Published in final edited form as:

Transl Stroke Res. 2011 September ; 2(3): 366–375. doi:10.1007/s12975-011-0078-0.

Oligogenesis and Oligodendrocyte Progenitor Maturation Vary in Different Brain Regions and Partially Correlate with Local Angiogenesis after Ischemic Stroke

Lidan Jiang,

Center for Cerebrovascular Research Department of Anesthesia and Perioperative Care
University of California, San Francisco 1001 Potrero Avenue Room 3C-38 San Francisco, CA
94110, USA

Fanxia Shen,

Center for Cerebrovascular Research Department of Anesthesia and Perioperative Care
University of California, San Francisco 1001 Potrero Avenue Room 3C-38 San Francisco, CA
94110, USA

Vincent Degos,

Center for Cerebrovascular Research Department of Anesthesia and Perioperative Care
University of California, San Francisco 1001 Potrero Avenue Room 3C-38 San Francisco, CA
94110, USA

Marcus Schonemann,

Center for Reproductive Science University of California, San Francisco 513 Parnassus Avenue
San Francisco, CA 94143

Samuel J. Pleasure,

Department of Neurology Programs in Neuroscience, Developmental Biology and Biomedical
Sciences UCSF Institute for Regeneration Medicine Room 448C, Rock Hall 1550 4th Street San
Francisco, CA 94158-2324

Synthia H. Mellon,

Center for Reproductive Science University of California, San Francisco 513 Parnassus Avenue
San Francisco, CA 94143

William L. Young, and

Center for Cerebrovascular Research Department of Anesthesia and Perioperative Care
University of California, San Francisco 1001 Potrero Avenue Room 3C-38 San Francisco, CA
94110, USA; Department of Neurological Surgery & Department of Neurology University of
California, San Francisco 1001 Potrero Avenue Room 3C-38 San Francisco, CA 94110, USA

Hua Su*

Center for Cerebrovascular Research Department of Anesthesia and Perioperative Care
University of California, San Francisco 1001 Potrero Avenue Room 3C-38 San Francisco, CA
94110, USA

Abstract

Oligogenesis plays an important role in functional recovery after ischemic stroke. We tested the hypothesis that oligogenesis and the maturation of oligodendrocyte progenitor cells (OPCs) vary in different brain regions using a rat transient middle cerebral artery occlusion (tMCAO) model. Compared to Day 1, olig2⁺ OPCs and oligodendrocytes (OLGs) increased in the peri-infarct basal

*Correspondence to: Phone: 415-206-3162 Fax: 415-206-8907 ccr@anesthesia.ucsf.edu .

ganglia (BG) 7 (44%) and 14 (61%) days after 2 hours of MCAO; OPCs (PDGFR α ⁺) and OLGs (CC1⁺) increased in this region 14 days after tMCAO by 139% and 126%, respectively. Although the olig2⁺ cells and OLGs did not increase significantly in the peri-infarct cortex (CTX), the OPCs increased in this region by 95% at Day 14 vs. Day 1 after tMCAO. The numbers of OPCs and OLGs remained low after an initial reduction at Day 1 in the peri-infarct corpus callosum (CC). Correlation analyses showed that the numbers of olig2⁺ cells ($r=0.73$, $P=0.03$) and OLGs ($r=0.74$, $P=0.02$) correlated with local vessel density; however, the number of OPCs did not correlate with vessel density ($r=0.43$, $P=0.24$). Our data show that oligogenesis and the maturation of OPCs differ in various brain regions and the difference in regional angiogenic response is one of the potential reasons.

Keywords

Transient middle cerebral artery occlusion; Rat model; Olig2 positive cells; CC1 positive cells; PDGFR α positive cells

Introduction

White matter damage contributes to cognitive decline. White matter is particularly susceptible to stroke [1-4], because white matter blood flow is lower than gray matter and there is little collateral blood supply in deep white matter [5, 6].

Oligodendrocytes (OLGs) are one of the major cell types in the white matter in the central nervous system (CNS). OLG-produced myelin enwraps axons facilitating axonal conduction. The differentiation and maturation of OLGs in the adult brain maintain white matter integrity. OLGs are sensitive to ischemic insult because they are vulnerable to energy failure. In white matter, the saltatory conduction is highly dependent on ATP supply. Pathological Ca²⁺ entry occurs in part because of increased intracellular Na⁺ and membrane depolarization in OLG after stroke [7]. OLG swelling and death occur within minutes to hours after ischemic insult [1, 8]. OLG death causes demyelination of neurofibers that leads to neurologic deficits in stroke patients [9-11]. The main factors involved in the vulnerability of OLG to ischemic injury are oxidative stress [12] and excitotoxicity through the N-methyl-d-aspartate (NMDA) receptor [9].

Mature OLGs in the adult mammalian central nervous system (CNS) are considered to be postmitotic and are unable to proliferate in response to injury [11]. However, there are abundant oligodendrocyte progenitor cells (OPCs) in the white matter of normal CNS. Animal studies show that OPC proliferation and maturation remyelinate the demyelinated neurofibers [13, 14].

Evidence of OLG regeneration after ischemia has been demonstrated in previous studies. In a rat transient middle cerebral artery occlusion model (tMCAO), the number of OLGs decreased initially (one and two days) and increased one and two weeks after reperfusion in the peri-infarct cortex (CTX) [15]. Myelin basic protein (BMP) messenger RNA (mRNA) increased in the ischemic peri-infarct zone one day after tMCAO in adult male of spontaneous hypertensive rats, and reached maximal levels seven days after injury [16]. The number of OPCs also increased one and two weeks after tMCAO [17, 18]. However, the ability of OLGs to regenerate in different brain regions has not been analyzed in the tMCAO model.

Arai and Lo have shown in vitro that cerebral endothelial cells secrete trophic factors to support OPCs proliferation, suggesting a link between angiogenesis and oligogenesis in

stroke recovery [19]. In this study, using immunocytochemical staining, we tested our hypothesis that oligogenesis and OPC maturation vary in different brain regions in a rat tMCAO model. To explore the potential local factors that influence oligogenesis and OPC maturation, we also analyzed the correlation between the increase of vessel densities and oligogenesis at the peri-infarct zone.

Materials and Methods

Transient Middle Cerebral Artery Occlusion (tMCAO) Model

Experimental procedures for using laboratory animals were approved by the Institutional Animal Care and Use Committee of the University of California, San Francisco. Adult male Sprague-Dawley rats (280 to 300 g; Charles River Laboratories, Wilmington, MA) were used. Focal cerebral ischemia was induced using the intraluminal MCAO method that was originally described by Koizumi et al. [20] and has since been modified by others [21-26]. Briefly, a 4-0 monofilament nylon was inserted from the external carotid artery into the lumen of the internal carotid artery until it blocked the origin of the middle cerebral artery, which is about 18-20 mm beyond the origin of the internal carotid artery. The intraluminal suture was carefully removed two hours later to reperfuse the brain. Brain samples were collected on Day 1, Day 7, and Day 14 after reperfusion, 3 rats for each time-point.

Histological and Immunohistochemical Staining

OLGs and OPCs were identified by immunostaining using antibodies specific to olig2 (a marker for both OLGs and OPCs) [27], PDGFR α (a marker for OPCs) [27-29], and CC1 (an antiadenomatous polyposis coli antibody that recognizes mature OLGs and astrocytes) [30-32]. Since some endothelial cells also express PDGFR α [33], the positive cells that overlaid with the vessel wall were not included in the cell-quantification.

Brain samples were fresh-frozen in dry ice and sectioned on a cryostat into 20 μ m thick sections. Cresyl fast violet staining was used to visualize the infarct region. TUNEL staining was performed using an ApopTag plus peroxidase in situ apoptosis detection kit (Millipore, Temecula, CA) according to the manufacturer's protocol. A rat anti-mouse myelin basic protein (MBP) antibody (1:1,500, Millipore, Temecula, CA) was used for MBP immunocytochemistry. After incubation with the primary antibody at 4°C overnight, tissue sections were incubated with biotinylated goat antimouse IgG (1:500 dilution; Vector Laboratory, CA) followed by ABC complex and 3,3'-diaminobenzidine (Vector Labs, CA) to visualize the positive MBP stain. The sections were then counterstained with hematoxylin.

For immunofluorescent staining, sections were incubated at 4°C overnight with the following primary antibodies: rabbit anti-mouse Ki67 (1:200, Abcam, Cambridge, MA), rabbit anti-rat Olig2 (1:800, Millipore, CA), rabbit anti PDGFR α (1:500, a gift from Dr. Michael Stallcup, Burnham Institute, San Diego, CA), mouse anti-rat CC1 (1:20 dilution, Calbiochem, NJ). After washing in PBT (0.1% Tween20 in PBS), sections were incubated at room temperature for 1 hour with Alexa Fluor 594-conjugated or Alexa Fluor 488-conjugated IgG (1:500, Invitrogen). Fluorescein-lycopersicin esculentum lectin (1:200 dilution, Vector Laboratory) was used to stain the endothelial cells. For the lectin staining, the sections were incubated overnight at 4°C and were mounted after washing. For negative controls, tissues were processed identically, omitting the primary antibody.

Quantification and Statistical Analysis

Two sections from each rat obtained between Bregma +1.5mm and Bregma 0, were used for quantifying each of the cell-types and vessel densities. The peri-infarct region was identified

using cresyl violet-stained sections adjacent to the immunostained sections (Figure 1A and Supplementary Figure 1). The 20X microscope objective fields chosen for quantification of cell numbers and vessel densities (number of vessels/20X field) are shown in Figure 1B. Since lectin can stain microglia and macrophage, we manually excluded these cells from the vessel counting by checking on the lectin and DAPI double-labeled sections. Numbers of TUNEL⁺, Ki67⁺ were counted in three 20X fields in the infarct core. The numbers of Olig2⁺, PDGFR α ⁺, and CC1⁺ cells, and vessels were counted in peri-infarct CTX (two fields), CC (one field) and BG (four fields, Figure 1B) using NIH Image 1.63 software.

Data are presented as means $\pm$ SD. Means were compared using one-way ANOVA analysis followed by Bonferroni post hoc correction. A P-value of < 0.05 was considered significant. The correlation between angiogenesis and oligogenesis was analyzed using Pearson's Correlation Coefficient assay.

Results

Cell-apoptosis and White Matter Injury in the Ischemic Core after MCAO

Many cells (146 ± 14.1 TUNEL positive cells/20X field) in the ischemic core were apoptotic one day after tMCAO (Figure 2). The number of TUNEL positive cells decreased over the next 14 days (39 ± 11 /field at Day 7, $P < 0.001$ vs Day 1; 16 ± 4.6 /field at Day 14, $P < 0.001$ vs Day 1). Few proliferating cells (Ki67 positive cells, 2 ± 1 /field) were detected in the ischemic core at Day 1 after MCAO. Ki67 positive cells increased over the next 14 days (Day 7: 8 ± 2.1 /field, $P = 0.04$ vs. Day 1; Day 14: 14 ± 4.7 /field, $P = 0.003$ vs. Day 1, Figure 2). These data indicate a pro-apoptotic environment in the early stage and pro-proliferation environment in the later stage in both gray and white matter in the ischemic core after tMCAO.

Distortion of myelinated fiber tracts was detected in the ischemic core in the CTX, CC, and BG after tMCAO. The pattern of MBP staining, which identifies mature OLGs and their fibers, became distorted at Day 1 after tMCAO (Figure 3A). The MBP staining pattern was unaltered in peri-infarct BG, CTX, and CC up to Day 14 after the reperfusion (Figure 3B). Thus, the tMCAO model we used had severe white matter injury in the ischemic core but not in the peri-infarct region.

Oligogenesis in the Peri-infarct Region After tMCAO

Oligodendrocyte precursor cell numbers increased in several regions within the peri-infarct area 7-14 days after tMCAO. In comparison to the contralateral regions, the olig2⁺ cells decreased in the peri-infarct BG: 0.9 ± 0.07 (ipsilateral/contralateral), CTX: 0.8 ± 0.2 , and CC: 0.8 ± 0.1 one day after tMCAO. Compared to Day 1, olig2⁺ cells increased in the BG by 44% at Day 7 (1.3 ± 0.09 , $P = 0.04$ vs. Day 1) and 61% at Day 14 after tMCAO (1.5 ± 0.23 , $P = 0.02$ vs. Day 1, Figure 4). In the peri-CTX, the number of olig2⁺ cells did not increase significantly at either Day 7 (1.1 ± 0.01 , $P = 0.165$ vs. Day 1) or Day 14 (1.1 ± 0.04 , $P = 0.165$ vs. Day 1) after tMCAO. The olig2⁺ cell numbers remained low in the peri-infarct CC at all the time-points tested after tMCAO (Figure 4).

There were fewer PDGFR α ⁺ OPCs in the peri-infarct zone compared to the similar region in the contralateral hemisphere at Day 1 after tMCAO. The ratios of PDGFR α ⁺ OPCs between ipsilateral and contralateral sides were 0.8 ± 0.22 in BG, 0.7 ± 0.18 in CTX, and 0.6 ± 0.32 in CC. Compared to Day 1, the PDGFR α ⁺ OPCs increased 139% in the peri-infarct BG (1.9 ± 0.27 vs. Day 1, $P = 0.04$) and 95% in the peri-infarct CTX (1.29 ± 0.11 vs. Day 1, $P = 0.01$) at Day 14 (Figure 5). The number of PDGFR α ⁺ OPCs did not increase in CC at Day 14 compared to Day 1. However, the significant increase of OLGs (CC1⁺) was observed in BG only at Day 14 (126%, 1.7 ± 0.15 , vs. Day 1: 0.7 ± 0.24 , $P = 0.006$) after tMCAO (Figure

6). The OLGs did not change significantly in CTX and CC at Day 7 and Day 14 after tMCAO. These data suggest that active oligogenesis and OPC maturation occur mostly in the peri-infarct BG after tMCAO.

Correlation between Oligogenesis/OPC Maturation and Local Angiogenesis

To analyze the potential mechanism underlying the difference in oligogenesis and OPC maturation in various peri-infarct regions, we quantified vascular density in the brain samples collected at Day 7 and Day 14 after tMCAO. Compared to corresponding contralateral regions, the vessel densities were 34% higher in the peri-infarct BG ($P=0.003$) and 23% higher in the peri-infarct CTX ($P=0.04$) at Day 14 after tMCAO, indicating active angiogenesis in these regions (Figure 7). The increase of olig2⁺ cells ($r=0.73$, $P=0.03$) and OLGs ($r=0.74$, $P=0.02$) correlated with the increase of vessel density (Figure 8). However, the number of OPCs (PDGFR α ⁺ cells) did not correlate with vessel density ($r=0.43$, $P=0.24$, Figure 8). Thus, greater increase of vessel density partially correlated with greater oligogenesis in the peri-infarct BG and CTX and with better maturation of OPC in the BG than CC. These data suggest that the difference in oligogenesis and OPC maturation in various brain regions is due in part to the difference of angiogenic response to tMCAO in these regions.

Discussion

Evidence of OLG regeneration after ischemia has been demonstrated in previous studies [15-18]. However, OLG regeneration in different brain regions has not been analyzed. In this study, we demonstrated that after tMCAO in the rat brain, oligogenic activity and OPC maturation differ in various brain regions and occur mostly in the peri-infarct BG. We also found that OLGs (Olig/CC1⁺) increased less (ratio of ipsilateral vs. contralateral) than PDGFR α ⁺ OPCs in the peri-infarct region at Day 7 and Day 14 after tMCAO (Figure 5 & 6), suggesting an impairment of OPC maturation in the ischemic brain.

Although we do not have direct evidence showing oligodendrocyte death preceding actual oligogenesis, we analyzed the total cell death and cell proliferation at different time points (Figure 2). Numerous apoptotic cells and few proliferating cells were detected in the ischemic region at Day 1. The number of apoptotic cells reduced and the number of proliferating cells increased over time. In addition, there were less OPCs and OLGs in the ipsilateral compared to the contralateral hemisphere at Day 1 after reperfusion. At Days 7 and 14, compared to the contralateral hemisphere, there were more olig2 and PDGFR α in the ipsilateral basal ganglia and cortex. All of these data indicate that oligodendroglial cell death preceded actual oligogenesis.

Impaired OPC maturation has also been shown by Miyamoto et al. [18] in a bilateral common carotid artery ligation (BCCAL) model. They found that while OPCs (PDGFR α ⁺) increased steadily between Day 7 and Day 14 after bilateral common carotid artery ligation (BCCAL), the density of mature OLGs (glutathione-S-transferase GST-pi⁺) increased significantly at Day 14 compared to the sham-operated control group, but then decreased to control level at Day 21. These data together with ours suggest that OPC-maturation is impaired in the ischemic-injured brain.

Contrary to our study in which the myelin density in the peri-infarct zone remained unchanged, others found a gradual increase of myelin density in the peri-infarct CTX [17]. Both studies relied on quantification of MBP expression after immunostaining, which may be assessed differently by investigators. More sophisticated quantification methods would help verify the changes of myelin density in the peri-infarct region.

The mechanisms underlying the difference in oligogenesis and OPC-maturation in various brain regions after rat tMCAO are not clearly understood. Arai and Lo showed in vitro that cerebral endothelial cells secrete trophic factors, such as FGF and BDNF that support the survival and proliferation of rat OPCs [19]. Their data suggest a novel oligovascular niche in the mammalian brain. In our in vivo study, we found that local oligogenesis and OPC maturation partially correlate with local angiogenic activity, supporting the notion of an oligovascular niche in the adult brain. Our data also indicate that there are additional local factors that participate in oligogenesis and OPC maturation after ischemic stroke, as the number of PDGFR α ⁺ did not correlate with local angiogenesis.

Zhang et al. showed that bone marrow cells (BMCs) stimulate oligogenesis in stroke lesions [34] and that BMCs exposure to the ischemic brain conditioned medium can produce trophic factors such as BDNF and FGF2 [35]. Thus, the recruitment of BMCs to the lesion site after ischemic stroke could be one of the additional local factors supporting oligogenesis and OPC maturation.

Gregersen et al. have shown that MBP mRNA expression is upregulated after rat tMCAO with a concomitant increased expression of growth-associated protein-43 (GAP-43) in neurons [16]. As GAP-43 is an axonal sprouting marker, their data indicate that neurons and OPCs/OLGs may cross-talk during the recovery stage of ischemic stroke. During the developmental stage, oligogenesis is dependent on homeodomain transcription factor expression such as Nkx6.1. These transcription factors are involved in the regulation of oligogenesis in a regional-dependent fashion [36]. As these transcription factors are also expressed in the adult brain, their role in the regional difference of oligogenesis and OPC maturation warrants further study.

Summary

In this study, we demonstrated that oligogenic activity and OPC maturation occur mostly in the peri-infarct BG after tMCAO in rats. Oligogenesis and OPC-maturation partially correlate with local angiogenic activity, suggesting that the difference in angiogenic levels in various brain regions is one of the underlying mechanisms. Data gathered from our study warrant future research into other local factors that might be involved in the regulation of oligogenesis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors thank Tony Pourmohamad, MS, for assistance with statistical analysis, Voltaire Gungab for assistance with manuscript preparation, and the other members of the UCSF BAVM Study Project (<http://avm.ucsf.edu>.) for their support.

This study was supported in part by grants R01 NS27713 (WLY), P01 NS44155 (WLY, HS), R21 NS070153 (HS) from the National Institutes of Health; AHA 10GRNT3130004 (HS) from the American Heart Association; and CIRM RC1-135 (SJP) from the California Institute of Regeneration Medicine. Lidian Jiang was partially supported by Dr. Feng Ling in the Department of Neurosurgery, Xuanwu Hospital, Capital Medical University, Beijing, China.

References

1. Pantoni L, Garcia JH, Gutierrez JA. Cerebral white matter is highly vulnerable to ischemia. *Stroke*. 1996; 27(9):1641–1646. [PubMed: 8784142]

2. Dewar D, Yam P, McCulloch J. Drug development for stroke: importance of protecting cerebral white matter. *Eur J Pharmacol.* 1999; 375(1-3):41–50. [PubMed: 10443563]
3. Volpe JJ. Cerebral white matter injury of the premature infant-more common than you think. *Pediatrics.* 2003; 112(1 Pt 1):176–180. [PubMed: 12837883]
4. Alix JJ. Recent biochemical advances in white matter ischaemia. *Eur Neurol.* 2006; 56(2):74–77. [PubMed: 16946618]
5. Arai K, Lo EH. Experimental models for analysis of oligodendrocyte pathophysiology in stroke. *Exp Transl Stroke Med.* 2009; 1:6. [PubMed: 20150984]
6. Lo EH, Dalkara T, Moskowitz MA. Mechanisms, challenges and opportunities in stroke. *Nat Rev Neurosci.* 2003; 4(5):399–415. [PubMed: 12728267]
7. Stys PK. Anoxic and ischemic injury of myelinated axons in CNS white matter: from mechanistic concepts to therapeutics. *J Cereb Blood Flow Metab.* 1998; 18(1):2–25. [PubMed: 9428302]
8. Yam PS, Patterson J, Graham DI, Takasago T, Dewar D, McCulloch J. Topographical and quantitative assessment of white matter injury following a focal ischaemic lesion in the rat brain. *Brain Res Brain Res Protoc.* 1998; 2(4):315–322. [PubMed: 9630700]
9. Micu I, Jiang Q, Coderre E, Ridsdale A, Zhang L, Woulfe J, et al. NMDA receptors mediate calcium accumulation in myelin during chemical ischaemia. *Nature.* 2006; 439(7079):988–992. [PubMed: 16372019]
10. Dewar D, Underhill SM, Goldberg MP. Oligodendrocytes and ischemic brain injury. *J Cereb Blood Flow Metab.* 2003; 23(3):263–274. [PubMed: 12621301]
11. McTigue DM, Tripathi RB. The life, death, and replacement of oligodendrocytes in the adult CNS. *J Neurochem.* 2008; 107(1):1–19. [PubMed: 18643793]
12. Arai K, Lo EH. Oligovascular signaling in white matter stroke. *Biol Pharm Bull.* 2009; 32(10):1639–1644. [PubMed: 19801821]
13. Carroll WM, Jennings AR. Early recruitment of oligodendrocyte precursors in CNS demyelination. *Brain.* 1994; 117(Pt 3):563–578. [PubMed: 8032866]
14. Gensert JM, Goldman JE. Endogenous progenitors remyelinate demyelinated axons in the adult CNS. *Neuron.* 1997; 19(1):197–203. [PubMed: 9247275]
15. Tanaka K, Nogawa S, Ito D, Suzuki S, Dembo T, Kosakai A, et al. Activation of NG2-positive oligodendrocyte progenitor cells during post-ischemic reperfusion in the rat brain. *Neuroreport.* 2001; 12(10):2169–2174. [PubMed: 11447328]
16. Gregersen R, Christensen T, Lehmann E, Diemer NH, Finsen B. Focal cerebral ischemia induces increased myelin basic protein and growth-associated protein-43 gene transcription in peri-infarct areas in the rat brain. *Exp Brain Res.* 2001; 138(3):384–392. [PubMed: 11460777]
17. Tanaka K, Nogawa S, Suzuki S, Dembo T, Kosakai A. Upregulation of oligodendrocyte progenitor cells associated with restoration of mature oligodendrocytes and myelination in peri-infarct area in the rat brain. *Brain Res.* 2003; 989(2):172–179. [PubMed: 14556938]
18. Miyamoto N, Tanaka R, Shimura H, Watanabe T, Mori H, Onodera M, et al. Phosphodiesterase III inhibition promotes differentiation and survival of oligodendrocyte progenitors and enhances regeneration of ischemic white matter lesions in the adult mammalian brain. *J Cereb Blood Flow Metab.* 2010; 30(2):299–310. [PubMed: 19826432]
19. Arai K, Lo EH. An oligovascular niche: cerebral endothelial cells promote the survival and proliferation of oligodendrocyte precursor cells. *J Neurosci.* 2009; 29(14):4351–4355. [PubMed: 19357263]
20. Koizumi J, Yoshida Y, Nakazawa T, Ooneda G. Experimental studies of ischemic brain edema. I: a new experimental model of cerebral embolism in rats in which recirculation can be introduced in the ischemic area. *Jpn J Stroke.* 1986; 8:1–8.
21. Fisher M. Recommendations for advancing development of acute stroke therapies: Stroke Therapy Academic Industry Roundtable 3. *Stroke.* 2003; 34(6):1539–1546. [PubMed: 12750546]
22. Sicard KM, Fisher M. Animal models of focal brain ischemia. *Exp Transl Stroke Med.* 2009; 1:7. [PubMed: 20150985]
23. Li F, Han SS, Tatlisumak T, Liu KF, Garcia JH, Sotak CH, et al. Reversal of acute apparent diffusion coefficient abnormalities and delayed neuronal death following transient focal cerebral ischemia in rats. *Ann Neurol.* 1999; 46(3):333–342. [PubMed: 10482264]

24. Li F, Han S, Tatlisumak T, Carano RA, Irie K, Sotak CH, et al. A new method to improve in-bore middle cerebral artery occlusion in rats: demonstration with diffusion- and perfusion-weighted imaging. *Stroke*. 1998; 29(8):1715–1719. [PubMed: 9707216]
25. Su H, Yang GY. Treatment of focal brain ischemia with viral vector-mediated gene transfer. *Methods Mol Biol*. 2011; 686:429–446. [PubMed: 21082386]
26. Spratt NJ, Fernandez J, Chen M, Rewell S, Cox S, van Raay L, et al. Modification of the method of thread manufacture improves stroke induction rate and reduces mortality after thread-occlusion of the middle cerebral artery in young or aged rats. *J Neurosci Methods*. 2006; 155(2):285–290. [PubMed: 16513179]
27. Nishiyama A, Komitova M, Suzuki R, Zhu X. Polydendrocytes (NG2 cells): multifunctional cells with lineage plasticity. *Nat Rev Neurosci*. 2009; 10(1):9–22. [PubMed: 19096367]
28. Gard AL, Pfeiffer SE. Two proliferative stages of the oligodendrocyte lineage (A2B5+O4– and O4+GalC–) under different mitogenic control. *Neuron*. 1990; 5(5):615–625. [PubMed: 2223090]
29. He Y, Cai W, Wang L, Chen P. A developmental study on the expression of PDGFalphaR immunoreactive cells in the brain of postnatal rats. *Neurosci Res*. 2009; 65(3):272–279. [PubMed: 19665498]
30. Iwai M, Stetler RA, Xing J, Hu X, Gao Y, Zhang W, et al. Enhanced oligodendrogenesis and recovery of neurological function by erythropoietin after neonatal hypoxic/ischemic brain injury. *Stroke*. 2010; 41(5):1032–1037. [PubMed: 20360553]
31. Bhat RV, Axt KJ, Fosnaugh JS, Smith KJ, Johnson KA, Hill DE, et al. Expression of the APC tumor suppressor protein in oligodendroglia. *Glia*. 1996; 17(2):169–174. [PubMed: 8776583]
32. Olivier P, Fontaine RH, Loron G, Van Steenwinckel J, Biran V, Massonneau V, et al. Melatonin promotes oligodendroglial maturation of injured white matter in neonatal rats. *PLoS One*. 2009; 4(9):e7128. [PubMed: 19771167]
33. Karvinen H, Rutanen J, Leppanen O, Lach R, Levonen AL, Eriksson U, et al. PDGF-C and -D and their receptors PDGFR-alpha and PDGFR-beta in atherosclerotic human arteries. *Eur J Clin Invest*. 2009; 39(4):320–327. [PubMed: 19292888]
34. Zhang J, Li Y, Zhang ZG, Lu M, Borneman J, Buller B, et al. Bone marrow stromal cells increase oligodendrogenesis after stroke. *J Cereb Blood Flow Metab*. 2009; 29(6):1166–1174. [PubMed: 19384336]
35. Qu R, Li Y, Gao Q, Shen L, Zhang J, Liu Z, et al. Neurotrophic and growth factor gene expression profiling of mouse bone marrow stromal cells induced by ischemic brain extracts. *Neuropathology*. 2007; 27(4):355–363. [PubMed: 17899689]
36. Liu R, Cai J, Hu X, Tan M, Qi Y, German M, et al. Region-specific and stage-dependent regulation of Olig gene expression and oligodendrogenesis by Nkx6.1 homeodomain transcription factor. *Development*. 2003; 130(25):6221–6231. [PubMed: 14602683]

Figure 1. Infarct area and regions selected for quantification

A. Representative picture of cresyl violet-stained section, obtained at Day 1 after tMCAO. The infarct area (unstained region) covers the CTX, CC and BG. The ipsilateral hemisphere of the infarct is slightly larger than the hemisphere on the contralateral side due to edema. **B.** Drawing shows the 20X microscope objective fields (squares) selected for quantification. Dotted line outlines the infarct area. Core: ischemic core. IBZ: ischemia border zone.

Figure 2. Cell apoptosis and proliferation in the ischemic core

A. Representative images of TUNEL (brown) and Ki67 (red) stained sections. The TUNEL-stained sections were counterstained with hematoxylin and the Ki67-stained sections with DAPI. Scale bar = 100 μ m. **B.** Bar graph shows quantifications of TUNEL- and Ki67-positive cells. Data are means $\pm$ SD. N=3 per group. *P< 0.05 and ** P< 0.01, vs. Day 1. D: day.

Figure 3. Changes in MBP staining patterns in the ischemic core and peri-infarct region
A. Images of the ischemic core. Brain sections from rats at Day 1 (1d), Day 7 (7d) and Day 14 (14d) after tMCAO were immunostained with an MBP antibody. **B.** Images of peri-infarct region. Scale bar=50 µm.

Figure 4. Changes in olig2⁺ OPCs and OLGs in the peri-infarct region

A. Images show olig2⁺ cells in the peri-infarct region. The cell nuclei were stained with DAPI (blue). Scale bar=100μm. **B.** Bar graph shows the quantifications of olig2⁺ cells in the peri-infarct region. *P< 0.05 and ** P< 0.01, vs. Day 1. Ipi: ipsilateral; con: contra lateral.

Figure 5. Changes in OPCs in the peri-infarct region

A. Photomicrographs showing OPCs (PDGFR α ⁺, green) in different brain regions. Scale bar=100 μ m. **B.** Bar graph shows quantification. Data are mean $\pm$ SD. N=3 per group. *P<0.05 and ** P<0.01, vs. Day 1. Ipi: ipsilateral; con: controlateral.

Figure 6. Changes in OLGs in the peri-infarct region

A. Photomicrographs showing olig2 (red) and CC1 (green) double positive OLGs. Scale bar=100 μ m. **B.** Bar graph shows quantification. Data are mean $\pm$ SD. N=3 per group. ** P< 0.05, vs. Day 1. Ipi: ipsilateral; con: controlateral.

Figure 7. Vascular density in the peri-infarct region

A. Photomicrographs of Lectin (green) stained sections. Scale bar=100μm. **B.** Bar graph shows the quantification of vessel density. Data are means ± SD. N=3 per group. *P< 0.05 vs. corresponding contralateral region. Ipi: ipsilateral; con: controlateral.

Figure 8. Correlation of oligogenesis and OPC maturation with local vascular density
 Graphs show the correlation of increases (the ratio of cell numbers in ipsilateral vs. contralateral) of olig2⁺ cells (A), PDGFR α ⁺ OPCs (B) and CC1 and olig2 double positive OLGs (C) with local vessel density. Ipi: ipsilateral; con: contralateral. BG has the highest vessel density and the highest increase of olig2⁺ cells and CC1⁺ OLGs among the three regions studied.