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CircPDS5B Reduction Improves Angiogenesis Following Ischemic Stroke by Regulating MicroRNA-223-3p/NOTCH2 Axis

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Abstract

Background and Objectives

Ischemic stroke (IS) is responsible for major causes of global death and disability, for which promoting angiogenesis is a promising therapeutic strategy. This study analyzed circular RNA PDS5B (circPDS5B) and its related mechanisms in angiogenesis in IS.

Methods

In the permanent middle cerebral artery occlusion (pMCAO) mouse model, circPDS5B, microRNA (miR)-223-3p, and NOTCH2 levels were checked. By testing neurologic function, neuronal apoptosis, and expression of angiogenesis-related proteins in pMCAO mice, the protective effects of circPDS5B knockdown were probed. In human brain microvascular endothelial cells (HBMECs) under oxygen-glucose deprivation (OGD) conditions, the effects of circPDS5B, miR-223-3p, and NOTCH2 on angiogenesis were studied by measuring cellular activities.

Results

The increase of circPDS5B and NOTCH2 expression and the decrease of miR-223-3p expression were examined in pMCAO mice. Reducing circPDS5B expression indicated protection against neurologic dysfunction, apoptosis, and angiogenesis impairment. For circPDS5B-depleted or miR-223-3p-restored HBMECs under OGD treatment, angiogenesis was promoted. MiR-223-3p inhibition–associated reduction of angiogenesis could be counteracted by knocking down NOTCH2. CircPDS5B depletion–induced angiogenesis in OGD-conditioned HBMECs was repressed after overexpressing NOTCH2.

Discussion

In IS, the expression of circPDS5B was upregulated, and miR-223-3p inhibited HBMECs activity and promoted NOTCH2 expression, thus promoting IS. CircPDS5B reduction improves angiogenesis following ischemic stroke by regulating microRNA-223-3p/NOTCH2 axis.

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Glossary

CCA = common carotid artery; **circPDS5B** = circular RNA PDS5B; **circPDS5B-WT and NOTCH2-WT** = wild-type circPDS5B and NOTCH2; **HBMEC** = human brain microvascular endothelial cell; **IS** = ischemic stroke; **MCA** = middle cerebral artery; **miRNA** = microRNA; **ncRNA** = noncoding RNA; **OGD** = oxygen-glucose deprivation; **pLVX-sh-circPDS5B** = lentiviral shRNA targeting circPDS5B; **pLVX-sh-NC** = lentiviral shRNA negative control; **pMCAO** = permanent middle cerebral artery occlusion; **si-circPDS5B/NOTCH2** = small interfering RNA.

Ischemic stroke (IS) is responsible for major causes of global death and disability, with ischemic stroke (IS) accounting for 80%.[1] IS is the result of occlusion of a cerebral artery with severe neurologic consequences.[2] Because of the complex pathophysiology and difficulty in recovery of neuronal function, current treatments are limited for IS.[3] Angiogenesis refers to the physiologic process by which existing capillaries or postcapillaries form new blood vessels,[4] which can promote collateral circulation, restore blood supply in ischemic areas, and reduce ischemic necrosis.[5] However, the molecular mechanisms regulating angiogenesis have not been fully elucidated.

Noncoding RNAs (ncRNAs), regulating gene expression in a posttranscriptional manner, have received extensive attention over the past decades and have been discussed in the pathophysiology of IS.[6] CircRNAs are not affected by RNA exonuclease and are featured by expression stability and degradation resistance, which can lead to new back-splicing or skipping events in pre-mRNA.[7] It is believed that circRNAs are often highly expressed in the CNS.[8] For example, circFUNDC1 is upregulated in patients with IS, serving a valuable indicator to evaluate the risk of stroke associated infection.[9] CircPDS5B has been observed to have elevated expression levels in patients with IS.[10]

MicroRNAs (miRNAs) are a group of ncRNAs that functions essentially in various diseases, including IS.[11] For example, miR-384-5p is downregulated in IS mice, and induction of miR-384-5p promotes IS angiogenesis,[12] and miR-493 is lowly expressed in patients with stroke and inhibits angiogenesis in IS rats.[13] MiR-223-3p is a widely studied miRNA that has been found to be involved in various diseases including sepsis,[14] cancer,[15] and cardiovascular disease.[16] A latest study has stated that miR-223-3p expression is elevated in a permanent middle cerebral artery occlusion (pMCAO) mouse model, suggesting a diagnostic value for IS.[17] And circRNAs can decoy miRNAs to regulate mRNA expression.[18] This study analyzed circular RNA PDS5B (circPDS5B) and its related mechanisms in angiogenesis in IS based on a mouse model of pMCAO and HBMEC model of OGD, hoping to earn a niche for IS therapy.

Methods

Ethical Statement

All procedures and animal care were approved by the Laboratory Animal Management and Use Committee of Shenzhen Jingtuo Biotechnology Co., Ltd, and performed according to the ARRIVE guidelines 2.0.

Induction of pMCAO

Fifty grown-up male C57BL/6 mice (7–8 weeks, 25–30 g), provided by Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China), were housed under a standard environment (22 ± 2°C, 55 ± 5% humidity, 12-hour light rhythm, and sufficient supply of food and water) and subjected to pMCAO surgery.[19] Briefly, after an intraperitoneal injection of pentobarbital sodium at 30 mg/kg, the mice were subjected to right common carotid artery (CCA) ligation using 5/0 sutures. The base of the parotid gland and the temporalis muscle's upper region were separated by an incision made in the lateral to orbit of the external auditory canal, exposing the cortical branch of the right middle cerebral artery (MCA). A 0.8-mm high-speed microdrill was used to perform a cranial otomy at the MCA's distal end to cauterize the MCA's cortical branches. Mice were placed in a thermostat to maintain body temperature throughout surgery. For sham-operated mice, neither ligation nor cauterization was performed. According to the experimental design, the group was compared, and a control group was set to eliminate the influence of irrelevant variables on the experimental results.

Neurologic function was scored 24 hours after surgery. Subsequently, mice were killed by euthanasia, and brain tissues were collected from 5 mice per group for apoptosis detection, and the remaining tissues were preserved at −80°C for extracting RNA or protein samples.

A lentiviral shRNA targeting circPDS5B (pLVX-sh-circPDS5B) was injected into the right ventricle 2 weeks before surgery[20] using stereotaxic equipment (RWD Life Science Co., Ltd, Shenzhen, China). Lentiviral shRNA negative control (pLVX-sh-NC) served as a control. The injection site was 0.22 cm deep into the dura, 0.1 cm to the right of the sagittal suture, and 0.03 cm posterior to the bregma. Using a Hamilton microsyringe, the lentivirus (5 μL, 3 × 108 CFU/mL) was injected at 1 μL/min and left on the needle for 5 minutes.

Neurologic Function Score

Neurobehavioral function was tested blindly using the Modified Neurological Severity Score (n = 10) as previously studied.[21] Higher scores indicated severer neurologic dysfunction.

Table 1 PCR Primer Sequences

	Primer sequence (5′ - 3′)
circPDS5B	Forward: 5′-TGTTCGCTTACTGGTAGCCT-3′
	Reverse: 5′-GACCCCAGGCGGATATGTAA-3′
miR-223-3p	Forward: 5′-ACACTCCAGCTGGGTGTCAGTTTGTCAAAT-3′
	Reverse: 5′-CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTT GAGTGGGGTAT-3′
β-actin	Forward: 5′-GAGCTATGAGCTGCCTGACG-3′ Reverse: 5′-AGCACTTGCGGTCCACGATG-3′
U6	Forward: 5′-CGAATTTGCGTGTCATCCTT-3′
	Reverse: 5′-CGAATTTGCGTGTCATCCTT-3′

TUNEL Staining

Brain tissue sections (5 μm) were dewaxed in xylene, dehydrated in ethanol, and tested by the TUNEL kit (C1089, Beyotime, Shanghai, China). The section was added with 20 μg/mL proteinase K solution (Solarbio, Beijing, China) for 15 minutes, dyed with 50 μL of TUNEL mixture for 60 minutes, counterstained with 4,6-diamidino-2-phenylindole, and imaged by a fluorescence microscope (Leica, Wetzlar, Germany).

OGD Treatment

HBMECs (Catalog #1000, ScienCell, CA) were spread in EBM-2 medium (Lonza, Basel, Switzerland) containing 40 μg/mL endothelial growth supplement and 10% FBS (ATCC 30-2020). Control HBMECs were conditioned to 24-hour normoxic incubation (5% CO_2 and 95% air), whereas OGD-treated HBMECs were conditioned to 24-hour hypoxic incubation (1% O_2, 5% CO_2, and 94% N_2). After that, HBMECs were maintained in serum-free medium (Teyebio, Shanghai, China).

Plasmid Modification

Small interfering RNA (si-circPDS5B/NOTCH2) or pcDNA 3.1-PDS5B/NOTCH2 targeting circPDS5B and NOTCH2 or miR-223-3p mimic/inhibitor (GenePharma, China) was transfected into HBMECs using Lipofectamine 2000 (Invitrogen). Si-NC, pcDNA 3.1, mimic NC, or inhibitor NC served as negative controls. The transfection efficiency was evaluated by quantitative PCR analysis at 48 hours post-transfection, and OGD was induced.

Quantitative PCR Analysis

After collecting total RNA samples from brain tissue and HBMECs using Trizol reagent (Invitrogen), protein concentration was examined by a NanoDrop ND-1000 Spectrophotometer (Agilent). With the first-strand cDNA reverse-transcribed through High Capacity cDNA Reverse Transcription Kit (Applied Biosystems), PCR was conducted by the mirVana qRT-PCR miRNA Detection Kit (Ambion) and SYBR Green PCR Kit (Thermo Fisher Scientific) in the 7500 Real-Time PCR System (Applied Biosystems). β-actin and U6 were the standard controls for mRNA and miRNA, respectively. The primer sequences are reported in Table 1.

Assessment of HBMEC Viability

Every 1×10^4 HBMECs were incubated with 20 μL of MTT solution (Sigma-Aldrich, CA) for 4 hours. Afterward, the supernatant was dissolved in 150 μL of DMSO, after which the optical density at 490 nm was measured on a microplate reader (Bio-Rad, CA).[22]

Apoptosis Detection

Trypsin-detached HBMECs were conditioned to analysis of apoptosis via the Annexin V-FITC Cell Death Detection Kit (BD Bioscience, NJ). HBMECs that were suspended in 100 μL of 1 × Annexin buffer were reacted with 5 μL of Annexin V-FITC and 1 μL of propidium iodide before detection on a FACScan (BD Biosciences).

Transwell Detection

A suspension of HBMECs (250 μL, 5×10^4) after starvation was suspended in DMEM to the upper Transwell chamber (Millipore, MA), and 500 μL of 10% FBS-DMEM was added to the lower chamber. Migrated cells fixed with methanol were stained with crystal violet before imaging under a phase contrast microscope (Olympus, Tokyo, Japan).

Tube Formation Experiments

Matrigel was plated on 96-well plates (100 μL/well) and solidified, on which HBMECs (2×10^4 cells/well) were incubated for 5 hours. The number of branches and nodules in the tubes was calculated.

Immunoblot Analysis

After collecting protein samples from cells and tissues through a protein extraction kit (Teyebio), protein concentration was determined by the BCA kit (Teyebio). Proteins that were separated by 15% SDS-polyacrylamide gel electrophoresis were transferred to a PVFD membrane and blocked with 5% nonfat milk and reacted overnight with primary antibodies against NOTCH2 (ab8926), VEGFA (ab46154), ANGPT2 (ab8452), and β-actin (ab8227, all from Abcam). Afterward, the secondary antibody (ab150081, Abcam) was combined with the membrane, followed by visualization of bands using an enhanced chemiluminescence kit (Teyebio) and assessment of data by ImageJ 1.46.

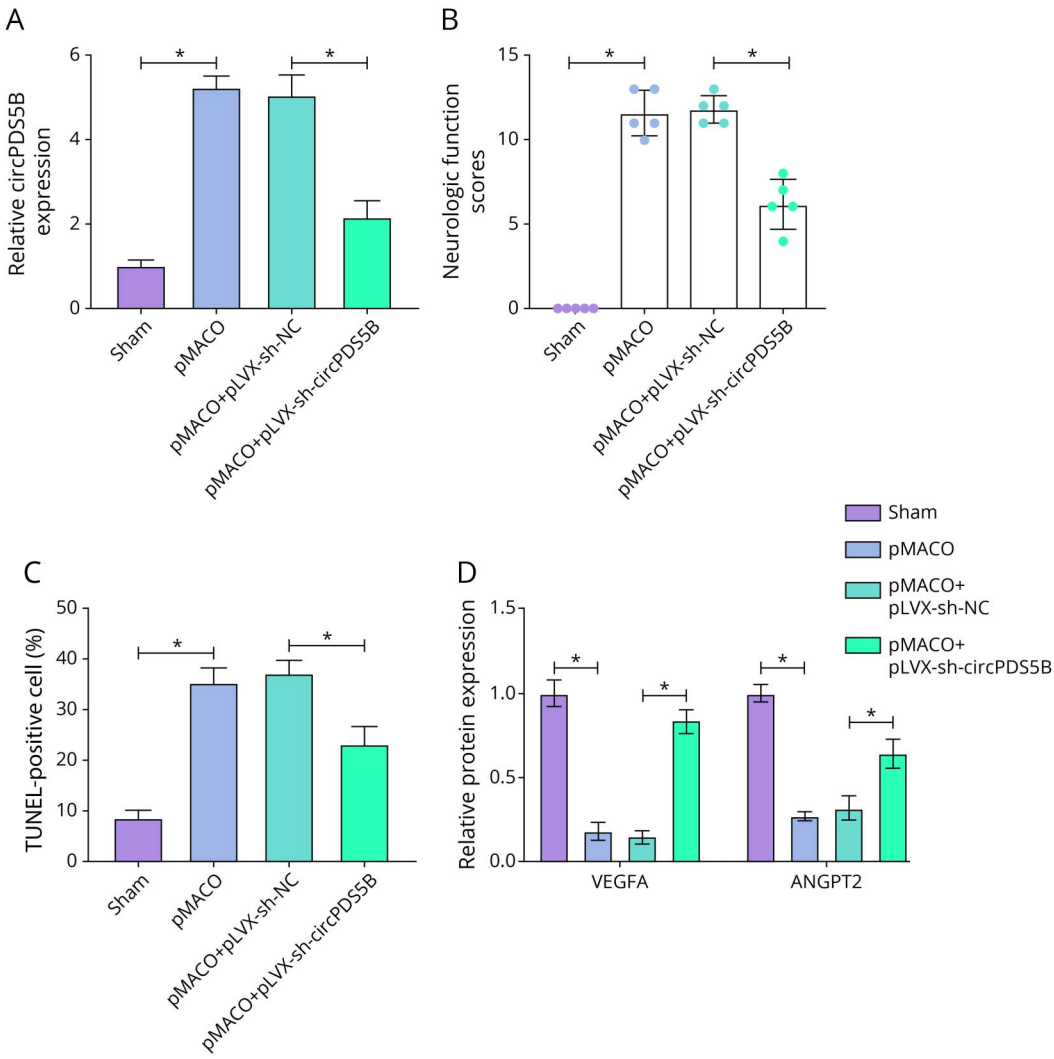
Luciferase Activity Analysis

Sequences of wild-type circPDS5B and NOTCH2 (circPDS5B-WT and NOTCH2-WT) or mutant circPDS5B and NOTCH2 (circPDS5B-MUT and NOTCH2-MUT, without miR-223-3p binding site) were amplified and inserted into the pmirGLO vector. Next, cells were transfected with the vector and miR-223-3p mimic or mimic NC for 48 hours and subjected to examination of luciferase activity in a Dual-Luciferase Reporter Assay System (Promega, WI, USA).

RIP Assay

RIP assay was performed using the EZ Magna RIP kit (Millipore). Cell lysate collected was incubated with anti-Ago2 or

Figure 1 Knockdown of circPDS5B Alleviates Ischemic Injury in pMCAO Mice



The mouse model of ischemic stroke was established by permanent middle cerebral artery occlusion (pMCAO) surgery, and pLVX-sh-circPDS5B was injected to interfere with circPDS5B expression (5 mice/group). (A) CircPDS5B expression in the brain tissue. (B) Neurologic function score. (C) Apoptosis rate. (D) Protein levels of VEGFA and ANGPT2 in the brain tissue of mice. *$p < 0.05$.

anti-IgG (Millipore) before binding to magnetic beads, which were conjugated with protein A/G (Thermo Fisher Scientific). After proteinase K treatment, the purified RNA was conditioned to quantitative PCR.

Data Analysis

Data were presented as mean ± SD. At least 3 biological replicates were performed for each experiment. Statistical analysis was performed using SPSS 17.0 software (SPSS, IL, USA), and 2 group data were assessed by a 2-tailed Student *t* test and multiple group data by 1-way analysis of variance plus Tukey post hoc test.[23] $p < 0.05$ indicated statistical significance.

Data Availability

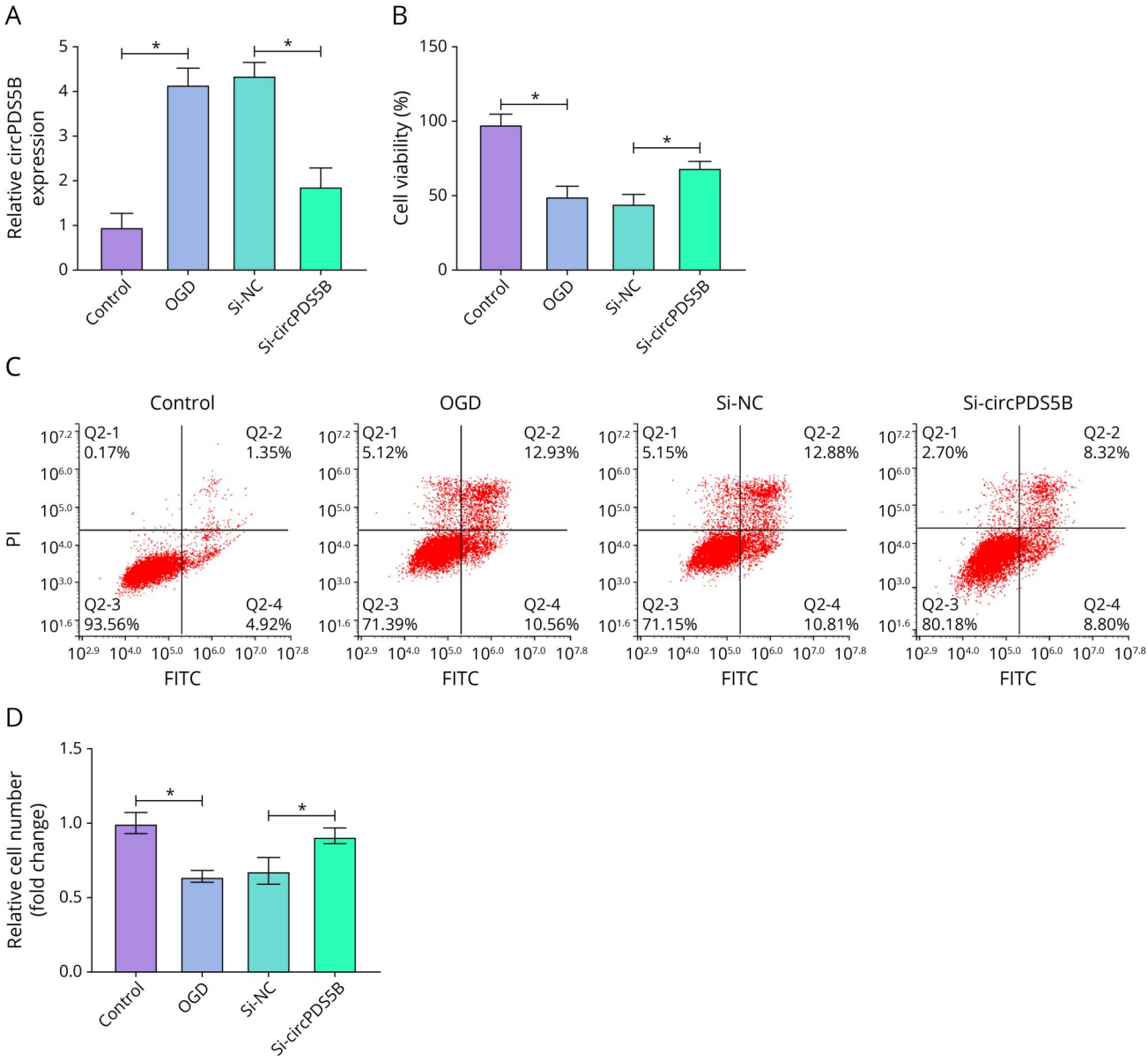
Data are available from the corresponding author on reasonable request.

Results

Knockdown of circPDS5B Alleviates Ischemic Injury in pMCAO Mice

We established a pMCAO mouse model and altered circPDS5B expression by injecting a lentiviral shRNA, pLVX-sh-circPDS5B. pMCAO surgery resulted in increased circPDS5B expression, whereas injection of pLVX-sh-circPDS5B suppressed circPDS5B expression (Figure 1A). The neurologic function scores of pMCAO mice were higher than those of sham mice, and circPDS5B inhibition improved neural damage in pMCAO mice (Figure 1B). Subsequently, apoptosis in the brain tissue was observed by TUNEL staining, as demonstrated by the results that pMCAO surgery enhanced the ratio of TUNEL-positive cells, and circPDS5B depletion reversed this phenomenon (Figure 1C, eFigure 1A, links.lww.com/NXG/A605). Next, immunoblot analysis of angiogenesis-related proteins indicated that pMCAO

Figure 2 Reduction of circPDS5B Promotes Angiogenesis in HBMECs Under OGD Treatment



Si-circPDS5B was transfected into human brain microvascular endothelial cells (HBMECs) before oxygen-glucose deprivation (OGD) induction. (A) CircPDS5B expression in HBMECs. (B) Viability. (C) Apoptosis rate. (D) Migration. *$p < 0.05$.

surgery caused the reduction of VEGFA and ANGPT2 protein expression, whereas circPDS5B silencing restored levels of these 2 proteins in the brain tissue (Figure 1D, eFigure 1B). Obviously, silencing circPDS5B improves angiogenesis in pMCAO mice.

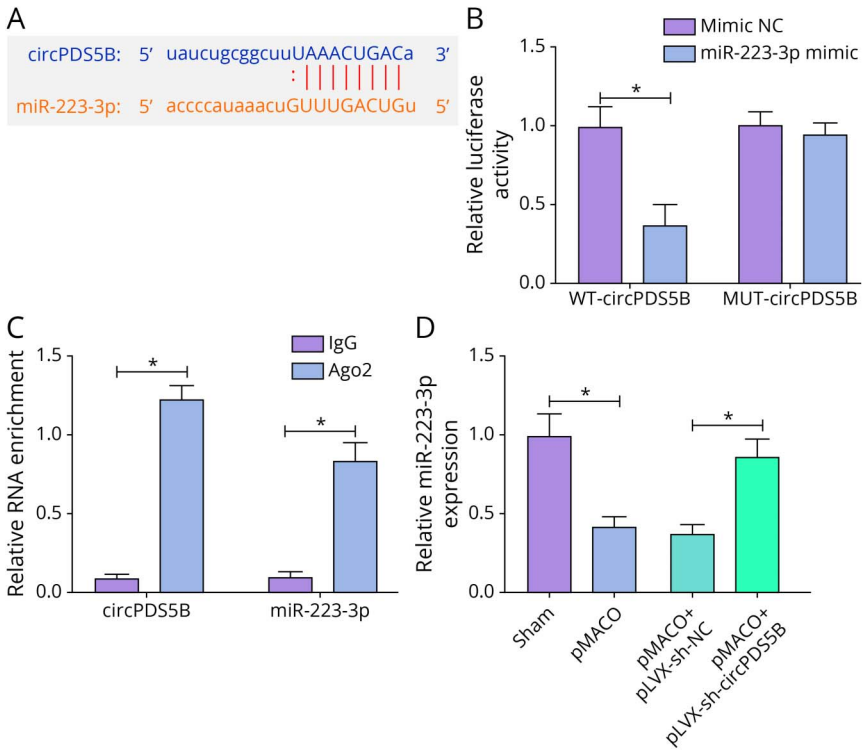
Reduction of circPDS5B Promotes Angiogenesis in HBMECs Under OGD Treatment

Next, we performed OGD to simulate ischemic injury in HBMECs. It was ensured that OGD treatment upgraded circPDS5B expression in HBMECs, and pretransfection with si-circPDS5B reduced circPDS5B levels (Figure 2A). HBMEC activity evaluation showed that OGD treatment HBMEC reduced cell viability, increased apoptosis rate, and inhibited cell migration. When circPDS5B expression was inhibited, OGD-induced changed in cell activity could be reversed (Figure 2, B–D, eFigure 2, A–C, links.lww.com/NXG/A605). Besides, assessment of angiogenesis proved the suppressive effects of OGD treatment on tube-forming ability of HBMECs and on VEGFA and ANGPT2 protein expression; however, depletion of circPDS5B could restore the angiogenic ability of OGD-conditioned HBMECs (eFigure 2D, eFigure 3A). In evidence, circPDS5B silencing improves angiogenesis in OGD-treated HBMECs.

CircPDS5B Targets miR-223-3p

When exploring downstream miRNAs of circPDS5B, the bioinformatics website starbase.sysu.edu.cn/ found that circPDS5B had a potential binding site for miR-223-3p

Figure 3 CircPDS5B Targets miR-223-3p



(A–C) The binding relationship between circPDS5B and miR-223-3p. (D) Effect of pLVX-sh-circPDS5B on miR-223-3p expression in mouse brain tissue. *$p < 0.05$.

(Figure 3A). Subsequently, their targeting relationship was verified by a dual-luciferase reporter experiment, as the outcomes indicated that the luciferase activity was decreased by cotransfection of WT-circPDS5B with miR-223-3p mimic but not that of MUT-circPDS5B with miR-223-3p mimic (Figure 3B). RIP experiments further confirmed their binding relationship, reflecting significant enrichment of Ago2-related RNA in both circPDS5B and miR-223-3p (Figure 3C). We then determined that decreased miR-223-3p in pMCAO mice and OGD-treated HBMECs could be restored after reduction of circPDS5B (Figure 3D, eFigure 3B, links.lww.com/NXG/A605). Shortly, circPDS5B mediates miR-223-3p expression.

CircPDS5B Affects Angiogenesis in OGD-Treated HBMECs by miR-223-3p

Before OGD treatment, miR-223-3p mimic/inhibitor and pcDNA 3.1-circPDS5B were transfected into HBMECs. Measurements of gene expression suggested the promoting effect of pcDNA 3.1-circPDS5B on circPDS5B expression, but nontargeted effect of miR-223-3p mimic/inhibitor on circPDS5B expression. Furthermore, both miR-223-3p inhibitor and pcDNA 3.1-circPDS5B reduced miR-223-3p expression, whereas miR-223-3p mimic suppressed the effect of pcDNA 3.1-circPDS5B (Figure 4B). Generally, circPDS5B affects HBMEC angiogenesis by miR-223-3p. Biological experiments evidenced that miR-223-3p inhibitor or pcDNA 3.1-circPDS5B reduced HBMECs viability (Figure 4C, eFigure 3C, links.lww.com/NXG/A605), increased apoptosis rate (Figure 4D), impaired migration (eFigure 3D, eFigure 4A) and tube formation quantity (eFigure 4B), and inhibited VEGFA and ANGPT2 protein expression (eFigure 4, C and D), whereas these effects of pcDNA 3.1-circPDS5B were prevented by miR-223-3p mimic.

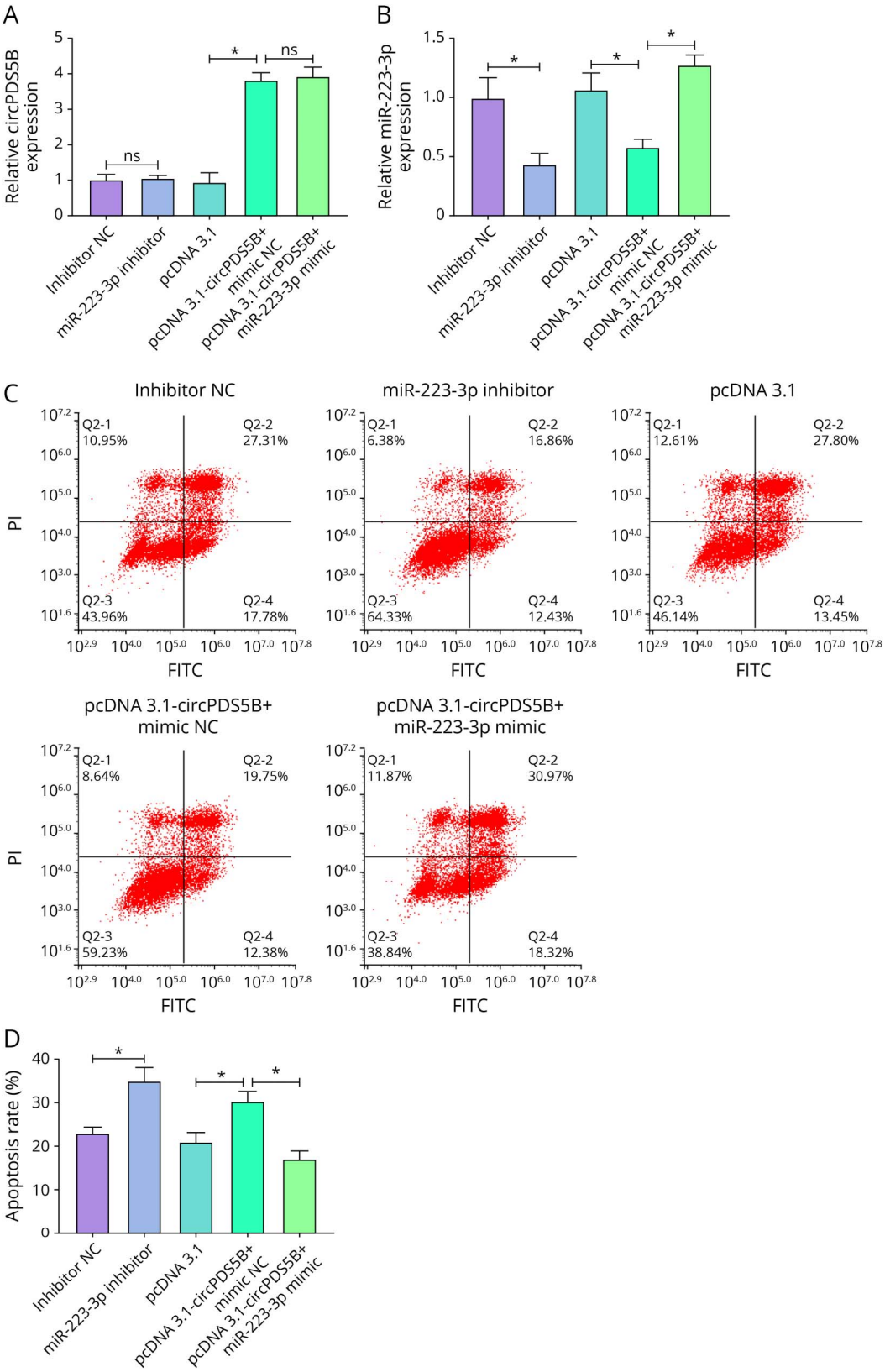
A Targeted Relation Between miR-223-3p and NOTCH2

Also, we searched for potential binding sites for NOTCH2 and miR-223-3p on bioinformatics website (Figure 5A). Subsequently, their targeting relationship was verified, with the results confirming that cotransfection of WT-NOTCH2 and miR-223-3p mimic reduced luciferase activity (Figure 5B); NOTCH2 and miR-223-3p were found to be enriched by Aog2 (Figure 5C). Subsequently, miR-223-3p-associated regulation of NOTCH2 expression was checked, as the outcomes demonstrated that NOTCH2 expression was increased in both pMCAO mice and OGD-treated HBMECs, whereas interference with miR-223-3p mimic lowered NOTCH2 protein expression in OGD-treated HBMECs. In total, there is a targeted relation between miR-223-3p and NOTCH2.

CircPDS5B/miR-223-3p/NOTCH2 Axis Affects Angiogenesis in OGD-Treated HBMECs

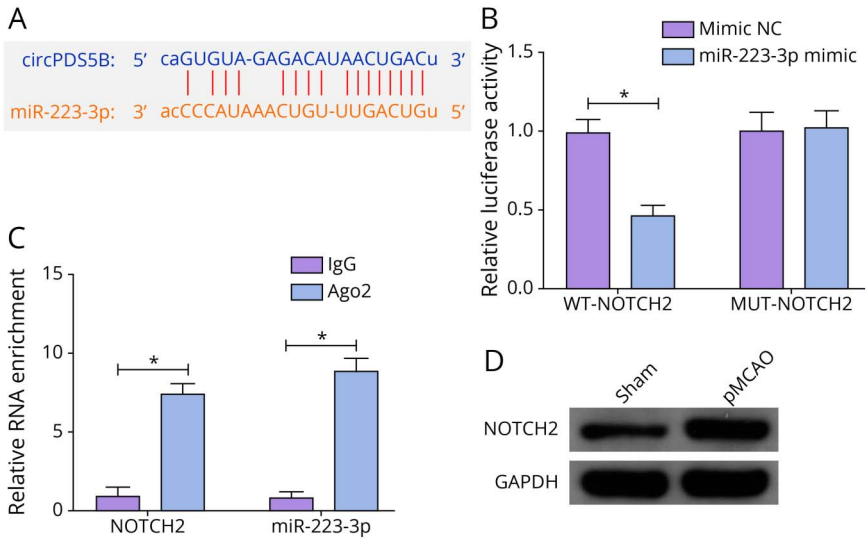
We cotransfected si-circPDS5B, miR-223-3p inhibitor, pcDNA 3.1-NOTCH2, and si-NOTCH2 into HBMECs before OGD induction. In the functional experiments, si-circPDS5B and miR-223-3p inhibitor decreased and promoted NOTCH2 protein levels and increased and decreased cell viability, respectively, but these effects were blocked by

Figure 4 CircPDS5B Affects Angiogenesis of OGD-Treated HBMECs by Regulating miR-223-3p



miR-223-3p mimic/inhibitor and pcDNA 3.1-circPDS5B were transfected into human brain microvascular endothelial cells (HBMECs) before oxygen-glucose deprivation (OGD) treatment. (A) CircPDS5B expression. (B) miR-223-3p expression. (C) Viability. (D) Apoptosis rate. *$p < 0.05$.

Figure 5 Targeted Relation Between miR-223-3p and NOTCH2



(A–C) Binding relation between NOTCH2 and miR-223-3p. (D) NOTCH2 expression in the brain tissue. *$p < 0.05$.

pcDNA 3.1-NOTCH2 and si-NOTCH2, respectively (Figure 6, A and B, eFigure 6A, links.lww.com/NXG/A605). Similarly, sicircPDS5B and miR-223-3p inhibitor inhibited and promoted apoptosis on the one hand, and increased and decreased migration and angiogenesis on the other, respectively. These effects were also blocked by pcDNA 3.1-NOTCH2 and si-NOTCH2, respectively (Figure 6, C and D, eFigure 6, B–D). Functional experiments also exhibited that the protein levels of VEGFA and ANGPT2 are the exact opposite of NOTCH2 protein levels (eFigure 7, A and B). Overall, circPDS5B affects angiogenesis in OGD-treated HBMECs by miR-223-3p/NOTCH2 axis.

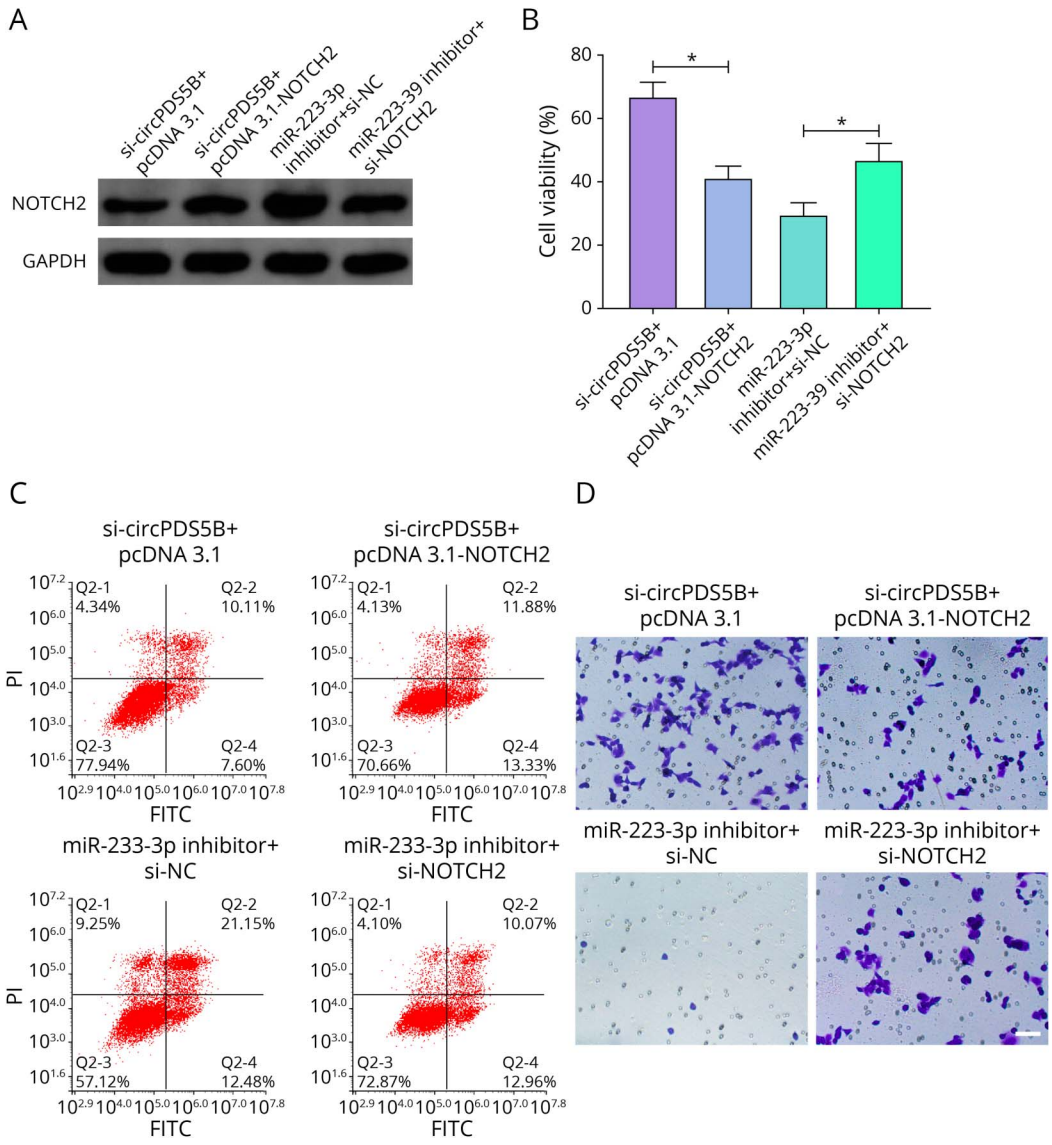
Discussion

In this research, we collected evidence to clarify that circPDS5B was upregulated in the brain tissue of pMCAO mice, and circPDS5B silencing ameliorated neurologic function, inhibited apoptosis, and promoted angiogenesis in the in vivo pMCAO model. The in vitro results also confirmed that reduction of circPDS5B increased the viability, migration, and angiogenesis and inhibited apoptosis of OGD-conditioned HBMECs.

CircRNAs are considered suitable biomarkers because of their high specificity and stability in the pathology of many diseases.[24] It has been elucidated that circRNAs can cross the blood-brain barrier and enter the bloodstream, and IS can further accelerate the process.[25] Although many circRNAs have been shown to be differentially expressed during IS, their biological functions are rarely studied. In addition, circRNAs often decoy miRNAs to regulate mRNA expression.[18] For example, It was found that circDLGAP4 ameliorated IS by regulating blood-brain barrier integrity–related endothelial-mesenchymal transition by sponging miR-143.[26] Yang Xu et al. have explained that circPHKA2 decreases the targeted control of superoxide dismutase 2 by miR-574-5p, thereafter attenuating injury of HBMECs under OGD treatment.[27] Notably, Zuo Li et al. have checked the increase of circPDS5B expression in patients with IS,[10] which is similar to our findings suggesting high levels of circPDS5B in pMCAO mouse brain tissue and OGD-treated HBMECs. In addition, we confirmed the findings with experimental data and proved that pMCAO-induced severe neurologic damage, increased apoptosis, and decreased expression of angiogenesis-related proteins (VEGFA and ANGPT2) could be improved when circPDS5B was knocked down. Angiogenesis is the physiologic process of dilating or diluting existing blood vessels to form new blood vessels, which is an essential recovery process after ischemia.[28] We further confirmed the role of circPDS5B in angiogenesis through cell experiments and obtained the following results: After OGD treatment, circPDS5B deletion can enhance the activity and migration ability of HBMECs, promote angiogenesis, and inhibit the apoptosis of HBMECs. In addition, we also confirmed that circPDS5B regulated NOTCH2 expression via miR-223-3p.

The research confirmed that miR-223-3p was downregulated in OGD-injured HBMECs and inhibited cell activities. MiR-223-3p restoration prevented overexpressed circPDS5B from further damaging OGD-injured HBMECs. Notably, miR-223-3p is almost exclusively derived from platelets or megakaryocytes in blood, and its biological activity is associated with aggregation and granule secretion.[29] MiR-223-3p is a potential diagnostic biomarker for various diseases, such as IS,[17] acute myocardial infarction,[30] and diabetic cerebral infarction.[31] Furthermore, exosomal miR-223-3p overexpression could attenuate cerebral ischemia.[32] Therefore, we speculate that quantifying miR-223-3p expression in IS models in follow-up studies may be beneficial to search for new IS biomarkers.

Figure 6 CircPDS5B Affects Angiogenesis of OGD-Treated HBMECs by miR-223-3p/NOTCH2 Axis



Si-circPDS5B, miR-223-3p inhibitor, pcDNA 3.1-NOTCH2, and si-NOTCH2 were cotransfected into human brain microvascular endothelial cells (HBMECs) before OGD treatment. (A) NOTCH2 expression. (B) Viability. (C) Apoptosis rate. (D) Migration. Scale bar = 50 μm. *$p < 0.05$.

NOTCH pathway is highly conserved and involved in many cellular processes[33] and congenital diseases, stroke, and cancers.[34] NOTCH2 is a membrane protein that, on ligand stimulation, releases its cytoplasmic domain as a transcription factor and is upregulated after neonatal IS.[35] Here, we checked that NOTCH2 was upregulated in both models, and miR-223-3p inhibition–associated reduction of angiogenesis could be counteracted by knocking down NOTCH2. CircPDS5B depletion–induced angiogenesis in OGD-conditioned HBMECs was repressed after overexpressing NOTCH2.

To shortly conclude, circPDS5B was upregulated in IS and promoted NOTCH2 expression by decoying miR-223-3p to inhibit HBMECs activities, thereby promoting IS. The results suggest that circPDS5BIS can act as a new diagnostic and therapeutic target for IS.

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Disclosure

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Zongyu Li, Master	Dehong People's Hospital, Mangshi, China	Drafting/revision of the manuscript for content, including medical writing for content, and major role in the acquisition of data
Guoyun Wang, Master	Shenzhen Qianhai Shekou Free Trade Zone Hospital, Shenzhen, China	Major role in the acquisition of data and analysis or interpretation of data
Xuzhen Li, Master	State Key Laboratory for Conservation and Utilization of Bio-Resources in Yunnan, Yunnan Agricultural University, Kunming, China; State Key Laboratory of Biological Big Data in Yunnan Province, Yunnan Agriculture University, Kunming, China	Analysis or interpretation of data
Feng Zhao, Master	Dehong People's Hospital, Mangshi, China	Major role in the acquisition of data
Yinming Jiao, PhD	Shenzhen Qianhai Shekou Free Trade Zone Hospital, Shenzhen, China	Drafting/revision of the manuscript for content, including medical writing for content; study concept or design; and analysis or interpretation of data

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