

Expression Patterns and Phenotypic Changes Regarding Stemness in Brain Pericytes in Health and Disease

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Abstract

Objective: Brain pericytes (PCs), which exist near endothelial cells, function in processes including maintenance of the blood-brain barrier and as stem cells. However, the relationship between expression patterns and phenotypic changes regarding stemness in brain PCs remains unclear. Thus, we investigated the properties of brain PCs in healthy and diseased mice.

Methods: We examined the expression of representative pericytic markers, such as neuron-glia antigen 2 (NG2) and alpha smooth muscle actin (α SMA), and of the stem cell marker Nestin, in developing and pathologic mouse brains.

Results: Brain PCs expressed NG2 and α SMA during the embryonic and postnatal stages of development, but rarely during adulthood. Although brain PCs exhibited Nestin expression during early development, it was scarce during adulthood, in parallel with a decrease in pericytic marker expression. However, upon brain injury following ischemic stroke, NG2 and α SMA were significantly induced in PCs in adult mice, concomitant with upregulated Nestin expression.

Conclusion: The expression of markers in brain PCs differs during development and between normal and pathologic conditions in addition to traits such as stemness. An understanding of these expression profiles will be useful for PC-based stem cell therapies in the future.

Keywords: Brain; Pericyte; Stemness; Development; Cerebral infarction; Ischemia

Introduction

Pericytes (PCs: from peri-around and cyte-cell) were discovered by Eberth more than 100 years ago [1]. In 1873, the French scientist Rouget named the perivascular cells adjacent to the endothelium of capillaries “Rouget” cells [2]. Because of their unique localization in perivascular regions, Zimmerman renamed Rouget cells “pericytes” in 1923 [3]. PCs have multiple functions in vessel maintenance, including in cell growth, differentiation, and migration [4,5].

In the central nervous system (CNS), brain PCs constitute the blood-brain barrier (BBB)/neurovascular unit (NVU) in combination with endothelial and neural cells (astrocytes and neurons). PCs regulate cerebral blood flow (CBF) and the permeability of the BBB/NVU [6,7]. PCs play a crucial role in maintaining the BBB/NVU, and their loss or dysfunction causes the breakdown of this unit [8,9]. However, increasing evidence shows that brain PCs also act as multipotent stem cells [10-12]. Thus, brain PCs play diverse and important roles, including serving as a source of stem cells, regulating CBF, and maintaining homeostasis of the BBB/NVU [13,14].

PCs are a heterogeneous cell population. Previous reports have shown that they express various markers, including neuron-glia antigen 2 (NG2), alpha smooth muscle actin (α SMA), platelet-derived growth factor receptor beta (PDGFR β), Cluster of differentiation (CD) 146, and Regulator of G protein signaling 5 [10,11,15-20]. Although no known marker is specific to PCs, we and others previously showed that PCs with multipotency express the stem cell marker Nestin, as

well as representative PC markers, such as NG2, α SMA, and PDGFR β [10-12,15,21]. In addition, we demonstrated that pericyte-derived multipotent stem cells can be obtained from adult brains following ischemic stroke, although they are not present in normal adult brains [11,12]. These results suggest that the properties of PCs differ between healthy and pathologic brains, and that pericytic markers may vary under different conditions. Because brain PCs can function as neural stem cells (NSC) that mediate the reparative process of damaged CNS [11,12], it is important to understand the fate of PCs in the CNS under various conditions when considering stem cell-based therapies that target endogenous NSCs.

In this study, we investigated whether the expression of pericytic markers and traits such as stemness change during development and under pathologic conditions induced by ischemic stroke.

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Materials and Methods

Animal studies

All procedures were performed in accordance with a protocol approved by the Animal Care Committee of the Hyogo College of Medicine, Hyogo, Japan, and adhered to the criteria outlined in the Animal Research: Reporting *in vivo* Experiments guidelines. Animals were given *ad libitum* access to food, and efforts were made to minimize the number of animals used and their suffering. Quantitative analyses were performed by investigators blinded to the experimental protocol and identities of the samples.

Preparation of tissue from developing brains

C57/BL6 mice (Clea Japan, Inc., Tokyo, Japan) at different developmental stages (Embryonic Day 17 [E17]; Postnatal Day 1 [P1]; P8; young adult at 8 weeks; and mature adult at 24 weeks) were prepared and sacrificed following anesthesia by intraperitoneal injection with sodium pentobarbital. Then, the brains were removed, fixed with periodate-lysine-paraformaldehyde (PLP), cryoprotected in 30% sucrose, and cut on a cryostat for immunohistochemical analysis.

Preparation of tissue from adult brains following ischemic stroke

C57/BL6 mice at 8 and 24 weeks of age were subjected to cerebral ischemia as described previously [11,12,22-24]. Permanent focal cerebral ischemia was produced by ligation and interruption of the distal portion of the left middle cerebral artery (MCA) [11,12,22-24]. Briefly, under halothane inhalation, the left MCA was isolated, electrocauterized, and disconnected just distal to its crossing of the olfactory tract (the distal M1 portion). On Day 4 after stroke, mice were

anesthetized with sodium pentobarbital and perfused transcardially with PLP. Then, the brains were removed, cryoprotected in 30% sucrose, and cut on a cryostat for immunohistochemical analysis.

Immunohistochemistry

Coronal brain sections were subjected to immunohistochemistry as described previously [11,22-24]. Samples were stained with antibodies against NG2 (EMD Millipore Corp., Billerica, MA, USA), α SMA (EMD Millipore Corp.), Nestin (EMD Millipore Corp.), PDGFR β (Santa Cruz Biotechnology, Inc., Dallas, TX, USA), and CD31 (BD Pharmingen, San Diego, CA, USA). Primary antibodies were visualized using Alexa Fluor 488- or 555-conjugated secondary antibodies (Molecular Probes, Eugene, OR, USA). Nuclei were stained with 4',6-diamidino-2-phenylindole (Kirkegaard and Perry Laboratories, Inc., Gaithersburg, MD, USA). Images of brain sections were captured using a confocal laser microscope (LSM780; Carl Zeiss AG, Oberkochen, Germany). NG2⁺, α SMA⁺, and Nestin⁺ cells were subjected to a semi-quantitative analysis as described previously [24,25].

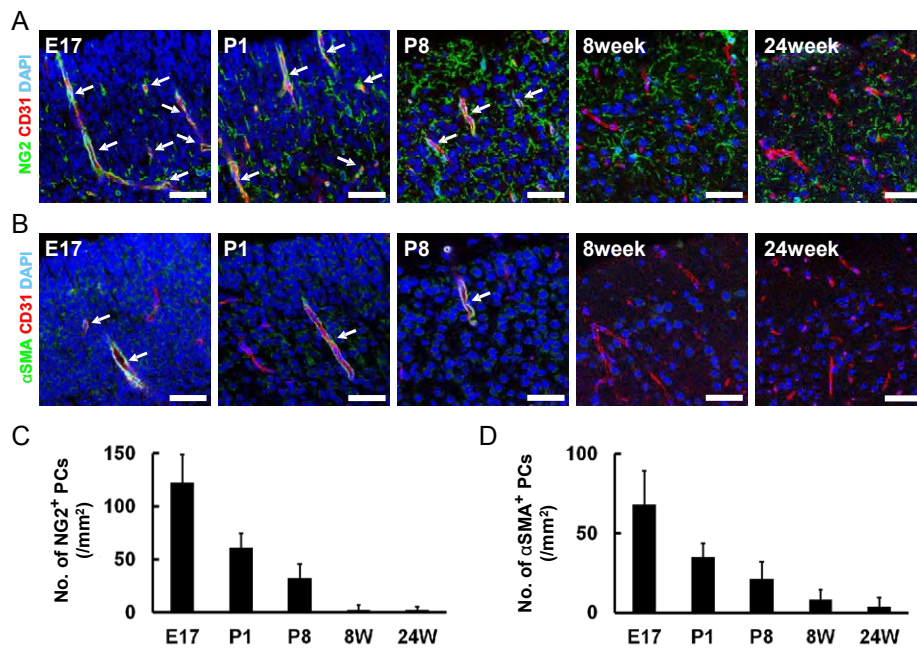
Statistical analysis

Data are presented as the mean \pm standard deviation. Statistical significance was calculated using the Student's t-test; a p value < 0.05 was considered to be statistically significant.

Results

Brain pericytes express pericytic markers during early development

To investigate whether NG2 is expressed in PCs near endothelial cells (ECs), sections of brains at different developmental stages (E17, P1, P8, and 8 and 24 weeks) were stained with antibodies against NG2



Most neuron-glia antigen 2⁺ (NG2⁺) cells were pericytes (PCs) near Cluster of differentiation (CD) 31⁺ endothelial cells (ECs) at the embryonic (Embryonic Day 17 [E17]) and early postnatal (Postnatal Day 1 [P1]) stages. However, NG2⁺ PCs gradually decreased postnatally (P8), and were rare during adulthood (8 and 24 weeks). NG2 (A: green); CD31 (A: red); 4',6-diamidino-2-phenylindole (DAPI; A: blue; arrows). Similarly, Alpha smooth muscle actin (α SMA) was present in PCs near CD31⁺ ECs in embryonic (E17) and postnatal (P1 and P8) brains, but was rare during adulthood (8 and 24 weeks). α SMA (B: green); CD31 (B: red); DAPI (B: blue; arrows). The numbers of NG2⁺ and α SMA⁺ PCs at each developmental stage are shown (C, D). Scale bars = 50 μ m (A, B).

Figure 1: Expression of pericytic markers by brain pericytes during development.

and CD31. During embryonic (E17; Figure 1A) and early postnatal (P1) stages (Figure 1A), most NG2⁺ cells were localized in perivascular areas near CD31⁺ ECs, suggesting that NG2 is mainly expressed in PCs. However, NG2⁺ PCs near CD31⁺ ECs gradually decreased postnatally (P8; Figure 1A), and were rare during adulthood (8 and 24 weeks; Figure 1A). However, NG2⁺ glia, which presumably included oligodendrocyte precursor cells (OPCs) [26] and microglia [27], were observed in areas remote from CD31⁺ ECs throughout development (E17, P1, P8, and 8 and 24 weeks; Figure 1A).

Using the alternative pericytic marker αSMA, we investigated whether αSMA is present in PCs during development. Brain sections were stained with antibodies against αSMA and CD31. In embryonic (E17) and postnatal (P1, and P8) brains, αSMA was present in PCs near CD31⁺ ECs (Figure 1B). Although the number of PCs expressing αSMA was smaller than that of PCs expressing NG2, αSMA expression was specifically observed in PCs near CD31⁺ ECs. However, αSMA⁺ cells were scarce during adulthood (8 and 24 weeks; Figure 1B).

The numbers of NG2⁺ (Figure 1C) and αSMA⁺ (Figure 1D) PCs near CD31⁺ ECs were analyzed in the cortical plate during early development (E17, P1, and P8) and in the cortex during adulthood (8 and 24 weeks). We found that brain PCs express representative pericytic markers, but that their expressions are restricted to early development.

Brain pericytes express a stem cell marker during early development

Because PCs with multipotency express the stem cell marker Nestin [10-12,15,21], we investigated the expression of Nestin during development. In embryonic (E17) and postnatal (P1 and P8) brains, some Nestin⁺ cells were observed among radial glia-like cells, as previously described [28]. However, Nestin expression was also present in PCs near CD31⁺ ECs during early development (E17, P1, and P8; Figure 2A). In addition, Nestin⁺ cells at E17 (Figure 2B), P1 and P8 (data not shown) co-expressed NG2, confirming their identity as PCs. However, Nestin expression by PCs was rare during adulthood (8 and 24 weeks; Figure 2A). The number of Nestin⁺ PCs near CD31⁺ ECs was analyzed in the cortical plate during early development and in the

cortex during adulthood (Figure 2C). We observed that the stem cell marker Nestin was mainly present in PCs during early development, including during embryonic and postnatal stages, corresponding to the period in which PCs express pericytic markers (Figures 1C and 1D).

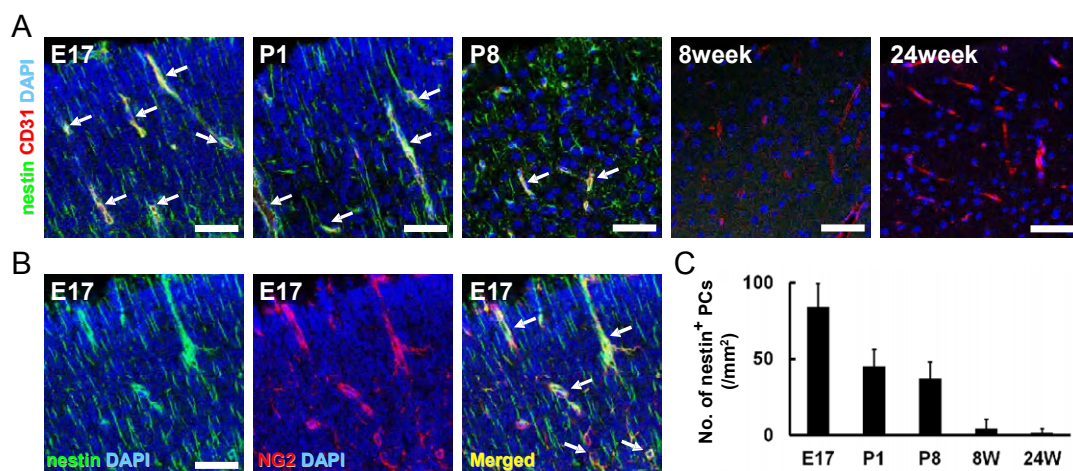
Adult brain pericytes re-express pericytic markers following ischemia

Our data show that the expression of pericytic markers by PCs decreases in adulthood. However, previous studies showed that, upon various stimuli, quiescent PCs become reactive [11,20,21,29], suggesting that PCs alter their traits in response to injuries. Thus, using adult mice subjected to cerebral infarction (Figures 3A and 3D), we investigated whether brain PCs re-express pericytic markers following ischemia. We found that NG2 was greatly induced within ischemic areas (Figures 3B and 3C), although NG2 was rarely observed within non-ischemic areas (Figure 3E). The majority of NG2⁺ cells ($82.1 \pm 5.5\%$) within ischemic areas were localized near CD31⁺ ECs (Figure 3C), indicating that NG2 is predominantly induced in PCs rather than glia in response to ischemia. At 8 and 24 weeks during adulthood, the number of NG2⁺ PCs near CD31⁺ ECs significantly increased within ischemic areas compared with in contralateral non-ischemic areas (Figure 3F).

Similarly, αSMA was significantly induced in PCs near CD31⁺ cells within ischemic areas (Figures 4A-4C and 4F), but was rare within non-ischemic areas (Figures 4D-4F). These results indicate that, even under severe ischemic conditions, not only brain ECs [24] but also PCs can survive and they become reactive and proliferate.

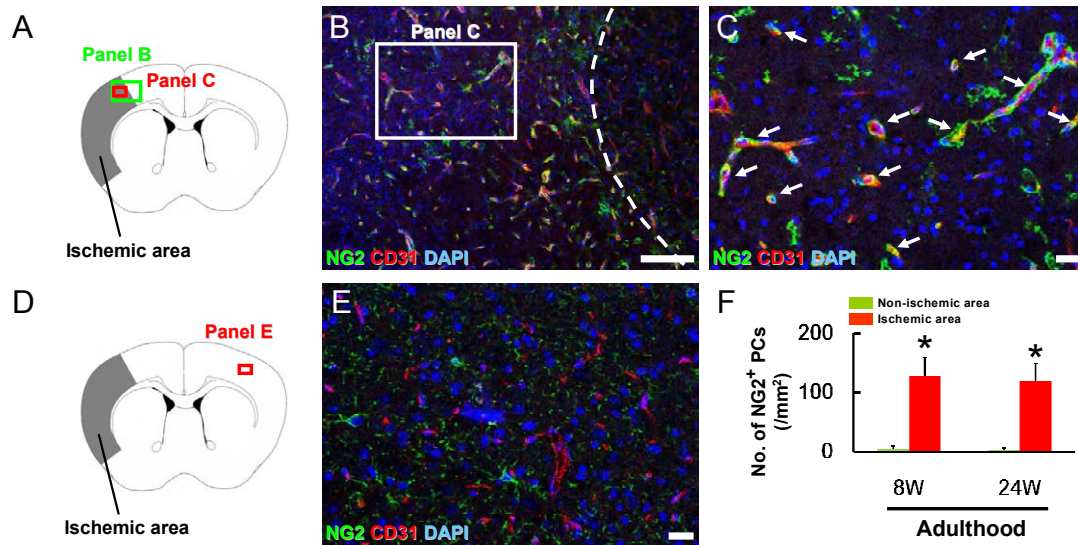
Adult brain pericytes re-express a stem cell marker following ischemia

Although our data show that adult brain PCs increase their representative markers in response to injuries, we recently found that brain PCs alter their traits and develop stemness following ischemic stroke [11,12]. Thus, using adult mice subjected to cerebral infarction (Figures 5A and 5D), we investigated whether PCs within ischemic areas express the stem cell marker Nestin. Consistent with our previous



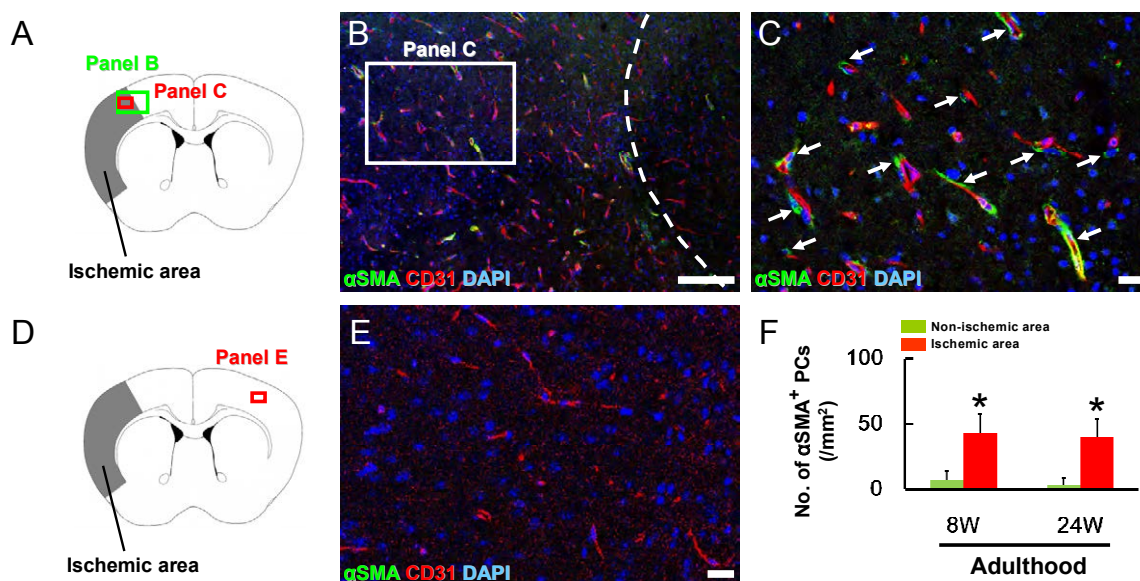
The stem cell marker Nestin was observed in pericytes (PCs) near Cluster of differentiation (CD) 31⁺ endothelial cells during early development (Embryonic Day 17 [E17], Postnatal Day 1 [P1], and P8), but was rare during adulthood (8 and 24 weeks). Nestin (A: green); CD31 (A: red); 4',6-diamidino-2-phenylindole (DAPI; A: blue; arrows). Some Nestin⁺ cells in brains at E17 co-expressed neuron-glia antigen 2 (NG2). Nestin (B: green); NG2 (B: red); DAPI (B: blue; arrows). The number of Nestin⁺ PCs at each developmental stage is shown (C). Scale bars = 50 μm (A, B).

Figure 2: Expression of the stem cell marker Nestin by brain pericytes during development.



In adult mice following ischemic stroke (A), neuron-glia antigen 2 (NG2) was highly induced in pericytes (PCs) near Cluster of differentiation (CD) 31⁺ cells within ischemic areas. NG2 (B, C: green); CD31 (B, C: red); 4',6-diamidino-2-phenylindole (DAPI; B, C: blue; arrows). In contrast, within contralateral non-ischemic areas (D), NG2⁺ cells were rarely PCs. NG2 (E: green); CD31 (E: red); DAPI (E: blue). At 8 and 24 weeks during adulthood, the number of NG2⁺ PCs was significantly increased within ischemic areas compared with in contralateral non-ischemic areas (F). *p<0.05 versus non-ischemic areas at each week (n = 3; F). Scale bars = 100 μ m (B) and 20 μ m (C, E).

Figure 3: Adult brain pericytes re-express neuron-glia antigen 2 following ischemic stroke.



In adult mice following ischemic stroke (A), Alpha smooth muscle actin (α SMA) was specifically induced in pericytes (PCs) near Cluster of differentiation (CD) 31⁺ cells within ischemic areas. α SMA (B, C: green); CD31 (B, C: red); 4',6-diamidino-2-phenylindole (DAPI; B, C: blue; arrows). In contrast, within contralateral non-ischemic areas (D), α SMA⁺ cells were rarely PCs. α SMA (E: green); CD31 (E: red); DAPI (E: blue). At 8 and 24 weeks during adulthood, the number of α SMA⁺ PCs was significantly increased within ischemic areas compared with in contralateral non-ischemic areas (F). *p<0.05 versus non-ischemic areas at each week (n = 3; F). Scale bars = 100 μ m (B) and 20 μ m (C, E).

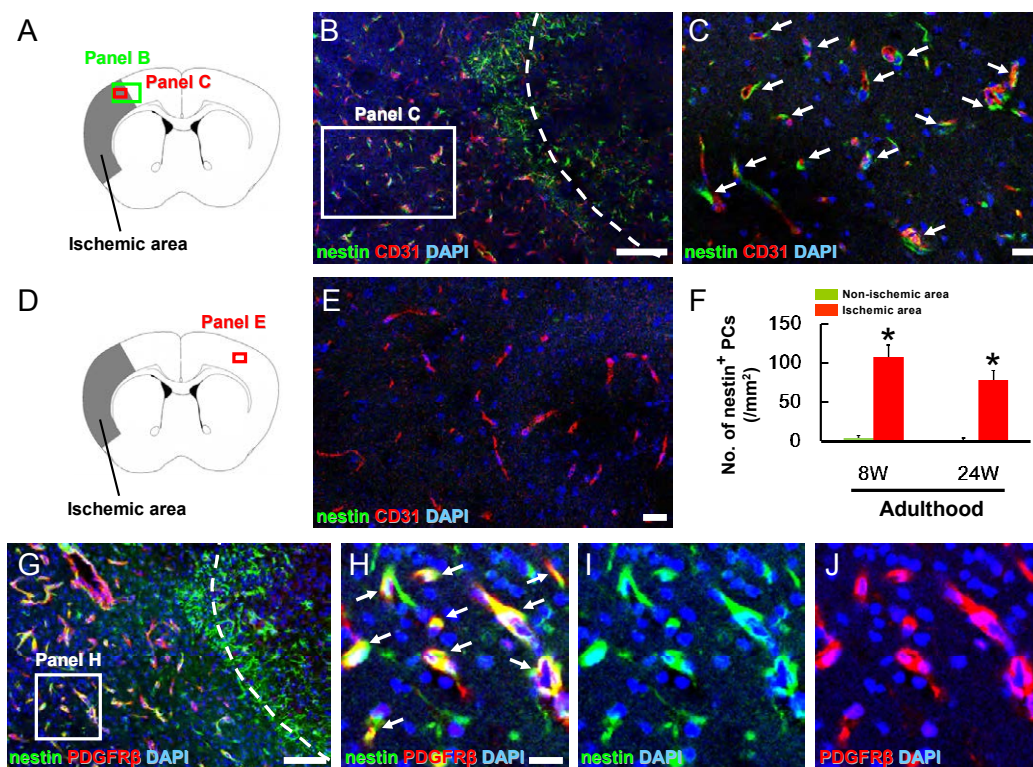
Figure 4: Adult brain pericytes re-express Alpha smooth muscle Actin following ischemic stroke.

reports [11,12,22], Nestin⁺ cells were significantly induced within ischemic areas (Figures 5B, 5C and 5F), but scarce within non-ischemic areas (Figures 5E and 5F). In addition, we found that most Nestin⁺ cells (60.7 \pm 10.1%) within ischemic areas expressed the pericytic marker PDGFR β (Figures 5G-5J). These results show that, under pathologic conditions, PCs re-express pericytic and also stem cell markers

normally decreased postnatally (Figures 6A and 6B). This indicates that marker expression by PCs is associated with traits such as stemness (Figures 6A and 6B).

Discussion

Although PCs were originally identified based on their distinctive



In adult mice following ischemic stroke (A), Nestin was specifically induced in pericytes (PCs) near Cluster of differentiation (CD) 31⁺ cells within ischemic areas. Nestin (B, C: green); CD31 (B, C: red); 4',6-diamidino-2-phenylindole (DAPI; B, C: blue; arrows). In contrast, within contralateral non-ischemic areas (D), Nestin⁺ cells were rarely PCs. Nestin (E: green); CD31 (E: red); DAPI (E: blue). At 8 and 24 weeks during adulthood, the number of Nestin⁺ PCs was significantly increased within ischemic areas compared with in contralateral non-ischemic areas (F). It was confirmed that most Nestin⁺ cells within ischemic areas expressed the pericytic marker platelet-derived growth factor receptor beta (PDGFRβ). Nestin (G, H, I: green); PDGFRβ (G, H, J: red); DAPI (G-J: blue). *p<0.05 versus non-ischemic areas at each week (n = 3; F). Scale bars = 100 μm (B, G) and 20 μm (C, E, H).

Figure 5: Adult brain pericytes re-express the stem cell marker Nestin following ischemic stroke

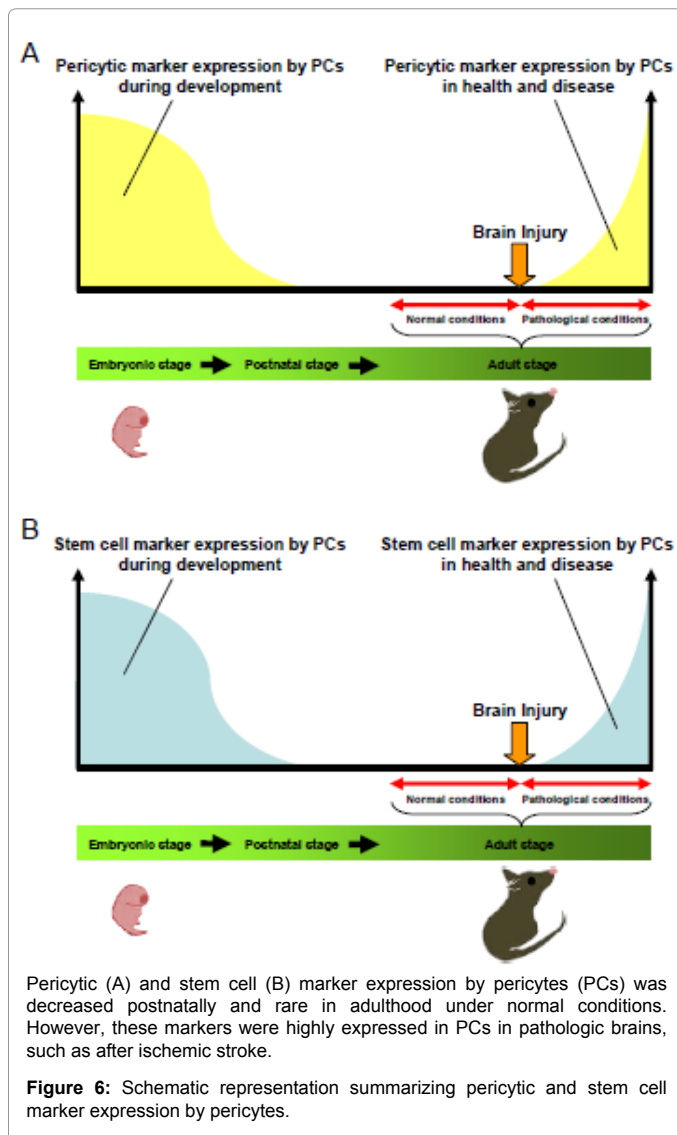
shape and location near ECs [1-3], they are now commonly recognized by various molecular markers, such as NG2, αSMA, and PDGFRβ [10,11,15-20].

NG2 is an integral membrane chondroitin sulfate proteoglycan expressed on the surface of PCs in various organs, including in the CNS [10,15]. Besides pericytic expression, NG2 is reportedly expressed in glial lineages, such as OPCs [26] and microglia [27], in the CNS. In this study, we found that NG2 was predominantly expressed in PCs rather than in glia during early embryonic stages. However, in postnatal and adult stages, NG2 expression gradually decreased in PCs and increased in glia. Although the relationship between PCs and glia remains unclear [14], increasing evidence shows that NG2⁺ cells have the potential to function as multipotent stem cells [27,30]. This suggests that PCs and/or glia can generate these cells. In this study, NG2 expression by PCs was diminished during development. However, NG2 was predominantly re-expressed in reactive PCs rather than in glia within ischemic regions following cerebral infarction. In addition, we previously demonstrated that NG2⁺ PCs obtained from ischemic regions produced multipotent stem cells [11,12], suggesting that PCs are the likely origin of multipotent stem cells.

αSMA is an isoform of the cytoskeletal protein Actin, usually expressed in smooth muscle lineages. Although αSMA is commonly used as a marker for PCs, αSMA expression by PCs varies in different tissues. αSMA is often absent in quiescent PCs in normal tissues, in particular in the CNS [31]. Consistently, we showed that αSMA

expression was downregulated during adulthood in the normal brain, although it was significantly expressed in early development. Quiescent PCs are slow-cycling during their resting stage, but become reactive and proliferate, migrate, and then differentiate into various cells in response to stimuli [11,20,21,29]. Under these conditions, various mediators, including growth factors, transcription factors, and stemness-related factors, are activated in PCs [32,33]. This suggests that, under pathologic conditions, PCs alter their phenotypes. In support of this viewpoint, this study shows that αSMA and NG2 were significantly re-expressed in PCs in injured areas following ischemic stroke, although they were rarely observed in adult brains under normal conditions. Although the factors that regulate αSMA expression remain unclear, PCs in tumors reportedly highly express this marker [16], implying that an abnormal relationship between PCs and ECs may alter the traits of PCs. Moreover, PCs from human brains begin to express αSMA in the presence of transforming growth factor-β1 [34].

The tyrosine kinase receptor PDGFRβ is a widely used molecule expressed in PCs. Because mice deficient in PDGFRβ or its ligand PDGFB exhibit significant reductions in and abnormalities of PCs [5], PDGFRβ is regarded as an essential regulator of the properties of PCs. Similar to NG2 and αSMA, we found that PDGFRβ is present in PCs during early development but rarely during adulthood (data not shown). However, PDGFRβ is highly re-expressed in adult brain PCs in response to ischemia. Although the mechanism underlying this re-expression remains unclear, PDGFB expression by ECs can activate



PDGFR β [35]. Thus, an endothelial source of PDGFB may play an important role in regulating and altering the properties of PCs during development and under pathologic conditions.

In the present study, we found that some pericytic markers were likely overlapped with an endothelial marker CD31 during development and under pathologic conditions. Although the precise relationship between PCs and ECs remains unclear, it is reported that both of them originate from certain common progenitors, such as Flk1⁺ vascular progenitor cells [36]. In addition, we recently demonstrated that adult brain PCs following ischemia acquired a complex phenotype of angioblasts, in addition to their original mesenchymal traits [12,37], similar to the finding showing that immature PCs during development have a mesenchymoangioblastic phenotype [38,39].

In this study, we found that the stem cell marker Nestin was significantly expressed in PCs during embryonic stages, although it was rarely present postnatally, as previously described [40,41]. However, Nestin was induced in reactive PCs following ischemia in adult mice, whereas it was rarely present in normal PCs in adult mice. These results show that traits of PCs, such as stemness, differ during development and under pathologic conditions. Although the mechanism by which

brain PCs acquire stemness remains unclear, we recently demonstrated that various stem cell and undifferentiated cell markers, such as Sox2, c-myc, and Klf4 were induced in adult brain PCs following ischemia, although they were rarely observed in adult brain PCs within non-ischemic areas [12,37]. In addition, we showed that brain PCs following ischemia acquire the multipotency through cellular reprogramming following ischemia/hypoxia [12,37]. Thus, it is likely that, under pathologic conditions, brain PCs re-acquire the stemness that is presumed to be lost postnatally [14,42], in association with the increased expression of pericytic markers. The exact relationship between expression of pericytic markers and traits should be clarified through further detailed studies because the present study based on immunohistochemical findings alone.

In conclusion, we show that the expression of representative pericytic markers, such as NG2 and α SMA, varies in brain PCs during development and between healthy and diseased states. Although marker expression by PCs decreases as they lose stemness, pericytic markers are re-induced as they re-acquire stemness in response to brain injuries, suggesting that marker expression by PCs is related to traits such as stemness. Thus, an understanding of the expression profiles of PCs may be useful for PC-based therapies, particularly because it may be possible to stimulate PCs to function as stem cells during development and at pathologic sites [12,14].

Acknowledgments

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References

- Eberth CJ (1871) *Handbuch der Lehre von den Geweben des Menschen und der Thiere*. Leipzig
- Rouget C (1873) *Memoire sur le developpement, la structures et les proprietes des capillaires sanguins et lymphatiques*. *Archs Physiol Norm Pathol* 5: 603-633.
- Zimmerman KW (1923) *Der feinere bau der blutcapillares*. *Z. Anat. Entwicklungsgesch* 68: 3-109.
- Hellstrom M, Gerhardt H, Kalen M, Li X, Eriksson U, et al. (2001) Lack of pericytes leads to endothelial hyperplasia and abnormal vascular morphogenesis. *J Cell Biol* 153: 543-553. [PubMed]
- Hellstrom M, Kalen M, Lindahl P, Abramsson A, Betsholtz C (1999) Role of PDGF-B and PDGFR-beta in recruitment of vascular smooth muscle cells and pericytes during embryonic blood vessel formation in the mouse. *Development* 126: 3047-3055. [PubMed]
- Hall CN, Reynell C, Gesslein B, Hamilton NB, Mishra A, et al. (2014) Capillary pericytes regulate cerebral blood flow in health and disease. *Nature* 508: 55-60. [PubMed]
- Armulik A, Genove G, Mae M, Nisancioglu MH, Wallgard E, et al. (2010) Pericytes regulate the blood-brain barrier. *Nature* 468: 557-561. [PubMed]
- Winkler EA, Bell RD, Zlokovic BV (2011) Central nervous system pericytes in health and disease. *Nat Neurosci* 14: 1398-1405. [PubMed]
- Bell RD, Winkler EA, Sagare AP, Singh I, LaRue B, et al. (2010) Pericytes control key neurovascular functions and neuronal phenotype in the adult brain and during brain aging. *Neuron* 68: 409-427. [PubMed]
- Dore-Duffy P, Katychew A, Wang X, Van Buren E (2006) CNS microvascular pericytes exhibit multipotential stem cell activity. *J Cereb Blood Flow Metab* 26: 613-624. [PubMed]
- Nakagomi T, Molnar Z, Nakano-Doi A, Taguchi A, Saino O, et al. (2011) Ischemia-induced neural stem/progenitor cells in the pia mater following cortical infarction. *Stem Cells Dev* 20: 2037-2051. [PubMed]
- Nakagomi T, Kubo S, Nakano-Doi A, Sakuma R, Lu S, et al. (2015) Brain vascular pericytes following ischemia have multipotential stem cell activity to differentiate into neural and vascular lineage cells. *Stem Cells* 33: 1962-1974. [PubMed]

13. Banerjee S, Bhat MA (2007) Neuron-glia interactions in blood-brain barrier formation. *Annu Rev Neurosci* 30: 235-258. [[PubMed](#)]
14. Nakagomi T, Nakano-Doi A, Kawamura M, Matsuyama T (2015) Do Vascular Pericytes Contribute to Neurovasculogenesis in the Central Nervous System as Multipotent Vascular Stem Cells? *Stem Cells Dev* 24: 1730-1739. [[PubMed](#)]
15. Birbrair A, Zhang T, Wang ZM, Messi ML, Enikolopov GN, et al. (2013) Skeletal muscle pericyte subtypes differ in their differentiation potential. *Stem Cell Res* 10: 67-84. [[PubMed](#)]
16. Morikawa S, Baluk P, Kaidoh T, Haskell A, Jain RK, et al. (2002) Abnormalities in pericytes on blood vessels and endothelial sprouts in tumors. *Am J Pathol* 160: 985-1000. [[PubMed](#)]
17. Mitchell TS, Bradley J, Robinson GS, Shima DT, Ng YS (2008) RGS5 expression is a quantitative measure of pericyte coverage of blood vessels. *Angiogenesis* 11: 141-151. [[PubMed](#)]
18. Birbrair A, Zhang T, Wang ZM, Messi ML, Olson JD, et al. (2014) Type-2 pericytes participate in normal and tumoral angiogenesis. *Am J Physiol Cell Physiol* 307: C25-38. [[PubMed](#)]
19. Karow M, Sanchez R, Schichor C, Masserdotti G, Ortega F, et al. (2012) Reprogramming of pericyte-derived cells of the adult human brain into induced neuronal cells. *Cell Stem Cell* 11: 471-476. [[PubMed](#)]
20. Nakagomi T, Molnar Z, Taguchi A, Nakano-Doi A, Lu S, et al. (2012) Leptomeningeal-derived doublecortin-expressing cells in poststroke brain. *Stem Cells Dev* 21: 2350-2354. [[PubMed](#)]
21. Kabara M, Kawabe J, Matsuki M, Hira Y, Minoshima A, et al. (2014) Immortalized multipotent pericytes derived from the vasa vasorum in the injured vasculature. A cellular tool for studies of vascular remodeling and regeneration. *Lab Invest* 94: 1340-1354. [[PubMed](#)]
22. Nakagomi T, Taguchi A, Fujimori Y, Saino O, Nakano-Doi A, et al. (2009) Isolation and characterization of neural stem/progenitor cells from post-stroke cerebral cortex in mice. *Eur J Neurosci* 29: 1842-1852. [[PubMed](#)]
23. Nakagomi N, Nakagomi T, Kubo S, Nakano-Doi A, Saino O, et al. (2009) Endothelial cells support survival, proliferation, and neuronal differentiation of transplanted adult ischemia-induced neural stem/progenitor cells after cerebral infarction. *Stem Cells* 27: 2185-2195. [[PubMed](#)]
24. Nakano-Doi A, Nakagomi T, Fujikawa M, Nakagomi N, Kubo S, et al. (2010) Bone marrow mononuclear cells promote proliferation of endogenous neural stem cells through vascular niches after cerebral infarction. *Stem Cells* 28: 1292-1302. [[PubMed](#)]
25. Saino O, Taguchi A, Nakagomi T, Nakano-Doi A, Kashiwamura S, et al. (2010) Immunodeficiency reduces neural stem/progenitor cell apoptosis and enhances neurogenesis in the cerebral cortex after stroke. *J Neurosci Res* 88: 2385-2397. [[PubMed](#)]
26. Nishiyama A, Komitova M, Suzuki R, Zhu X (2009) Polydendrocytes (NG2 cells): multifunctional cells with lineage plasticity. *Nat Rev Neurosci* 10: 9-22. [[PubMed](#)]
27. Yokoyama A, Sakamoto A, Kameda K, Imai Y, Tanaka J (2006) NG2 proteoglycan-expressing microglia as multipotent neural progenitors in normal and pathologic brains. *Glia* 53: 754-768. [[PubMed](#)]
28. Murdoch B, Roskams AJ (2008) A novel embryonic nestin-expressing radial glia-like progenitor gives rise to zonally restricted olfactory and vomeronasal neurons. *J Neurosci* 28: 4271-4282. [[PubMed](#)]
29. Dore-Duffy P, Owen C, Balabanov R, Murphy S, Beaumont T, et al. (2000) Pericyte migration from the vascular wall in response to traumatic brain injury. *Microvasc Res* 60: 55-69. [[PubMed](#)]
30. Belachew S, Chittajallu R, Aguirre AA, Yuan X, Kirby M, et al. (2003) Postnatal NG2 proteoglycan-expressing progenitor cells are intrinsically multipotent and generate functional neurons. *J Cell Biol* 161: 169-186. [[PubMed](#)]
31. Gerhardt H, Betsholtz C (2003) Endothelial-pericyte interactions in angiogenesis. *Cell Tissue Res* 314: 15-23. [[PubMed](#)]
32. Tsang WP, Shu Y, Kwok PL, Zhang F, Lee KK, et al. (2013) CD146+ human umbilical cord perivascular cells maintain stemness under hypoxia and as a cell source for skeletal regeneration. *PLoS One* 8: e76153. [[PubMed](#)]
33. Chen CW, Okada M, Proto JD, Gao X, Sekiya N, et al. (2013) Human pericytes for ischemic heart repair. *Stem Cells* 31: 305-316. [[PubMed](#)]
34. Verbeek MM, Otte-Holler I, Wesseling P, Ruiter DJ, de Waal RM (1994) Induction of alpha-smooth muscle actin expression in cultured human brain pericytes by transforming growth factor-beta 1. *Am J Pathol* 144: 372-382. [[PubMed](#)]
35. Abramsson A, Lindblom P, Betsholtz C (2003) Endothelial and nonendothelial sources of PDGF-B regulate pericyte recruitment and influence vascular pattern formation in tumors. *J Clin Invest* 112: 1142-1151. [[PubMed](#)]
36. Yamashita J, Itoh H, Hirashima M, Ogawa M, Nishikawa S, et al. (2000) Fli-1-positive cells derived from embryonic stem cells serve as vascular progenitors. *Nature* 408: 92-96. [[PubMed](#)]
37. Sakuma R, Kawahara M, Nakano-Doi A, Takahashi A, Tanaka Y, et al. (2016) Brain pericytes serve as microglia-generating multipotent vascular stem cells following ischemic stroke. *J Neuroinflammation* 13: 57. [[PubMed](#)]
38. Tigges U, Hyer EG, Scharf J, Stallcup WB (2008) FGF2-dependent neovascularization of subcutaneous Matrigel plugs is initiated by bone marrow-derived pericytes and macrophages. *Development* 135: 523-532. [[PubMed](#)]
39. Rolny C, Nilsson I, Magnusson P, Armulik A, Jakobsson L, et al. (2006) Platelet-derived growth factor receptor-beta promotes early endothelial cell differentiation. *Blood* 108: 1877-1886. [[PubMed](#)]
40. Mignone JL, Kukekov V, Chiang AS, Steindler D, Enikolopov G (2004) Neural stem and progenitor cells in nestin-GFP transgenic mice. *J Comp Neurol* 469: 311-324. [[PubMed](#)]
41. Bifari F, Decimo I, Chiamulera C, Bersan E, Malpeli G, et al. (2009) Novel stem/progenitor cells with neuronal differentiation potential reside in the leptomeningeal niche. *J Cell Mol Med* 13: 3195-3208. [[PubMed](#)]
42. Nakagomi T, Nakano-Doi A, Matsuyama T (2015) Leptomeninges: a novel stem cell niche harboring ischemia-induced neural progenitors. *Histol Histopathol* 30: 391-399. [[PubMed](#)]