**Regular Article**

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**Transient Receptor Potential Melastatin 3 Is Functionally Expressed in Oligodendrocyte Precursor Cells and Is Upregulated in Ischemic Demyelinated Lesions**

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Oligodendrocyte precursor cells (OPCs) are glial cells that differentiate into oligodendrocytes and myelinate axons. The number of OPCs is reportedly increased in brain lesions in some demyelinating diseases and during ischemia; however, these cells also secrete cytokines and elicit both protective and deleterious effects in response to brain injury. The mechanism regulating the behaviors of OPCs in physiological and pathological conditions must be elucidated to control these cells and to treat demyelinating diseases. Here, we focused on transient receptor potential melastatin 3 (TRPM3), a Ca2+-permeable channel that is activated by the neurosteroid pregnenolone sulfate (PS) and body temperature. Trpm3+/Pdgfra+ OPCs were detected in the cerebral cortex (CTX) and corpus callosum (CC) of P4 and adult rats by in situ hybridization. Trpm3 expression was detected in primary cultured rat OPCs and was increased by treatment with tumor necrosis factor α (TNFα). Application of PS (30–100µM) increased the Ca2+ concentration in OPCs and this effect was inhibited by co-treatment with the TRP channel blocker Gd3+ or the TRPM3 inhibitor iso-sakuranetin (10µM). Stimulation of TRPM3 with PS (50µM) did not affect the differentiation or migration of OPCs. The number of Trpm3+ OPCs was markedly increased in demyelinated lesions in an endothelin-1 (ET-1)-induced ischemic rat model. In conclusion, TRPM3 is functionally expressed in OPCs in vivo and in vitro and is upregulated in inflammatory conditions such as ischemic insults and TNFα treatment, implying that TRPM3 is involved in the regulation of specific behaviors of OPCs in pathological conditions.

**Key words** oligodendrocyte precursor cell; ischemia; Ca2+ imaging; demyelination; transient receptor potential melastatin 3

**INTRODUCTION**

Oligodendrocyte precursor cells (OPCs) are glial cells that differentiate into mature oligodendrocytes and form myelin sheaths around axons. The proliferation, migration, and differentiation of OPCs are altered in many brain pathologies.1) Although OPCs rapidly proliferate in lesions following brain injury,2) they can also secrete cytokines and elicit both protective and deleterious effects in response to brain injury. The mechanism regulating the behaviors of OPCs in physiological and pathological conditions must be elucidated to control these cells and to treat demyelinating diseases. Here, we focused on transient receptor potential melastatin 3 (TRPM3), a Ca2+-permeable channel that is activated by the neurosteroid pregnenolone sulfate (PS) and body temperature. Trpm3+/Pdgfra+ OPCs were detected in the cerebral cortex (CTX) and corpus callosum (CC) of P4 and adult rats by in situ hybridization. Trpm3 expression was detected in primary cultured rat OPCs and was increased by treatment with tumor necrosis factor α (TNFα). Application of PS (30–100µM) increased the Ca2+ concentration in OPCs and this effect was inhibited by co-treatment with the TRP channel blocker Gd3+ or the TRPM3 inhibitor iso-sakuranetin (10µM). Stimulation of TRPM3 with PS (50µM) did not affect the differentiation or migration of OPCs. The number of Trpm3+ OPCs was markedly increased in demyelinated lesions in an endothelin-1 (ET-1)-induced ischemic rat model. In conclusion, TRPM3 is functionally expressed in OPCs in vivo and in vitro and is upregulated in inflammatory conditions such as ischemic insults and TNFα treatment, implying that TRPM3 is involved in the regulation of specific behaviors of OPCs in pathological conditions.

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MATERIALS AND METHODS

Reagents  Unless otherwise noted, all reagents were purchased from NacalaiTesque (Kyoto, Japan). PS was obtained from Sigma-Aldrich (St. Louis, MO, U.S.A.) and isosakuranetin was obtained from Extractsynhese (Genay, France). Recombinant human vascular endothelial growth factor 165 (100-20), recombinant rat TNFα (400-14), and recombinant rat interleukin 1β (IL1β; 400-01B) were purchased from PeproTech (U.S.A.).

Primary Culture of OPCs  All experiments were conducted in accordance with the ethical guidelines set down by the Kyoto University Animal Research Committee. Primary cultures of OPCs were prepared from cerebral cortices of 0–2-d-old Wistar/ST rats (Japan SLC, Shizuoka, Japan) as previously reported with slight modifications. Briefly, dissociated cells were plated onto poly-l-ornithine-coated 75 cm² flasks in Dulbecco’s modified Eagle’s medium (D5796, Sigma-Aldrich) containing 10% heat-inactivated fetal bovine serum and 1% penicillin/streptomycin mixed solution (Nacalai Tesque) and maintained at 37 °C in a humidified atmosphere containing 5% CO₂. After 10–15 d, OPCs were purified from the mixed glial culture using a two-step procedure. First, microglia were removed by shaking at 130–150 rpm for 1.5 h. After changing the medium, OPCs were detached by shaking at 220–230 rpm for 15–17 h. To remove astrocytes, the supernatant was cultured on non-coated 10 cm dishes for 1 h at 37 °C in 5% CO₂. The final cell suspension was replated onto poly-l-ornithine-coated 10 mm glass coverslips in 35-mm dishes and maintained in Neurobasal medium (Life Technologies, U.S.A.) supplemented with 1 x B27 (Life Technologies), glutamine (2 mM), 1% penicillin/streptomycin mixed solution, platelet-derived growth factor-AA (PDGF-AA) (10 ng/mL; PeproTech), and basic fibroblast growth factor (10 ng/mL; PeproTech), which was used as proliferation medium. To analyze the differentiation of OPCs, 2d after replating, proliferation medium was replaced by Neurobasal medium supplemented with 1 x B27, glutamine (2 mM), 1% penicillin/streptomycin mixed solution, triiodo-l-thyronine (30 ng/mL; Sigma-Aldrich), and ciliary neurotrophic factor (10 ng/mL; PeproTech), which was used as differentiation medium.

RT-PCR  Total RNA was extracted from OPCs cultured in a 35 mm dish using a Nucleo Spin RNA Kit (TaKaRa Bio, Shiga, Japan). First-strand cDNA was prepared from total RNA using a ReverTra Ace qPCR RT Kit (Toyobo, Osaka, Japan). Rat Trpm3 cDNA was amplified by PCR using Blend Taq (Toyobo) and the following primer pair: 5'-AGC AGT GGC CGG TAG GGTC CGG GCA CCA CGG GG-3' and 5'-GAG GTT GGA GCG GCT CAA GGT ACG CAC CAT CCA-3' for rat Ldha. 5'-CTCTGGAAGAAGAGCAGTC-3' and 5'-GAT GAG TTT AGT AGC CGA CAC CCA-3' for rat 18S rRNA, 5'-GAT GAG TTT AGT AGC CGA CAC CCA-3' and 5'-CAT CGG CAC GTG TCT GTG GTC A-3' for rat Pdgfra, 5'-CTCTGGAAGAAGAGCAGTC-3' and 5'-GAT GAG TTT AGT AGC CGA CAC CCA-3' for rat Tgtp.

Fluorometric Ca²⁺ Imaging  Ca²⁺ imaging was conducted as previously reported with slight modifications. OPCs on coverslips were loaded for 45–55 min with 5 µM fura 2 acetoxymethyl ester (fura 2-AM; Dojindo, Kumamoto, Japan) prepared in Krebs–Ringer buffer (140 m NaCl, 5 mM KCl, 1 mM MgCl₂, 2 mM CaCl₂, 10 mM glucose, and 10 mM N-(2-hydroxyethyl)piperazine-N′-2-ethanesulfonic acid (HEPES), adjusted to pH 7.4) containing 0.005% Cremophor EL (Sigma-Aldrich). Fluorescence images were captured at room temperature every 2 s with the AQUACOSMOS/ORCA-AG imaging system (Hamamatsu Photonics, Shizuoka, Japan) using alternating excitation wavelengths of 340 and 380 nm and an emission wavelength of 510 nm. The ratio of the fluorescence intensity obtained with excitation/emission wavelengths of 340 nm/510 nm (F340) to that obtained with excitation/emission wavelengths of 380/510 nm (F380), so-called F340/F380, was calculated to quantify the intracellular calcium concentration ([Ca²⁺]i).

Scratch-Wound Assay  Scratch-wound assay was conducted as previously reported with slight modifications. When OPCs in 35 mm dishes were 70–80% confluent, a wound was created using a cell scraper (BM Bio, Tokyo, Japan). Cultures were washed twice with PBS, medium containing PS or vehicle (dimethyl sulfoxide) was added, and cells were allowed to migrate for 12–24 h. Three-phase contrast images were acquired per dish using a DIAPHOT 300 inverted phase contrast microscope (Nikon, Tokyo, Japan) and a DS-U1 DIGITAL SIGHT camera system (Nikon). The number of cells that migrated into the scratched area was counted. The average number of migrated cells in one dish was regarded as n = 1 and three dishes were used per group (n = 3).

Quantitative (q)RT-PCR  After reverse transcription of total mRNA into cDNA using a ReverTra Ace qPCR RT Kit, real-time quantitative PCR was performed using the StepOne Real-Time PCR System (Life Technologies, Waltham, MA, U.S.A.) and THUNDERBIRD SYBR qPCR Mix (Toyobo). The primer sets were as follows: 5'-CGG TCC AAC AGA TTCTACCTC-3' and 5'-ACC GC GGTT CTA TTT TTT TGTTG-3' for rat 18S ribosomal RNA (rRNA), 5'-AGG CTC AAG GGT ATT TTCTGG-3' and 5'-CGG CTC TTCTC TTCTT GGAG AT-3' for rat Trpm3, 5'-GAT AGT TTT AGT AGC CGA CACC ACA-3' and 5'-CATTG GAC GT TAA CTG TTGTG TCTCA-3' for rat Pdgfra, 5'-CTCTGGAAGAAGAGCAGTC-3' and 5'-TGTC TCTCTTCTCCCA CGACTA-3' for rat Mbp (myelin basic protein), 5'-TAA GGC CAA CGGT GAA AAG-3' and 5'-TAC ATGGC TGG GTGGT TTG-3' for rat Actb (beta-actin) and 5'-TCC AGT GTG TAA TGT CAC CG-3' and 5'-CAT CAC TAT TCA ATC GAT CTCT TAC-3' for rat Ldha (lactate dehydrogenase A). The PCR conditions were as follows: 95 °C for 10 min, followed by 45 cycles of 95 °C for 15 s and 60 °C for 60 s. Expression levels of each gene were normalized against that of 18S rRNA or Actb (only in hypoxic experiment), which was measured in parallel in each sample.

Oxygen Deprivation of OPCs  Three days after replating, medium was changed to fresh proliferation medium and OPCs were placed in hypoxic conditions (2% O₂, 5% CO₂, 93% air) at 37 °C using Personal CO₂ Multi Gas Incubator (APM-30D; ASTEC, Fukuoka, Japan) for 12 h, or in normoxic condition (5% CO₂, 95% air) for 12 h as control. After hypoxia treatment, OPCs were used to extract total RNA immediately. Actb was used as internal control gene in hypoxic experiment because 18S rRNA expression was unexpectedly increased in 2% O₂ condition.

Stereotactic Injection of ET-1  All procedures were performed in accordance with the guidelines for Animal Experimentation at Gunma University Graduate School of Medicine and were approved by Gunma University Ethics Committee. ET-1 was injected as previously described with Sprague-Dawley rats.
Trpm3 and Pdgfra mRNA in the CTX and CC of P4 and adult rats. (A) TRPM3 and Pdgfra mRNA in the CTX and CC of P4 and adult rats. Scale bar, 200 μm. (Color figure can be accessed in the online version.)

RESULTS

TRPM3 Is Expressed in OPCs in P4 and Adult Rats

Mature oligodendrocytes reportedly express TRPM3. To determine whether this is also true of OPCs, we performed double fluorescent in situ hybridization. We detected Trpm3 and Pdgfra (an OPC marker) in the cerebral cortex (CTX, gray matter) and corpus callosum (CC, white matter) of P4 and adult rats (Fig. 1). Most Pdgfra+ OPCs (more than 80%, arrowheads) expressed Trpm3 in P4 rats. The number of Trpm3+/Pdgfra− cells did not differ between the CTX and CC (Fig. 1). Many Pdgfra+ cells were also Trpm3+ (more than 80%, arrowheads) in the CTX and CC of adult rats (Fig. 1). These results indicate that TRPM3 is expressed in most OPCs in vivo.

TRPM3 Is Functionally Expressed in Primary Cultured OPCs

Next, we examined whether TRPM3 is functionally expressed in primary cultured rat OPCs. We detected Trpm3 mRNA in cultured rat OPCs by RT-PCR (Fig. 2A). To investigate whether TRPM3 is functional in these cells, we measured changes in \([Ca^{2+}]i\) using a fura 2-AM-based fluorometric \(Ca^{2+}\) imaging assay. Treatment with the neurosteroid PS, which directly activates TRPM3, increased \([Ca^{2+}]i\), in a concentration-dependent manner (Figs. 2B–D). Co-treatment with the non-selective TRP channel blocker Gd3+ (100 μM, Fig. 2E) or the TRPM3 antagonist isosakuranetin (20 μM, Fig. 2F) almost completely inhibited \(Ca^{2+}\) influx induced by PS treatment (50 μM). These results indicate that TRPM3 is functionally expressed in cultured rat OPCs and plays an important role in influx of extracellular \(Ca^{2+}\) across the plasma membrane.

TRPM3 Is Not Involved in the Migration or Differentiation of OPCs

We assessed if TRPM3 plays a role in the differentiation of OPCs. Culture of OPCs in proliferation medium containing PS (50 μM) for 48 h did not change the mRNA levels of the immature OPC marker Pdgfra (Fig. 3A) or the mature oligodendrocyte marker Mbp (Fig. 3B). Cells cultured in differentiation medium were used as a negative control for Pdgfra expression and as a positive control for Mbp expression. These results indicate that activation of TRPM3 does not affect the differentiation of OPCs.

Next, we examined the effect of TRPM3 on the migration of OPCs by performing a scratch-wound assay. The number of
migrated OPCs at 12 and 24 h after scratching did not differ between the PS-treated and control groups (Figs. 3C–E). However, the number of OPCs in the scratched area at 24 h was higher in the group treated with vascular endothelial growth factor-A (VEGF-A), which was used as a positive control, than in the control group. These results suggest that TRPM3 is not involved in the migration of OPCs.

The Number of TRPM3+ OPCs Is Increased in Demyelinated Lesions in an ET-1-Induced Ischemic Rat Model

Finally, we explored the pathophysiological roles of OPCs. We previously reported that stereotactic injection of ET-1 into the internal capsule causes severe demyelination and that proliferation of OPCs in the scratched area at 24 h was higher in the group treated with vascular endothelial growth factor-A (VEGF-A), which was used as a positive control, than in the control group. These results suggest that TRPM3 is not involved in the migration of OPCs.

**The Number of TRPM3+ OPCs Is Increased in Demyelinated Lesions in an ET-1-Induced Ischemic Rat Model**

Finally, we explored the pathophysiological roles of OPCs. We previously reported that stereotactic injection of ET-1 into the internal capsule causes severe demyelination and that proliferation of OPCs close to demyelinated regions is increased at 7 d after injection. We detected Trp3 mRNA in this demyelinated model. Consistent with our previous report, the number of NG2+ OPCs in the internal capsule was significantly higher in the ET-1-injected group than in the sham group at 7 d after injection (Figs. 4A, B, upper panels). Most Trp3 signals colocalized with NG2 signals. The number of Trp3+ OPCs significantly increased after demyelination in the internal capsule, whereas the number of Trp3+/NG2− cells was increased in ET-1 injected group (Fig. 4B, upper panels), indicating that Trp3 expression was also increased in not only OPCs but also other brain cells or infiltrated immune cells. Fluorescent halation made it difficult to distinguish individual cells in FISH sections. Therefore, we also detected Trp3 by alkaline phosphatase-based in situ hybridization. The number of Trp3 signals was increased in the ET-1-injected group (Fig. 4B, lower panels). On the other hands, the number of Trp3 signals was not changed in hippocampus of ET-1-injected group (Fig. 4C). To explore the mechanism of Trp3 upregulation, we investigated whether inflammatory cytokines, whose levels increase during ischemia, affect Trp3 expression in primary cultured rat OPCs. Trp3 expression was significantly increased by TNFα (30 ng/mL) treatment and was increased by IL1β (30 ng/mL) treatment, although this effect was not sig-

**Fig. 2. TRPM3 Is Functionally Expressed in Cultured Rat OPCs**

(A) RT-PCR analysis of Trp3 in OPCs. The marker (M) shows a 100 bp DNA ladder. (B–F) Measurement of [Ca2+]i in OPCs using fura 2-AM (B–D) Ca2+ response elicited by application of the indicated concentrations (30–100 µM) of PS. (E) Changes in [Ca2+]i after co-application of PS (50 µM) and the non-selective TRP channel blocker Gd3+ (100 µM). (F) Changes in [Ca2+]i after co-application of PS (50 µM) and the selective TRPM3 antagonist isosakuranetin (10 µM, Iso).

**Fig. 3. Stimulation of TRPM3 by PS Does Not Affect the Differentiation or Migration of OPCs**

(A, B) OPCs were cultured in control proliferation medium, proliferation medium containing PS (50 µM), or differentiation medium for 48 h. mRNA expression of the OPC marker Pdgfra (A) and the mature oligodendrocyte marker Mbp (B) was analyzed by quantitative RT-PCR. ***p < 0.001 vs. control (ctrl); n = 8. (C–E) Migration of OPCs was evaluated by a scratch-wound assay. After scratching, cells were treated with vehicle, PS (50 µM), or VEGF-A (200 ng/mL) and allowed to migrate for 24 h. (C) Number of OPCs that had migrated into the scratched area at 12 h (C) and 24 h (D) after scratching. *p < 0.05 vs. control (ctrl, 24 h); n = 3. (E) Representative images of OPCs acquired at 0 h (upper) and 24 h (lower) after scratching. The black line indicates the scratch edge. Scale bar, 100 µm.
DISCUSSION

This study demonstrates that TRPM3 is functionally expressed in platelet-derived growth factor receptor (PDGFR) α+ OPCs in rat CTX and CC and in primary cultured OPCs (Figs. 1, 2). In addition, the TRPM3 agonist PS evoked Ca^{2+} influx (Fig. 3), suggesting that TRPM3 plays a role in Ca^{2+} signaling in OPCs. TRPM3 is functionally expressed in mature oligodendrocytes. Our data demonstrate that TRPM3 is also expressed in immature OPCs, indicating that this channel is involved in the function of the oligodendrocyte lineage from early stages.

TRPM3 in the brain can be activated by PS, sphingosine, hypo-osmotic stress and mild body temperature. PS is a neurosteroid produced in the brain and is present at a nanomolar concentration in cerebrospinal fluid. Although a micromolar concentration of PS was needed to activate TRPM3 in this study and a previous report, the PS concentration is increased at specific sites and in certain conditions, such as anxiety-depressive disorders and hyperthyroidism. Sulfa-
tion of pregnenolone is catalyzed by SULT2A1, SULT2B1a, and SULT2B1b, which belong to subfamilies of cytosolic sulfotransferases. Given that neurons can convert cholesterol into pregnenolone and express SULT2B1b, endogenous PS produced by neurons may activate TRPM3 in OPCs. This indicates that TRPM3 in OPCs is an important factor to exert neuron-glial interaction. In addition, the number of Trpm3+ OPCs was increased in ischemic demyelinated lesions (Fig. 4). Damaged neurons in these lesions may produce PS that activates TRPM3 in OPCs and this cell-to-cell communication may affect cellular behaviors in ischemic areas of the brain.

Sphingosine is an endogenous activator of TRPM3 and can induce Ca^{2+} entry into mature oligodendrocytes via TRPM3. This compound is synthesized from ceramide by ceramidase and is phosphorylated to produce sphingosine 1-phosphate. Given that levels of ceramide, sphingosine, and sphingosine 1-phosphate are increased in the ischemic brain, sphingosine 1-phosphate is increased in ischemic demyelinated lesions (Fig. 4). TRPM3 in proliferating OPCs may be more activated in ischemic lesions than in the normal brain. In addition, body temperature and hypo-osmotic stress are another endogenous activators of TRPM3 because opening of this channel increases between 30 and 40°C and 200 and 300 mOsm/L, respectively. Consequently, elevated mRNA expression of Trpm3 should result in increased Ca^{2+} entry even in the absence of chemical agonists.

In spite of the limited understanding of the endogenous activators, there have been advances in knowledge of endogenous inhibitory mechanisms of TRPM3. Recently, TRPM3 activation is reported to be inhibited by βγ subunits of G-protein that is released from activated G-protein coupled receptors and also inhibited by phospholipase C (PLC) activation. In HEK cells expressing both TRPM3 and platelet-derived growth factor receptor (PDGFR) β, PS-induced TRPM3 current was significantly inhibited by PDGF via PLCγ activation. In our study, cultured OPC highly expressed PDGFRα and almost all experiments except for Ca^{2+} imaging were performed using culture media containing PDGF-AA. It is conceivable that strong PDGFα stimulation with PDGF may activate PLC and inhibit PS-induced TRPM3 activation, resulting in no effects of PS on the migration and differentiation of OPCs. We could not perform experiments using...
PDGF-AA-free culture media because PDGF-AA is critically important for the cellular function of OPCs, therefore further experiments will be needed to clarify the inhibitory effect of PDGF-AA. In addition, the increase of Pdgfb expression was reported in ET-1-induced lacunar infarction model,\textsuperscript{40} and therefore, secreted PDGF may have an effect on OPCs in the lesion. Moreover, brain endothelial cell-derived PDGF-BB enhances blood-brain barrier integrity through a mechanism involving PDGF-BB/PDGFRα signaling in OPCs.\textsuperscript{42} Considering that TRPM3 is inhibited by PDGF-BB,\textsuperscript{39} these relationships are likely to be very complex at the ET-1 injected site. Further research will be needed to understand the functional linkage between TRPM3 and PDGF-BB/PDGFRα signaling in OPCs of the lesion area.

Injection of ET-1 into the internal capsule causes contraction of small vessels and injury of white matter, which is similar to lacunar infarction.\textsuperscript{43} The number of OPCs, most of which were Trpm3\textsuperscript{+}, was increased in demyelinated lesions in the ET-1-injected group. Given that Trpm3 was upregulated in response to TNFα, Ca\textsuperscript{2+} signals through TRPM3 may alter the behaviors of OPCs at sites of inflammation. Demyelination occurs in the ET-1-induced ischemic model, while axons are not damaged.\textsuperscript{35} Therefore, modulation of the expression or opening of TRPM3 may facilitate the proper function of OPCs and assist the development of therapies that promote remyelination. Our in vitro data showed that TRPM3 agonist PS did not change the differentiation or migration under non-inflammatory condition. TRPM3 might contribute to changes in specific cellular behaviors, such as proliferation or cytokine production in inflammatory conditions because the number of Trpm3\textsuperscript{+} OPCs was increased in demyelinated lesions in an ischemic rat model. Further in vitro and in vivo investigations are required to reveal the pathophysiological roles of TRPM3 in OPCs.

What impact does upregulation of TRPM3 in OPCs in demyelinated lesions have on the pathology of ischemia? In general, it is thought that proliferating OPCs in lesions can differentiate into mature oligodendrocytes. The L-type voltage-dependent Ca\textsuperscript{2+} channel Cav1.2 in OPCs is reported to promote remyelination\textsuperscript{44}, therefore, Ca\textsuperscript{2+} influx via TRPM3 might enhance remyelination and recovery from ischemia. Activation of TRPM3 did not affect the differentiation of OPCs cultured in proliferation medium; however, it may affect the differentiation of these cells if they are cultured in differentiation medium or maintained in the presence of inflammatory cytokines. Further in vitro examinations are required to verify which cellular functions are affected by activation of TRPM3. Surprisingly, OPCs can also elicit deleterious effects, such as disruption of the blood–brain barrier via matrix metalloproteinase-9 release in response to brain injury.\textsuperscript{35} It is recently reported that OPCs in inflammatory demyelination model lose their differentiation capacity and instead gain antigen-presenting capacity and propagate inflammation.\textsuperscript{43} If OPCs elicit deleterious effects upon activation of TRPM3, upregulation of TRPM3 may exacerbate brain injury after ischemia. Regulating the amount of Ca\textsuperscript{2+} influx is also critical for proper cellular function. While AMPA receptors in OPCs are physiologically important for oligodendrocyte survival,\textsuperscript{36} excessive Ca\textsuperscript{2+} influx via AMPA receptors in oligodendrocytes exacerbates demyelination in multiple sclerosis model.\textsuperscript{35} In the ischemia experiment (Fig. 4), enhanced TRPM3 expression in OPCs may disrupt intracellular Ca\textsuperscript{2+} homeostasis, leading to dysfunction of the cells. Further investigations of TRPM3-knockout mice are required to elucidate the importance of TRPM3 in OPCs.

In conclusion, this study demonstrates that TRPM3 is functionally expressed in OPCs in vivo and in vitro. TRPM3 is upregulated in proliferating OPCs in ischemic demyelinated lesions and in cultured OPCs treated with the inflammatory cytokine TNFα. We suggest that TRPM3 in OPCs plays important roles in brain injury and inflammation.

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**Conflict of Interest** The authors declare no conflict of interest.

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