson MR, Polito A, Levine JM, Reynolds R. NG2-expressing glial progenitor cells: an abundant and widespread population of cycling cells in the adult rat CNS. *Mol Cell Neurosci*. 2003;24:476-488.
3. Nishiyama A, Komitova M, Suzuki R, Zhu X. Polydendrocytes (NG2 cells): multifunctional cells with lineage plasticity. *Nat Rev Neurosci*. 2009;10:9-22.
4. Levine JM, Card JP. Light and electron microscopic localization of a cell surface antigen (NG2) in the rat cerebellum: association with smooth protoplasmic astrocytes. *J Neurosci*. 1987;7:2711-2720.
5. Levine JM, Stallcup WB. Plasticity of developing cerebellar cells in vitro studied with antibodies against the NG2 antigen. *J Neurosci*. 1987;7:2721-2731.
6. Reynolds R, Hardy R. Oligodendroglial progenitors labeled with the O4 antibody persist in the adult rat cerebral cortex in vivo. *J Neurosci Res*. 1997;47:455-470.
7. Ozerdem U, Grako KA, Dahlin-Huppe K, Monosov E, Stallcup WB. NG2 proteoglycan is expressed exclusively by mural cells during vascular morphogenesis. *Dev Dyn*. 2001;222:218-227.
8. Dimou L, Gallo V. NG2-glia and their functions in the central nervous system. *Glia*. 2015;63:1429-1451.
9. Levine J. The reactions and role of NG2 glia in spinal cord injury. *Brain Res*. 2016;1638:199-208.
10. De Biase LM, Nishiyama A, Bergles DE. Excitability and synaptic communication within the oligodendrocyte lineage. *J Neurosci*. 2010;30:3600-3611.
11. Levine JM, Reynolds R, Fawcett JW. The oligodendrocyte precursor cell in health and disease. *Trends Neurosci*. 2001;24:39-47.
12. Tong XP, Li XY, Zhou B, et al. Ca(2+) signaling evoked by activation of Na(+) channels and Na(+)/Ca(2+) exchangers is required for GABA-induced NG2 cell migration. *J Cell Biol*. 2009;186:113-128.
13. Lin SC, Bergles DE. Synaptic signaling between GABAergic interneurons and oligodendrocyte precursor cells in the hippocampus. *Nat Neurosci*. 2004;7:24-32.
14. Ge WP, Yang XJ, Zhang Z, et al. Long-term potentiation of neuron-glia synapses mediated by Ca2+-permeable AMPA receptors. *Science*. 2006;312:1533-1537.
15. Bergles DE, Roberts JD, Somogyi P, Jahr CE. Glutamatergic synapses on oligodendrocyte precursor cells in the hippocampus. *Nature*. 2000;405:187-191.
16. Longa EZ, Weinstein PR, Carlson S, Cummins R. Reversible middle cerebral artery occlusion without craniectomy in rats. *Stroke*. 1989;20:84-91.
17. Sommer CJ. Ischemic stroke: experimental models and reality. *Acta Neuropathol*. 2017;133:245-261.
18. Kessaris N, Fogarty M, Iannarelli P, Grist M, Wegner M, Richardson WD. Competing waves of oligodendrocytes in the forebrain and postnatal elimination of an embryonic lineage. *Nat Neurosci*. 2006;9:173-179.

19. Aguirre A, Gallo V. Reduced EGFR signaling in progenitor cells of the adult subventricular zone attenuates oligodendrogenesis after demyelination. *Neuron Glia Biol*. 2007;3:209-220.
20. Menn B, Garcia-Verdugo JM, Yaschine C, Gonzalez-Perez O, Rowitch D, Alvarez-Buylla A. Origin of oligodendrocytes in the subventricular zone of the adult brain. *J Neurosci*. 2006;26:7907-7918.
21. Ortega F, Gascon S, Masserdotti G, et al. Oligodendrogliogenic and neurogenic adult subependymal zone neural stem cells constitute distinct lineages and exhibit differential responsiveness to Wnt signalling. *Nat Cell Biol*. 2013;15:602-613.
22. Psachoulia K, Jamen F, Young KM, Richardson WD. Cell cycle dynamics of NG2 cells in the postnatal and ageing brain. *Neuron Glia Biol*. 2009;5:57-67.
23. Dimou L, Simon C, Kirchhoff F, Takebayashi H, Gotz M. Progeny of Olig2-expressing progenitors in the gray and white matter of the adult mouse cerebral cortex. *J Neurosci*. 2008;28:10434-10442.
24. Kang SH, Fukaya M, Yang JK, Rothstein JD, Bergles DE. NG2+ CNS glial progenitors remain committed to the oligodendrocyte lineage in postnatal life and following neurodegeneration. *Neuron*. 2010;68:668-681.
25. Simon C, Gotz M, Dimou L. Progenitors in the adult cerebral cortex: cell cycle properties and regulation by physiological stimuli and injury. *Glia*. 2011;59:869-881.
26. Vigano F, Mobius W, Gotz M, Dimou L. Transplantation reveals regional differences in oligodendrocyte differentiation in the adult brain. *Nat Neurosci*. 2013;16:1370-1372.
27. Battiste J, Helms AW, Kim EJ, et al. Ascl1 defines sequentially generated lineage-restricted neuronal and oligodendrocyte precursor cells in the spinal cord. *Development*. 2007;134:285-293.
28. Parras CM, Hunt C, Sugimori M, Nakafuku M, Rowitch D, Guillemot F. The proneural gene Mash1 specifies an early population of telencephalic oligodendrocytes. *J Neurosci*. 2007;27:4233-4242.
29. Boda E, Vigano F, Rosa P, et al. The GPR17 receptor in NG2 expressing cells: focus on in vivo cell maturation and participation in acute trauma and chronic damage. *Glia*. 2011;59:1958-1973.
30. Zhang Y, Chen K, Sloan SA, et al. An RNA-sequencing transcriptome and splicing database of glia, neurons, and vascular cells of the cerebral cortex. *J Neurosci*. 2014;34:11929-11947.
31. Buffo A, Vosko MR, Erturk D, et al. Expression pattern of the transcription factor Olig2 in response to brain injuries: implications for neuronal repair. *Proc Natl Acad Sci U S A*. 2005;102:18183-18188.
32. Levine JM. Increased expression of the NG2 chondroitin-sulfate proteoglycan after brain injury. *J Neurosci*. 1994;14:4716-4730.
33. McTigue DM, Wei P, Stokes BT. Proliferation of NG2-positive cells and altered oligodendrocyte numbers in the contused rat spinal cord. *J Neurosci*. 2001;21:3392-3400.
34. Zhang R, Chopp M, Zhang ZG. Oligodendrogenesis after cerebral ischemia. *Front Cell Neurosci*. 2013;7:201.
35. Tanaka K, Nogawa S, Ito D, et al. Activation of NG2-positive oligodendrocyte progenitor cells during post-ischemic reperfusion in the rat brain. *NeuroReport*. 2001;12:2169-2174.
36. Ahrendsen JT, Grewal HS, Hickey SP, et al. Juvenile striatal white matter is resistant to ischemia-induced damage. *Glia*. 2016;64:1972-1986.
37. Di Bello IC, Dawson MR, Levine JM, Reynolds R. Generation of oligodendroglial progenitors in acute inflammatory demyelinating lesions of the rat brain stem is associated with demyelination rather than inflammation. *J Neurocytol*. 1999;28:365-381.
38. Lecca D, Trincavelli ML, Gelosa P, et al. The recently identified P2Y-like receptor GPR17 is a sensor of brain damage and a new target for brain repair. *PLoS ONE*. 2008;3:e3579.
39. Zhao B, Zhao CZ, Zhang XY, et al. The new P2Y-like receptor G protein-coupled receptor 17 mediates acute neuronal injury and late microgliosis after focal cerebral ischemia in rats. *Neuroscience*. 2012;202:42-57.
40. Sontheimer H, Kettenmann H. Heterogeneity of potassium currents in cultured oligodendrocytes. *Glia*. 1988;1:415-420.
41. Knutson P, Ghiani CA, Zhou JM, Gallo V, McBain CJ. K^+ channel expression and cell proliferation are regulated by intracellular sodium and membrane depolarization in oligodendrocyte progenitor cells. *J Neurosci*. 1997;17:2669-2682.
42. Chen P, Wang L, Deng Q, Ruan H, Cai W. Alteration in rectification of potassium channels in perinatal hypoxia ischemia brain damage. *J Neurophysiol*. 2015;113:592-600.
43. Tang X, Taniguchi K, Kofuji P. Heterogeneity of Kir4.1 channel expression in glia revealed by mouse transgenesis. *Glia*. 2009;57:1706-1715.
44. Tong X, Ao Y, Faas GC, et al. Astrocyte Kir4.1 ion channel deficits contribute to neuronal dysfunction in Huntington's disease model mice. *Nat Neurosci*. 2014;17:694-703.
45. Pivonkova H, Benesova J, Butenko O, Chvatal A, Anderova M. Impact of global cerebral ischemia on K^+ channel expression and membrane properties of glial cells in the rat hippocampus. *Neurochem Int*. 2010;57:783-794.
46. Oka A, Belliveau MJ, Rosenberg PA, Volpe JJ. Vulnerability of oligodendroglia to glutamate: pharmacology, mechanisms, and prevention. *J Neurosci*. 1993;13:1441-1453.
47. Deng W, Rosenberg PA, Volpe JJ, Jensen FE. Calcium-permeable AMPA/kainate receptors mediate toxicity and preconditioning by oxygen-glucose deprivation in oligodendrocyte precursors. *Proc Natl Acad Sci USA*. 2003;100:6801-6806.
48. Velde CV, Cizeau J, Dubik D, et al. BNIP3 and genetic control of necrosis-like cell death through the mitochondrial permeability transition pore. *Mol Cell Biol*. 2000;20:5454-5468.
49. Li C, Guan T, Chen X, et al. BNIP3 mediates pre-myelinating oligodendrocyte cell death in hypoxia and ischemia. *J Neurochem*. 2013;127:426-433.
50. Lee DR, Helps SC, Gibbins IL, Nilsson M, Sims NR. Losses of NG2 and NeuN immunoreactivity but not astrocytic markers during early reperfusion following severe focal cerebral ischemia. *Brain Res*. 2003;989:221-230.
51. Tatsumi K, Takebayashi H, Manabe T, et al. Genetic fate mapping of Olig2 progenitors in the injured adult cerebral cortex reveals preferential differentiation into astrocytes. *J Neurosci Res*. 2008;86:3494-3502.
52. Komitova M, Serwanski DR, Lu QR, Nishiyama A. NG2 cells are not a major source of reactive astrocytes after neocortical stab wound injury. *Glia*. 2011;59:800-809.
53. Heinrich C, Bergami M, Gascon S, et al. Sox2-mediated conversion of NG2 glia into induced neurons in the injured adult cerebral cortex. *Stem Cell Reports*. 2014;3:1000-1014.
54. Nishiyama A, Boshans L, Goncalves CM, Wegrzyn J, Patel KD. Lineage, fate, and fate potential of NG2-glia. *Brain Res*. 2016;1638:116-128.
55. Sugimoto K, Nishioka R, Ikeda A, et al. Activated microglia in a rat stroke model express NG2 proteoglycan in peri-infarct tissue through the involvement of TGF-beta1. *Glia*. 2014;62:185-198.
56. Choudhury ME, Sugimoto K, Kubo M, et al. A cytokine mixture of GM-CSF and IL-3 that induces a neuroprotective phenotype of microglia leading to amelioration of (6-OHDA)-induced parkinsonism of rats. *Brain Behav*. 2011;1:26-43.
57. Kitamura Y, Inden M, Minamino H, Abe M, Takata K, Taniguchi T. The 6-hydroxydopamine-induced nigrostriatal neurodegeneration produces microglia-like NG2 glial cells in the rat substantia nigra. *Glia*. 2010;58:1686-1700.
58. Gao Q, Lu J, Huo Y, Baby N, Ling EA, Dheen ST. NG2, a member of chondroitin sulfate proteoglycans family mediates the inflammatory response of activated microglia. *Neuroscience*. 2010;165:386-394.
59. Kucharova K, Chang Y, Boor A, Yong VW, Stallcup WB. Reduced inflammation accompanies diminished myelin damage and repair in the NG2 null mouse spinal cord. *J Neuroinflammation*. 2011;8:158.

60. Grako KA, Stallcup WB. Participation of the NG2 proteoglycan in rat aortic smooth muscle cell responses to platelet-derived growth factor. *Exp Cell Res*. 1995;221:231-240.
61. Burg MA, Nishiyama A, Stallcup WB. A central segment of the NG2 proteoglycan is critical for the ability of glioma cells to bind and migrate toward type VI collagen. *Exp Cell Res*. 1997;235:254-264.
62. He X, Li Y, Lu H, Zhang Z, Wang Y, Yang GY. Netrin-1 overexpression promotes white matter repairing and remodeling after focal cerebral ischemia in mice. *J Cereb Blood Flow Metab*. 2013;33:1921-1927.
63. Li Y, Tang G, Liu Y, et al. CXCL12 gene therapy ameliorates ischemia-induced white matter injury in mouse brain. *Stem Cells Transl Med*. 2015;4:1122-1130.
64. Guo Z, Zhang L, Wu Z, Chen Y, Wang F, Chen G. In vivo direct reprogramming of reactive glial cells into functional neurons after brain injury and in an Alzheimer's disease model. *Cell Stem Cell*. 2014;14:188-202.
65. Huang JK, Franklin RJ. Current status of myelin replacement therapies in multiple sclerosis. *Prog Brain Res*. 2012;201:219-231.
66. Vidaurre OG, Liu J, Haines J, Sandoval J, Nowakowski R, Casaccia P. An integrated approach to design novel therapeutic interventions for demyelinating disorders. *Eur J Neurosci*. 2012;35:1879-1886.

How to cite this article: Song F-E, Huang J-L, Lin S-H, Wang S, Ma G-F, Tong X-P. Roles of NG2-glia in ischemic stroke. *CNS Neurosci Ther*. 2017;23:547–553. https://doi.org/10.1111/cns.12690