# miR-145 Regulates Diabetes-Bone Marrow Stromal Cell-Induced Neurorestorative Effects in Diabetes Stroke Rats

CHENGCHENG CUI,[a,b,*] XINCHUN YE,[a,*] MICHAEL CHOPP,[b,c] POORNIMA VENKAT,[b,c] ALEX ZACHAREK,[b] TAO YAN,[b] RUIZHOU NING,[b] PENG YU,[b] GUIYUN CUI,[a] JIELI CHEN[b,d]

[a]Department of Neurology, The Affiliated Hospital of Xuzhou Medical College, Xuzhou, People's Republic of China; [b]Department of Neurology, Henry Ford Hospital, Detroit, Michigan, USA; [c]Department of Physics, Oakland University, Rochester, Michigan, USA; [d]Department of Geriatrics, Tianjin Geriatrics Institute, Tianjin Medical University General Hospital, Tianjin, People's Republic of China

*Contributed equally.

Correspondence: Jieli Chen, M.D., Henry Ford Hospital, Neurology Research, E&R Building, 3091, Detroit, Michigan 48202, USA. Telephone: 313-916-1991; E-Mail: jieli@neuro.hfh.edu; or Guiyun Cui, Ph.D., The Affiliated Hospital of Xuzhou Medical College, Department of Neurology, Xuzhou, Jiangsu-221004, People's Republic of China. Telephone: 86 516 8580 2129; E-Mail: guiyuncui99@126.com

## ABSTRACT

In rats with type 1 diabetes (T1DM), the therapeutic effects and underlying mechanisms of action of stroke treatment were compared between bone-marrow stromal cells (BMSCs) derived from T1DM rats (DM-BMSCs) and BMSCs derived from normal rats (Nor-BMSCs). The novel role of microRNA-145 (miR-145) in mediating DM-BMSC treatment-induced benefits was also investigated. T1DM rats ($n = 8$ per group) underwent 2 hours of middle cerebral artery occlusion (MCAo) and were treated 24 hours later with the one of the following ($5 \times 10^6$ cells administered i.v.): (a) phosphate-buffered saline (PBS); (b) Nor-BMSCs; (c) DM-BMSCs; (d) DM-BMSCs with miR-145 overexpression (miR-145+/+DM-BMSCs); or (e) Nor-BMSCs with miR-145 knockdown. Evaluation of functional outcome, vascular and white-matter remodeling and microRNA expression was made, and in vitro studies were performed. In vitro, DM-BMSCs exhibited decreased miR-145 expression and increased survival compared with Nor-BMSCs. Capillary tube formation and axonal outgrowth in cultured primary cortical neurons were significantly increased by DM-BMSC-conditioned medium compared with Nor-BMSCs, and significantly decreased by miR-145+/+DM-BMSC-conditioned medium compared with DM-BMSCs. In T1DM rats in which stroke had been induced (T1DM stroke rats), DM-BMSC treatment significantly improved functional outcome, increased vascular and white matter remodeling, decreased serum miR-145 expression, and increased expression of the miR-145 target genes adenosine triphosphate-binding cassette transporter 1 (*ABCA1*) and insulin-like growth factor 1 receptor (*IGFR1*), compared with Nor-BMSCs or PBS treatment. However, miR-145+/+DM-BMSCs significantly increased serum miR-145 expression and decreased brain *ABCA1* and IGFR1 expression, as well as attenuated DM-BMSC-induced neurorestorative effects in T1DM-MCAo rats. DM-BMSCs exhibited decreased miR-145 expression. In T1DM-MCAo rats, DM-BMSC treatment improved functional outcome and promoted neurorestorative effects. The miR-145/ABCA1/IGFR1 pathway may contribute to the enhanced DM-BMSCs' functional and neurorestorative effects in T1DM stroke rats. STEM CELLS TRANSLATIONAL MEDICINE *2016;5:1656–1667*

## SIGNIFICANCE

In rats with type 1 diabetes (T1DM), the therapeutic effects and underlying mechanisms of action of stroke treatment were compared between bone-marrow stromal cells (BMSCs) derived from T1DM rats (DM-BMSCs) and BMSCs derived from normal rats (Nor-BMSCs). In vitro, DM-BMSCs and derived exosomes decreased miR-145 expression and increased DM-BMSC survival, capillary tube formation, and axonal outgrowth, compared with Nor-BMSCs; these effects were decreased by DM-BMSCs in which miR-145 was overexpressed. In vivo, compared with Nor-BMSC or phosphate-buffered saline treatment, DM-BMSC treatment improved functional outcome and vascular and white matter remodeling, decreased serum miR-145 expression, and increased expression of the miR-145 target genes *ABCA1* and *IGFR1*. microRNA-145 mediated the benefits induced by DM-BMSC treatment.

## INTRODUCTION

Diabetes induces an assortment of vascular pathologies, along with increased vascular permeability, that contribute to high morbidity associated with ischemic stroke [1]. Exacerbated white matter and axonal injuries found in diabetic stroke mice contribute to worse poststroke outcome [2]. Diabetes alters metabolism and complicates stroke pathology, making it more difficult to treat the diabetic ischemic brain. With approximately 30% of all stroke patients suffering from diabetes, there

is a compelling need to develop stroke treatments specifically targeting the diabetic population.

Intravenous administration of bone marrow stromal cells (BMSCs) derived from normal rats (Nor-BMSCs) amplifies angiogenesis and vascular stabilization, and improves neurological functional outcome after stroke in the nondiabetic population [3]. BMSCs can migrate to injured brain tissue [4], stimulate release of growth and trophic factors by parenchymal brain cells, and enhance functional recovery and neurorestorative effects, such as neurogenesis, angiogenesis, and arteriogenesis [5]. However, treatment of stroke in type 1 diabetic (T1DM) rats, using Nor-BMSCs at 24 hours poststroke, does not improve functional outcome after stroke but increases blood brain barrier (BBB) leakage, brain hemorrhagic transformation, and inflammatory factor expression in the ischemic brain [6]. In addition, T1DM rats subjected to stroke exhibit significantly worse outcome compared with stroke in nondiabetic rats, and tissue plasminogen activator treatment of stroke induces beneficial effects in non-DM rats, but not in T1DM rats in which stroke was induced (T1DM stroke rats) [7].

MicroRNAs (miRs) are small noncoding RNA sequences that are emerging as key players in the pathogenesis of diabetes and hyperglycemia-induced vascular damage [5]. Circulating miR-145 significantly increases within 24 hours of cerebral ischemia, and the circulating miR-145 level is positively correlated with elevated serum inflammatory factor interleukin-6 [8]. Inhibition of miR-145 using anti-miR was found to yield moderate neuroprotective effects after transient focal ischemia [9]. miR-145 has several target genes, such as adenosine triphosphate-binding cassette transporter 1 (*ABCA1*) and [10] insulin-like growth factor 1 (*IGF1*) [11]. *ABCA1* plays an important role in regulating cellular cholesterol and phospholipids by mediating their efflux to lipid-poor apolipoproteins [12] and maintaining high-density lipoprotein cholesterol homeostasis in the central nervous system [10]. IGF1 exerts an important role in central nervous system development by regulating survival, proliferation, and differentiation of brain cells [13]; neurodegeneration; and neural plasticity [14].

Whether BMSCs regulate miR-145 expression and their target gene expression in the ischemic brain and whether inhibition of miR-145 expression mediates DM-BMSC-induced neurorestorative effects in T1DM has not been investigated [15]. In the present study, we investigated the therapeutic effects and underlying mechanisms of action of the treatment of T1DM stroke rats with DM-BMSCs.

## MATERIALS AND METHODS

### T1DM Induction

A single intraperitoneal injection of streptozotocin (60 mg/kg; Merck, St. Louis, MO, http://www.sigmaaldrich.com) was used to induce T1DM in adult male Wistar rats (Charles River Laboratories International, Wilmington, MA, http://www.criver.com) [16, 17]. Two weeks later, rats with a fasting blood glucose level >300 mg/dl, measured using a glucose analyzer (Accu-Chek Compact System; Roche Diagnostics, Basel, Switzerland, http://www.roche.com), were identified as diabetic and subjected to 2 hours of middle cerebral artery occlusion (MCAo).

### MCAo Model and Experimental Groups

Transient (2-hour) MCAo was performed on anesthetized T1DM rats via intraluminal vascular occlusion, as previously described

[3, 18]. Rats were randomized ($n$ = 8 per group) and treated 24 hours poststroke via tail vein injection with $5 \times 10^6$ cells of one of the following: (a) phosphate-buffered saline (PBS); vehicle control); (b) BMSCs derived from normal nondiabetic rats (Nor-BMSCs); (c) BMSCs derived from T1DM rats (DM-BMSCs); (d) DM-BMSCs with miR-145 overexpression (miR-145+/+DM-BMSCs); or (e) Nor-BMSCs with miR145 knockdown (miR145$-$/$-$Nor-BMSCs). Blood glucose was measured in T1DM rats before MCAo, treatment and sacrifice. Rats were killed at 14 days after MCAo, for immunostaining quantification analysis. The mortality rates were 30% in DM-MCAo groups and 9% in DM-BMSC-treated group.

### Neurological Functional Tests

An investigator blinded to the experimental groups performed a battery of functional tests, including a modified neurological severity score, foot-fault test, and adhesive removal test before MCAo and on days 1, 7, and 14 after MCAo, as previously described [3, 19].

### Histological and Immunohistochemical Assessment

Rats were killed 14 days after MCAo. All brains were fixed by transcardial perfusion with 0.9% saline, followed by perfusion and immersion in 4% paraformaldehyde, and then embedded in paraffin. Seven coronal sections of tissue were processed and stained with hematoxylin and eosin for calculation of brain infarct volume. A standard block was obtained from the center of the lesion (bregma, approximately 1 mm $\times$ 1 mm). A series of 6-$\mu$m-thick sections was cut from the block. Every 10th coronal section for a total 5 sections was used for immunohistochemical staining. Antibody against $\alpha$-smooth muscle actin ($\alpha$-SMA) a smooth muscle cell marker to identify arteries (1:800; Agilent Technologies, Glostrup, Denmark, http://www.dako.com); von Willebrand factor (vWF), a blood vessel marker (1:400; Agilent Technologies); SMI-31, a phosphorylated monoclonal antibody that acts as a neurofilament marker (1:1,000; Biolegend, Dedham, MA, http://www.biolegend.com/); zonula occluden-1 (ZO-1), a tight-junction protein (rabbit polyclonal IgG; 1:50; Thermo Fisher Scientific Life Sciences, Waltham, MA, http://www.thermofisher.com); Bielschowsky silver stain (an axon marker); Luxol fast-blue stain (a myelin marker); fluorescein isothiocyanate conjugated albumin (FITC-albumin), a polyclonal antibody (1:500; Abcam, Cambridge, U.K., http://www.abcam.com); ABCA1 (1:200; Novus Biologicals, Minneapolis, MN, http://www.novusbio.com); and IGFR1 (1:100; Santa Cruz Biotechnology, Dallas, TX, http://www.scbt.com) were used. Control experiments consisted of staining brain coronal tissue sections as outlined above, but nonimmune serum was substituted for the primary antibody.

### Immunostaining Quantification

For quantitative measurements, 5 slides from each brain, with each slide containing 8 fields of view from the ischemic boundary zone, were digitized under a $\times$20 objective (Olympus BX40; Olympus, Tokyo, Japan, http://www.olympus-global.com) using a 3-charge-coupled device color video camera (Sony DXC-970MD; Sony, Tokyo, Japan, https://www.sony.com) interfaced with an MCID image analysis system (GE Healthcare, Chicago, IL, http://www.gelifesciences.com) [20]. Positive areas of Bielschowsky-silver and Luxol fast-blue staining were measured in the striatal white matter bundles of the ischemic boundary zone. Immunoreactive

positive areas of FITC-albumin, SMI-31, ABCA1, and IGFR-1 were measured in the ischemic boundary zone, and ZO-1-positive areas in 20 enlarged, thin-walled vessels in the ischemic boundary zone were quantified [21]. Vascular density was measured in the ischemic boundary zone using vWF immunostaining and presented as the number of vessels per unit area. To measure arterial density, $\alpha$-SMA-stained vessels were counted and analyzed with regard to small and large vessels ($\geq 10\,\mu$m diameter) and the internal arterial diameter was measured. Wall thickness of the 10 largest arteries was measured.

## BMSC Culture

Bone marrow was isolated from normal rats and from DM rats, 2 weeks post-T1DM induction, and cultured in BMSC growth media (HyClone Classical Liquid Media; minimal essential medium-$\alpha$; with L-glutamine and ribonucleosides and deoxyribonucleosides; 20% fetal bovine serum [FBS], and 1% antibiotic/antimycotic), as previously described [22]. To test the differences between Nor-BMSCs and DM-BMSCs in cell survival and miRs expression levels, four passages of Nor-BMSCs and DM-BMSCs and their conditioned media were isolated and used. In our previous studies, as well as studies by others, identification and characterization of BMSCs using flow cytometry analysis were performed on cells from passages 3 to 5. CD29 and CD44 are BMSC surface markers, whereas CD14 exists in monocytes and macrophages, and CD34 is a hematopoietic stem cell marker. In our previous studies, we have found that cultured cells expressed CD29 (95.3%), CD44 (98.1%), and lacked CD14 (1.9%) and CD34 (1.3%) [23]. Therefore, high-purity BMSCs can be harvested simply and effectively by their adherent characteristics. The present study used the same BMSC culture methods and, as such, flow cytometry analysis was not repeated.

## Smooth Muscle Cell and Mouse Brain Endothelial Cell Culture

Smooth muscle cells and mouse brain endothelial cells (MBECs) [24] were cultured in $\alpha$−Dulbecco's modified Eagle medium (DMEM; Thermo Fisher Scientific Life Sciences) with 10% FBS and 1% antibiotic/antimycotic (Thermo Fisher Scientific Life Sciences).

## Oligodendrocyte Cell Culture

A premature oligodendrocyte cell line was cultured in a medium consisting of 5.4 g of DMEM/F12 high-glucose powder, 1.8 g of dextrose anhydrous, 1.69 g of HEPES, and 1.08 g sodium bicarbonate (all Thermo Fisher Scientific Life Sciences), and 45 mg gentamicin (Gemini Bio-Products, West Sacramento, CA, http://www.gembio.com) in 450 ml of double-distilled H$_2$O with 10% FBS and 1% antibiotic/antimyotic.

## In Vitro Experimental Groups

Smooth muscle cells, MBECs, and oligodendrocytes were all treated with the following: (a) a nontreated control, (b) Nor-BMSC-conditioned medium, and (c) DM-BMSC-conditioned medium. Smooth muscle cell, MBEC, and oligodendrocyte proliferation and cell death were measured using lactate dehydrogenase (LDH) and 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4 sulfophenyl)-2H-tetrazolium (MTS) assays.

## MTS Assay

An MTS assay was performed using the CellTiter 96 Aqueous One Solution Cell Proliferation MTS Assay (Promega, Madison, WI, https://www.promega.com). At 48 hours after treatment, MTS was added and, following incubation, absorbance was recorded at 490 nm.

## LDH Assay

An LDH assay was performed using the CytoTox 96 nonradioactive cytotoxicity assay kit (Promega), following standard protocol. Briefly, cells were cultured in a 96-well plate for 24 hours and then the cells in half the wells for each experimental group were lysed to give total LDH, while medium only was collected from the other set of wells for secreted LDH. The medium was added to a fresh 96-well plate, followed by the addition of substrate solution. Following incubation, absorbance was recorded at 490 nm.

## miR Measurements

For the measurement of miRs in cultured Nor-BMSCs and DM-BMSCs, and in blood serum and brain tissue, samples were lysed in Qiazol reagent and the total RNA was isolated using the miR-Neasy mini kit (both from Qiagen, Hilden, Germany, https://www.qiagen.com). Using real-time polymerase chain reaction (PCR), we detected the levels of miR-145, miR-181c, and mir-146b. Briefly, miRNAs were reverse transcribed with the miRNA reverse transcription kit (Thermo Fisher Scientific Life Sciences) and PCR amplification was performed with the TaqMan miRNA assay kit specific for mature miRNA sequences (Thermo Fisher Scientific Life Sciences), according to the manufacturer's protocol, with U6 snRNA as an internal control.

## Real-Time PCR

Cultured cells were harvested and total RNA was isolated with TRIzol (Thermo Fisher Scientific Life Sciences). cDNA was made using TaqMan microRNA reverse transcription kit (Qiagen). Quantitative PCR was performed using the TaqMan real time PCR method and run on a ViiA7 system (Thermo Fisher Scientific Life Sciences). Each sample was tested in triplicate for analysis of relative expression data using the 2,5-dimethoxy-4-propylthiophenethylamine (2-$\Delta\Delta$CT) method.

## Overexpression or Knockdown of miR-145 in DM-BMSCs

DM-BMSCs were harvested and resuspended in 95 $\mu$l of Ingenio electroporation solution (Mirus Bio, Madison, WI, https://www.mirusbio.com) and 5 $\mu$l of 20-$\mu$M miR-145 mimic (GE Dharmacon) or miR-145 inhibitor (GE Healthcare). The cell suspension was then placed in an Ingenio cuvette (Mirus Bio) and electroporated in a Lonza Nucleofector (Lonza, Basel, Switzerland, http://www.lonza.com) using program C-17. Cells were then placed in growth media (Hyclone) with 20% FBS and 1% antibiotic/antimycotic (all from Thermo Fisher Scientific Life Sciences) for 2 days. The cells and condition medium were isolated for cell injection or for in vitro measurements.

## Capillary-Like Tube Formation Assay

Matrigel (0.1 ml) was added per well in a 96-well plate; when it gelled, MBECs suspended in serum-free DMEM were added
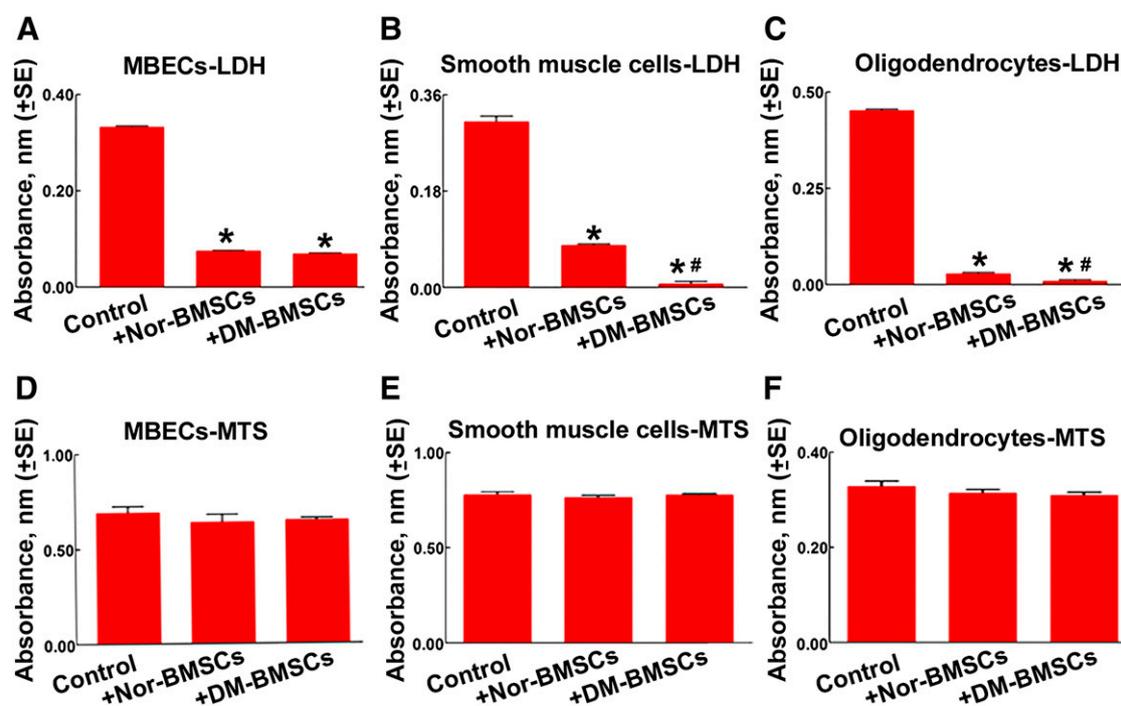
**Figure 1.** DM-BMSC- and Nor-BMSC-conditioned medium treatment did not promote cell proliferation but significantly decreased cell death of MBECs, smooth muscle cells, and oligodendrocytes compared with the nontreated control. DM-BMSC treatment significantly decreased smooth muscle cell and oligodendrocyte cell death compared with Nor-BMSC treatment. LDH assay quantification data of BMSCs in MBECs **(A)**, smooth muscle cells **(B)**, and oligodendrocytes **(C)**; $n = 3$ per group. MTS assay quantification data are presented for BMSCs in MBECs **(D)**, smooth muscle cells **(E)**, and oligodendrocytes **(F)**. *, $p < .05$ versus control; #, $p < .05$ versus Nor-BMSCs. Abbreviations: DM-BMSC, bone-marrow stromal cell derived from rats with type 1 diabetes; LDH, lactate dehydrogenase; MBEC, mouse brain endothelial cell; MTS, 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4 sulfophenyl)-2H-tetrazolium; Nor-BMSC, bone-marrow stromal cell derived from normal rats.

and incubated for 3 hours. Matrigel wells were digitized under a ×10 objective using a video camera interfaced with the MCID computer image analysis system (GE Healthcare). Tracks of endothelial cells were measured. The total length of tube formation was presented. The experimental groups were (a) MBECs control, no treatment; (b) +50% of DM-BMSC-conditioned medium; (c) +50% of miR-145+/+DM-BMSC-conditioned medium; and (d) +50% of miR-145−/−DM-BMSC-conditioned medium.

### Primary Cortical Neuron Axonal Outgrowth Assay

Axonal outgrowth was measured, as previously described [25]. Briefly, primary cortical neurons were harvested from pregnant (embryonic day 18) Wistar rats. A microfluidic chamber (Standard Neuron Device; Xona Microfluidics, Temecula, CA, http://xonamicrofluidics.com) was used to separate axons from neuronal soma [26]. The experimental groups were (a) control, no treatment; (b) +50% of DM-BMSC-conditioned medium; (c) +50% of miR-145+/+DM-BMSC-conditioned medium; and (d) +50% of miR-145−/−DM-BMSC-conditioned medium.

### Statistical Analysis

One-way analysis of variance was used for the evaluation of functional outcome and histology. "Contract/estimate" statement was used to test the group difference. If an overall treatment group effect was detected at $p < .05$, pairwise comparisons were made. All data are presented as mean ± SE.

### DM-BMSC Treatment of Smooth Muscle Cells, MBECs, and Oligodendrocytes Significantly Improved Cell Survival Compared With Nor-BMSCs and Nontreatment Control

To test if DM-BMSCs secrete factors that promote survival and proliferation of brain cells, LDH and MTS assays were performed. Compared with the nontreatment control, treatment with DM-BMSC- and Nor-BMSC-conditioned media significantly decreased death of MBECs, smooth muscle cells, and oligodendrocytes (Fig. 1A–1C). Treatment with DM-BMSC-conditioned medium significantly decreased smooth muscle cell and oligodendrocyte death compared with treatment with Nor-BMSC-conditioned medium (Fig. 1A–C). No significant difference in MBEC, smooth muscle cell, and oligodendrocyte proliferation was observed between those treated with Nor-BMSC- and DM-BMSC-conditioned media (Fig. 1D–1F). The data indicate that DM-BMSC-conditioned medium decreased smooth muscle cell and oligodendrocyte cell death compared with Nor-BMSC-conditioned medium.

### Treatment of Stroke in T1DM Rats With DM-BMSCs Significantly Improved Functional Outcome, Which Is Mediated by a Decrease in miR-145 Expression

To test whether DM-BMSC treatment of stroke in T1DM rats has a better therapeutic effect than Nor-BMSCs, a battery of functional tests was performed. Figure 2A and 2B shows that DM-BMSC
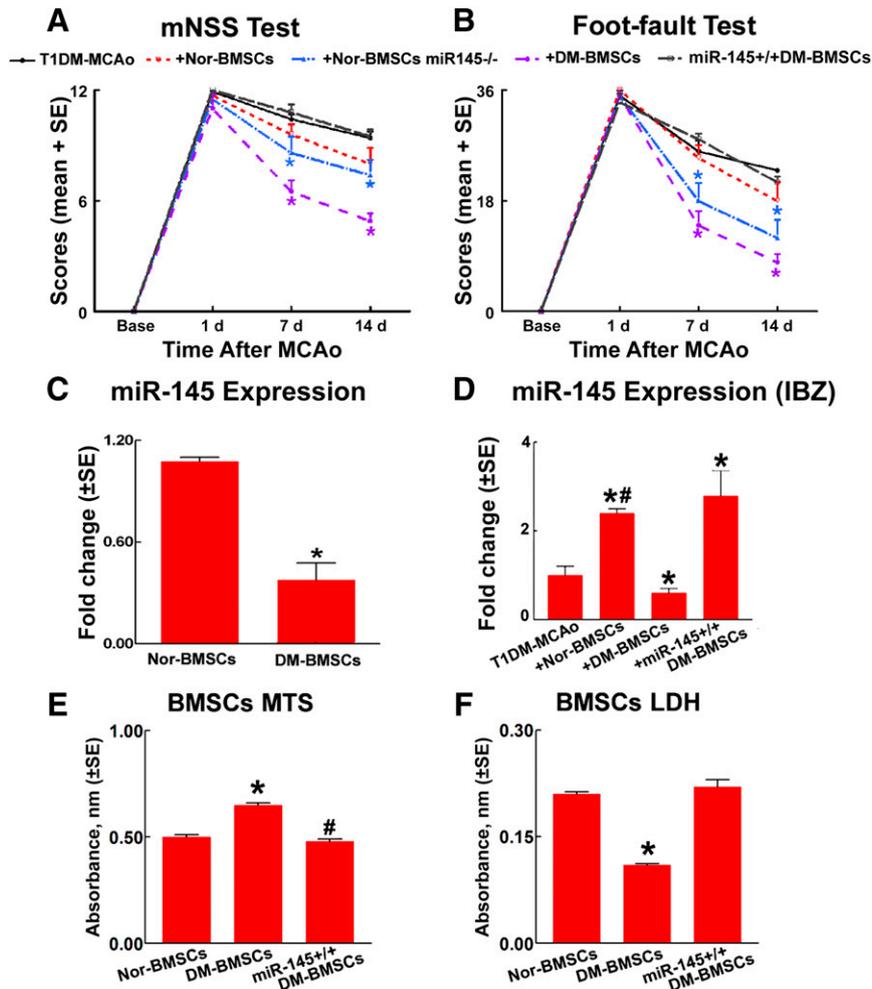
**Figure 2.** Treatment of stroke with DM-BMSCs significantly improved functional outcome in type 1 diabetes (T1DM) stroke rats compared with Nor-BMSC- or phosphate-buffered saline (PBS)-treated T1DM-MCAo rats. Treatment of stroke with Nor-BMSCs did not improve functional outcome in T1DM-MCAo rats. Overexpression of miR-145 in DM-BMSCs attenuated DM-BMSC-induced functional outcome after stroke in T1DM rats. Knockout of miR-145 in Nor-BMSCs improved functional outcome after T1DM-MCAo, compared with T1DM-MCAo rats. **(A):** mNSS and Foot-fault test **(B)** (*n* = 8 per group for both). ∗, *p* < .05 versus T1DM-MCAo. **(C):** miR-145 expression was significantly decreased in cultured DM-BMSCs compared with Nor-BMSCs (*n* = 3 per group). ∗, *p* < .05 versus Nor-BMSCs. **(D):** DM-BMSCs significantly decreased miR-145 expression in the ischemic boundary zone of T1DM stroke rats, compared with PBS-treated T1DM-MCAo rats (*n* = 8 per group). ∗, *p* < .05 versus T1DM-MCAo; #, *p* < .05 versus +Nor-BMSCs. **(E, F):** Cultured miR-145+/+DM-BMSCs showed lower cell proliferation and higher cell death compared with DM-BMSCs in BMSC MTS assay **(E)** and BMSC LDH assay quantification data **(F)** (*n* = 3 per group for both). ∗, *p* < .05 versus Nor-BMSCs; #, *p* < .05 versus DM-BMSCs. Abbreviations: DM-BMSC, bone-marrow stromal cell derived from rats with type 1 diabetes; IBZ, ischemic border zone; LDH, lactate dehydrogenase; MCAo, middle cerebral artery occlusion; miR-145+/+DM-BMSC, miR-145 overexpression in bone-marrow stromal cell derived from rats with type 1 diabetes; mNSS, modified neurological severity score; MTS, 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4 sulfophenyl)-2H-tetrazolium; Nor-BMSC, bone-marrow stromal cell derived from normal rats.

treatment significantly improved functional recovery starting at 7 days after stroke, as indicated by the modified neurological severity score and foot-fault test compared with PBS-treated and Nor-BMSC-treated T1DM-MCAo rats. Consistent with our previous studies [6], Nor-BMSC treatment of stroke in T1DM rats did not improve functional outcome compared with the PBS-treated T1DM-MCAo control.

### DM-BMSCs Exhibited Decreased Expression Level miR-145 Compared With Nor-BMSCs

To gain insight into possible mechanisms of DM-BMSC-conditioned medium-induced increase in cell survival, miR expression was measured. Previous studies have reported that miR-145 inhibits cell

proliferation and increases apoptosis [27], and miR-181c and miR-146b are regulated by diabetes; therefore, expression of these miRs was measured in cultured BMSCs. We found that DM-BMSC treatment significantly decreased miR-145 expression but did not alter miR-181c and miR-146b expression compared with Nor-BMSCs (Fig. 2C).

### Increased Proliferation and Survival Rate of DM-BMSCs Were Mediated by miR-145 Expression

To test whether miR-145 mediates BMSCs survival and proliferation, LDH and MTS assays and miR-145 overexpression measurements in DM-BMSCs were conducted (Fig. 2E, 2F). DM-BMSCs exhibited significantly increased cell proliferation and survival
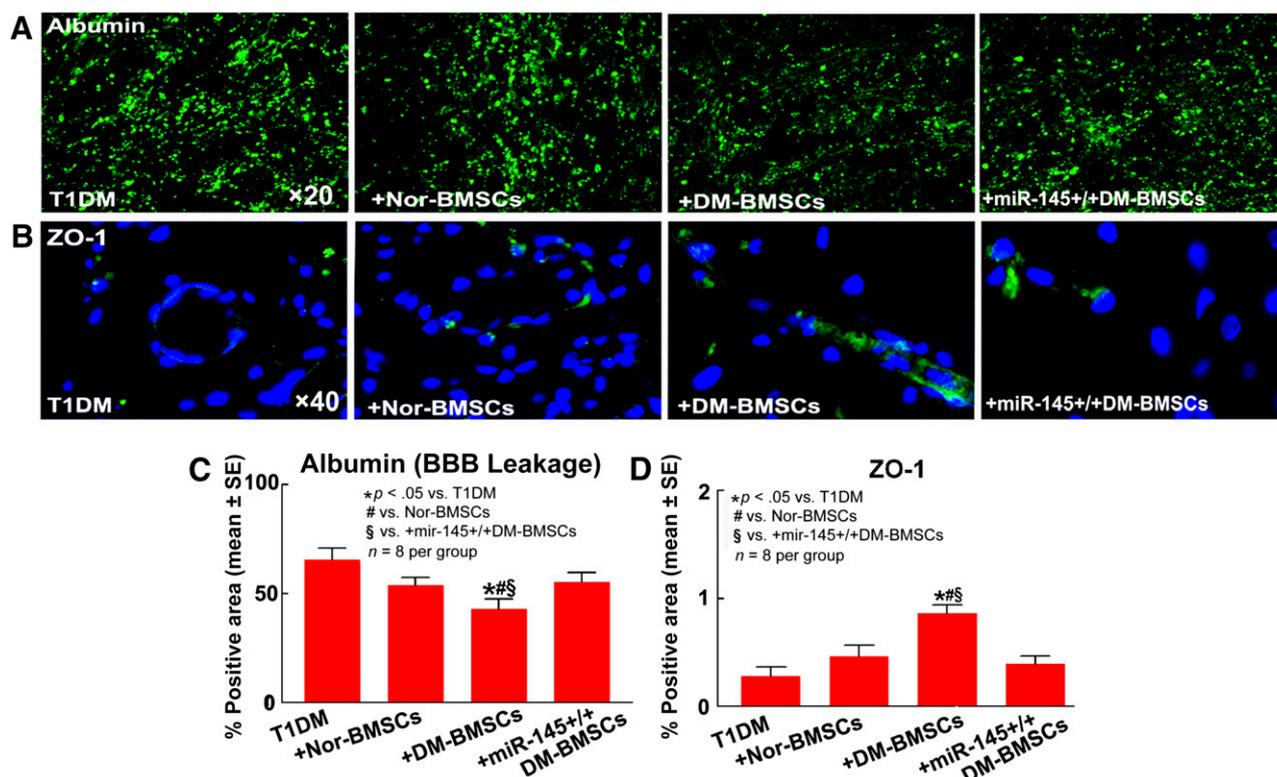
**Figure 3.** Fluorescein isothiocyanate conjugated albumin (FITC-albumin) and ZO-1 antibody immunoreactive positive areas seen on micrographs and quantified in bar graphs. Treatment of T1DM stroke with DM-BMSCs, but not Nor-BMSCs, phosphate-buffered saline, or miR-145+/+DM-BMSCs, decreased BBB leakage (as seen with FITC-albumin) **(A, C)**, and increased tight-junction protein ZO-1 expression **(B, D)**. $n = 8$ per group, *, $p < .05$ versus T1DM; #, $p < .05$ versus Nor-BMSCs; §, $p < .05$ versus miR-145+/+DM-BMSCs. Abbreviations: BBB, blood-brain barrier; miR-145+/+DM-BMSC, miR-145 overexpression in bone-marrow stromal cell derived from rats with type 1 diabetes; Nor-BMSC, bone-marrow stromal cell derived from normal rats; T1DM, type 1 diabetes; ZO-1, zonula occluden-1.

compared with Nor-BMSCs. miR-145 overexpression in DM-BMSC significantly attenuated DM-BMSC-induced enhanced survival and proliferation effects.

To test if miR-145 mediates DM-BMSC-induced functional benefit, overexpression of miR-145 in DM-BMSCs and knockout of miR-145 in Nor-BMSCs were evaluated. Treatment of T1DM-MCAo rats with miR-145+/+DM-BMSC significantly attenuated DM-BMSC treatment-induced functional outcome benefit, whereas knockout of miR-145 in Nor-BMSCs significantly improved functional outcome.

To test if decreasing miR-145 may contribute to DM-BMSC-induced neurorestorative effects after stroke in T1DM rats, miR-145 expression was measured in the ischemic boundary zone. In T1DM-MCAo rats treated with DM-BMSCs, miR-145 expression in the ischemic brain was significantly decreased compared with PBS- or Nor-BMSC-treated T1DM rats (Fig. 2D). There were no significant differences in blood glucose level and lesion volume among T1DM-MCAo rats treated with PBS, Nor-BMSCs, DM-BMSCs, and miR-145+/+DM-BMSCs (supplemental online Tables 1 and 2).

### Treatment of Stroke in T1DM Rats With DM-BMSCs Significantly Decreased BBB Leakage, Increased Tight-Junction Protein ZO-1 Expression, and Promoted Vascular Remodeling Mediated by miR-145 Expression

To test the mechanisms of DM-BMSC-induced neurorestorative effects, BBB permeability via FITC- albumin infiltration into the

injured brain was measured. Figure 3A and 3B shows that treatment of stroke in T1DM rats with DM-BMSCs significantly decreased BBB leakage and increased tight-junction protein ZO-1 expression in the ischemic brain compared with Nor-BMSC-treated rats or PBS-treated T1DM-MCAo rats.

However, in T1DM-MCAo rats, treatment with miR-145 +/+DM-BMSCs decreased DM-BMSC-induced beneficial effects, increased BBB leakage, and decreased ZO-1 expression, compared with the DM-BMSC treatment group.

To evaluate vascular remodeling, $\alpha$-SMA and vWF immunostaining were used. We found that DM-BMSC treatment of T1DM-MCAo rats significantly increased arteriole density, arteriole diameter, and vessel density compared with those measurements in PBS-treated T1DM-MCAo rats. Treatment of T1DM-MCAo with miR-145+/+DM-BMSCs significantly decreased DM-BMSC treatment-induced vascular remodeling and decreased arteriole density, arteriole diameter, and vessel density compared with DM-BMSC-treatment group (Fig. 4A, 4B).

### DM-BMSC Treatment of Stroke in T1DM Rats Significantly Promoted White Matter Remodeling Mediated by Decrease in miR-145 Expression

To investigate whether DM-BMSC treatment regulates white matter remodeling after stroke in T1DM rats, axonal, myelin, and neurofilament densities were measured. Treatment of stroke in T1DM rats using DM-BMSCs and Nor-BMSCs significantly
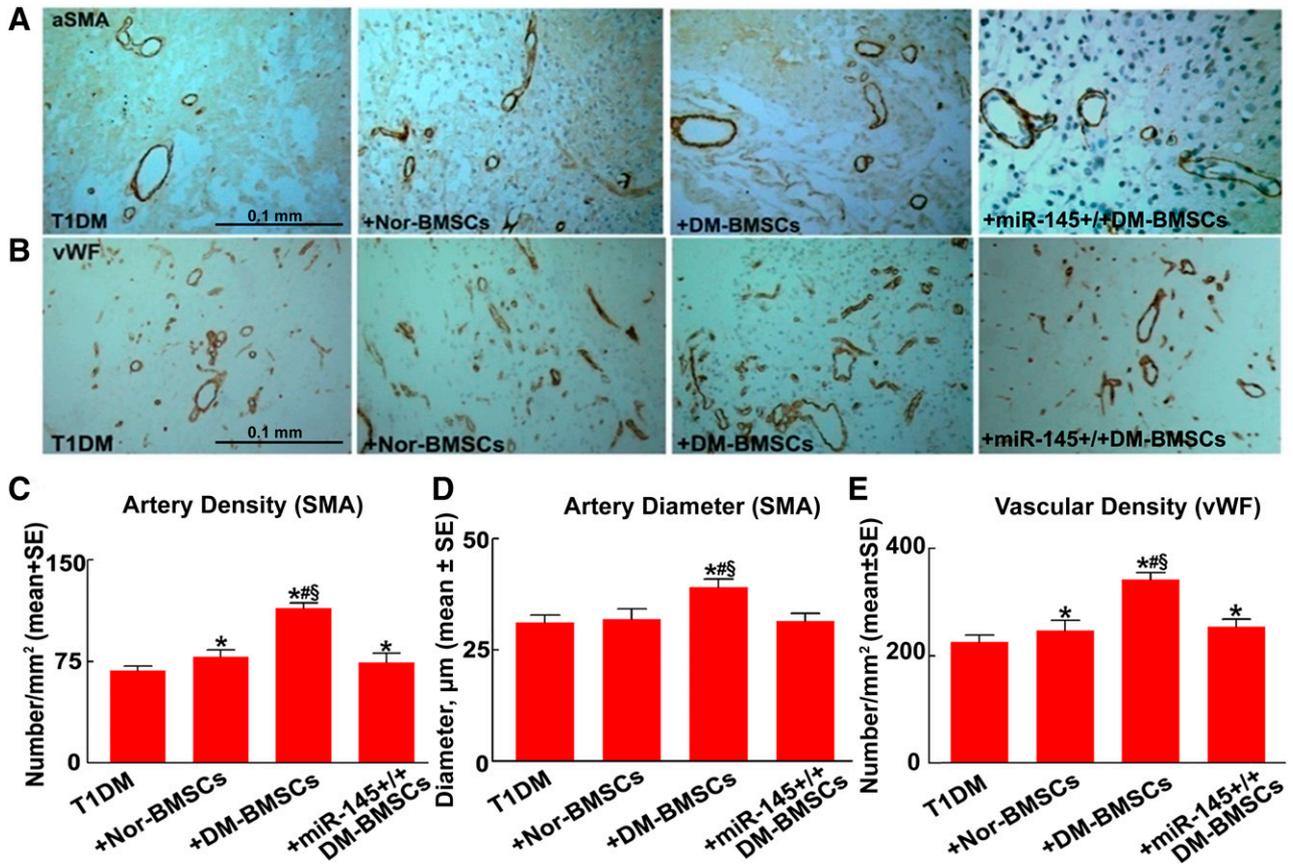
**Figure 4.** Treatment of T1DM stroke with DM-BMSCs, but not Nor-BMSCs, phosphate-buffered saline, or miR-145+/+DM-BMSCs **(A, B)**, improved arterial density **(C)** and diameter **(D)** and improved vascular density **(E)** in the ischemic boundary zone. $n = 8$ per group. ∗, $p < .05$ versus T1DM; #, $p < .05$ versus Nor-BMSCs; §, $p < .05$ versus miR-145+/+DM-BMSCs. Abbreviations: DM-BMSC, bone-marrow stromal cell derived from rats with type 1 diabetes; miR-145+/+DM-BMSC, miR-145 overexpression in bone-marrow stromal cell derived from rats with type 1 diabetes; Nor-BMSC, bone-marrow stromal cell derived from normal rats; $\alpha$-SMA, $\alpha$-smooth muscle actin; T1DM, type 1 diabetes; vWF, von Willebrand factor.

increased axon density (Bielschowsky silver stain), myelin density (Luxol fast-blue stain), and neurofilament density (SMI-31 antibody) in the ischemic boundary zone compared with PBS-treated T1DM-MCAo rats (Fig. 5A–5C). DM-BMSC treatment induced significantly increased white matter remodeling compared with the Nor-BMSC-treatment group, whereas miR-145+/+DM-BMSC treatment significantly decreased DM-BMSC-induced white matter remodeling (Fig. 5).

### DM-BMSCs Significantly Increased Expression of miR-145 Target Genes *ABCA1* and *IGFR1*

To investigate how decreasing miR-145 promotes DM-BMSC-induced neurorestorative effects, expression of the miR-145 target genes *ABCA1* and *IGFR1* was measured. Treatment with DM-BMSCs increased *ABCA1* and *IGFR1* expression in the ischemic boundary zone compared with PBS-treated and Nor-BMSC-treated T1DM-MCAo rats (Fig. 6A, 6B). However, treatment with miR-145+/+DM-BMSCs significantly decreased *ABCA1* and *IGFR1* expression compared with DM-BMSC treatment in T1DM-MCAo rats. This indicates that decreasing miR-145 may upregulate expression of its target genes *ABCA1* and *IGFR1* in the ischemic brain, which may contribute to DM-BMSC-induced neurorestorative effects in T1DM stroke rats.

### Decrease in miR-145 Expression Promoted Capillary Tube Formation and Axonal Outgrowth

To test whether miR-145 mediates DM-BMSC-induced angiogenesis and axonal outgrowth, overexpression and knockdown of miR-145 in DM-BMSCs were assessed. An MBEC capillary tube formation assay and axonal outgrowth assay were performed. The efficacy of miR-145 overexpression or knockdown in DM-BMSCs is shown in Figure 7A. We found that DM-BMSC-conditioned medium significantly increased MBEC capillary tube formation and primary cortical neuron axonal outgrowth compared with the nontreated control (Fig. 7B, 7C). Knockdown of miR-145 in DM-BMSCs induced significantly greater capillary tube formation and axonal outgrowth than did the DM-BMSC-conditioned medium. However, overexpression of miR-145 in DM-BMSCs significantly decreased DM-BMSC-induced capillary tube formation and axonal outgrowth compared with the group treated with DM-BMSC-conditioned medium. This suggests that miR-145 may regulate DM-BMSC-induced angiogenesis and axonal outgrowth.

### DISCUSSION

Treatment of T1DM stroke rats with DM-BMSCs improved neurological functional outcome, decreased BBB leakage, increased
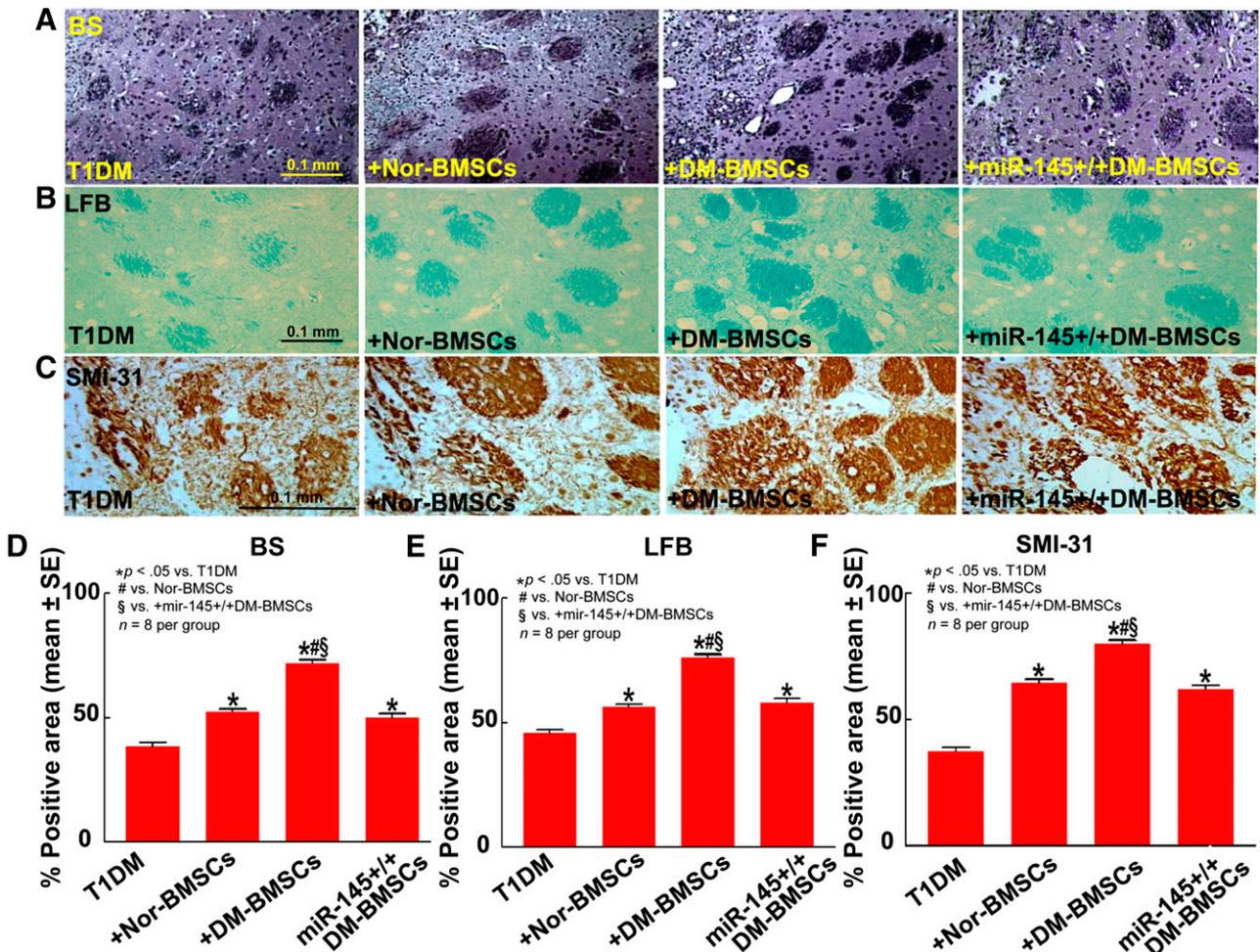
**Figure 5.** Results of treatment of T1DM stroke rats with DM-BMSCs and Nor-BMSCs on axon density, myelin density, and neurofilament-SMI-31 expression as seen in micrographs and quantified in bar graphs. Treatment of T1DM stroke rats with DM-BMSCs and Nor-BMSCs promoted white matter remodeling, identified by increase in axon density **(A, D)**, myelin density **(B, E)**, and neurofilament-SMI-31 expression **(C, F)** in the ischemic boundary zone brain compared with phosphate-buffered saline-treated T1DM rats in which stroke had been induced. DM-BMSC treatment significantly enhanced white matter remodeling compared with Nor-BMSC treatment in T1DM stroke rats, and miR-145+/+DM-BMSC treatment decreased DM-BMSC-induced beneficial effects. $n$ = 8 per group. ∗, $p < .05$ versus T1DM; #, $p < .05$ versus Nor-BMSCs; §, $p < .05$ versus miR-145+/+DM-BMSCs. Abbreviations: BS, Bielschowsky silver stain; DM-BMSC, bone-marrow stromal cell derived from rats with type 1 diabetes; LFB, Luxol fast-blue stain; miR-145+/+DM-BMSC, miR-145 overexpression in bone-marrow stromal cell derived from rats with type 1 diabetes; Nor-BMSC, bone-marrow stromal cell derived from normal rats; T1DM, type 1 diabetes.

tight-junction protein expression, increased capillary tube formation, promoted vascular remodeling, enhanced axonal outgrowth, and promoted white matter remodeling compared with Nor-BMSC- or PBS-treated T1DM-MCAo rats. Treatment of T1DM stroke rats with Nor-BMSCs did not promote neurological outcome or neurorestorative effects. These findings are consistent with our previous results showing that Nor-BMSC treatment in T1DM rats did not promote neurorestoration but instead had adverse effects, including increased mortality, BBB leakage, and increased hemorrhage [6]. Our data indicate that treatment of T1DM-MCAo rats with DM-BMSCs significantly increased neurorestorative effects compared with treatment with Nor-BMSCs. This study shows, for the first time, to our knowledge, that treatment of stroke in T1DM rats with BMSC derived from T1DM rats significantly promoted neurorestorative effects, which may be mediated by downregulation of miR-145 and the subsequent increase in expression of its target genes *ABCA1* and *IGFR1*.

It has been previously reported that under hyperglycemic and ischemic conditions, BMSCs are genetically stable [22]. In addition, activation of cell proliferation under conditions of hypoxia and hyperglycemia has been demonstrated [28]. Glucose also stimulates the proliferation of pancreatic $\beta$-cells in rodents and humans [29]. In our previous studies, we found that BMSCs derived from ischemic stroke animals or that are subjected to ischemic preconditioning significantly increased growth factor expression, and treatment of stroke with ischemic BMSCs induced enhanced functional outcome compared with normal BMSC cells [22]. Hypoxic preconditioning of BMSCs is an effective approach to enhance their therapeutic effect for diabetes and osteonecrosis [30, 31]. Similar to hypoxia preconditioned BMSCs, we found that BMSCs isolated from short-term diabetic rats exhibited increased cell proliferation and decreased cell death.

We have investigated the underlying mechanisms and also compared the efficacy of Nor-BMSCs and DM-BMSCs as
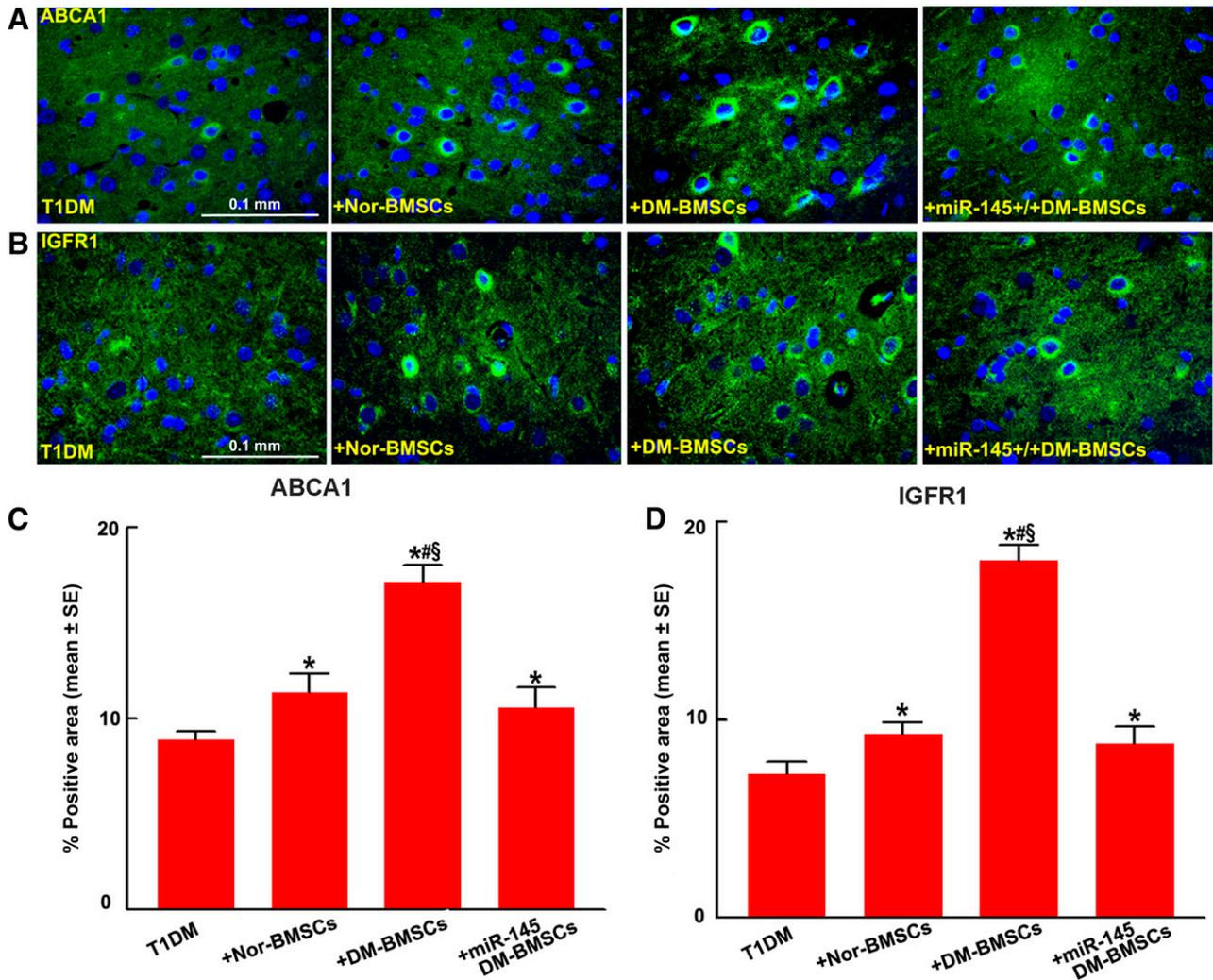
**Figure 6.** Results of treatment of T1DM stroke rats with DM-BMSCs and Nor-BMSCs on ABCA1 and IGF1R expression. Treatment of T1DM stroke with DM-BMSCs, Nor-BMSCs, and miR-145+/+DM-BMSCs significantly increased ABCA1 **(A, C)** and IGF1R **(B, D)** expression compared with phosphate-buffered saline-treated T1DM rats in which stroke had been induced. DM-BMSCs significantly increased ABCA1 and IGFR1 expression compared with Nor-BMSC- and miR-145+/+ DM-BMSC-treatment groups. $n = 8$ per group. $*$, $p < .05$ versus T1DM; #, $p < .05$ versus Nor-BMSCs; §, $p < .05$ versus miR-145+/+DM-BMSCs. Abbreviations: ABCA1, adenosine triphosphate-binding cassette transporter 1; DM-BMSC, bone-marrow stromal cell derived from rats with type 1 diabetes; IGFR-1, insulin-like growth factor 1; miR-145+/+DM-BMSC, miR-145 overexpression in bone-marrow stromal cell derived from rats with type 1 diabetes; Nor-BMSC, bone-marrow stromal cell derived from normal rats; T1DM, type 1 diabetes.

treatment of stroke in T1DM rats. The BBB is constituted by impermeable tight junctions between endothelial cells forming capillaries and venules in the central nervous system, and plays an important role in mediating neurological functional outcome [32, 33]. The disruption of the BBB occurs acutely after stroke and facilitates invasion of inflammatory factors, neurotoxins, and pathogens. Although BBB permeability usually increases within 7 days poststroke in non-DM animals, it can extend up to 14 days or longer in DM animals [30] and facilitate prolonged entry of inflammatory factors, which, in turn, increase BBB permeability. Increasing tight-junction protein decreases interendothelial cell leakage. DM-BMSC treatment in T1DM stroke rats decreased BBB leakage and increased ZO-1 tight-junction protein expression compared with PBS or Nor-BMSC treatment of T1DM-MCAo rats. Reduction of BBB leakage has been associated with improved

functional outcome in stroke, particularly in diabetic stroke rats [17, 31].

Several factors mediate functional reorganization and neurological recovery after stroke, including vascular remodeling, white matter remodeling, nerve regeneration, and synaptogenesis [5]. Treatment with DM-BMSCs improves vascular remodeling and increases the number of vessels and arteries in the ischemic brain. The presence of more microvessels in the ischemic hemisphere relative to the contralateral hemisphere is significantly correlated with longer survival [32]. A majority of neurorestorative agents that are therapeutically beneficial increase angiogenesis and neurogenesis, thereby expanding or initiating neurovascular niches for neuronal remodeling [33]. Increased capillary tube formation induced by DM-BMSCs can augment angiogenesis, which can, in turn, facilitate neurogenesis, as well as induce neurorestorative effects, resulting in improved survival after stroke [34].
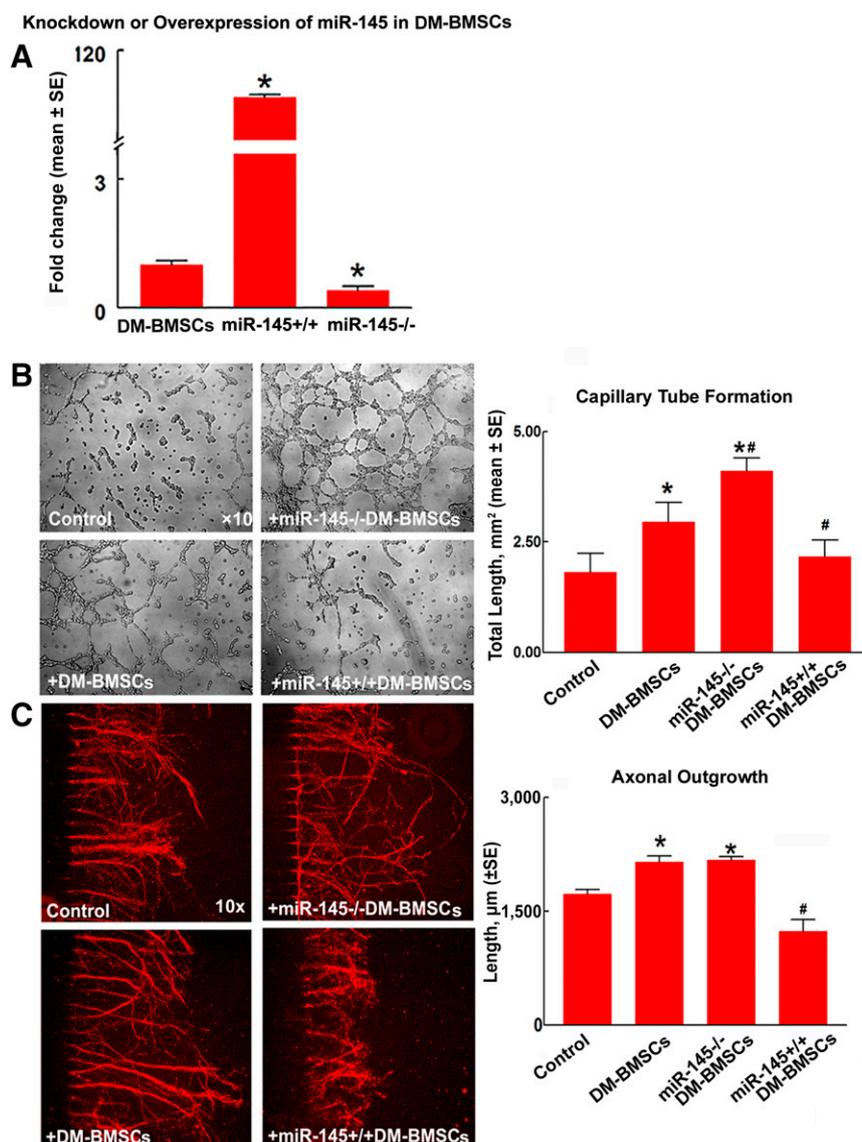
**Figure 7.** Knockdown or overexpression of miR-145 in DM-BMSCs. **(A):** Bar graph of efficacy of knockdown or overexpression of miR145 in DM-BMSCs. $n = 3$ per group. *, $p < .05$. **(B, C):** Treatment with DM-BMSC-conditioned medium and miR-145$-/-$DM-BMSC-conditioned medium both significantly promoted mouse brain endothelial cell capillary tube formation and increased primary cortical neuron axonal outgrowth, compared with the nontreatment control. Compared with DM-BMSC-conditioned medium, miR-145$-/-$DM-BMSCs significantly enhanced and miR-145+/+DM-BMSC-conditioned medium significantly attenuated capillary tube formation **(B)** Primary cortical neuron axon outgrowth **(C)** assay and quantification data. $n = 6$ per group. *, $p < .05$ versus control; #, $p < .05$ versus DM-BMSCs. Abbreviations: DM-BMSC, bone-marrow stromal cell derived from rats with type 1 diabetes; miR-145+/+, miR-145 overexpression; miR-145$-/-$, miR-145 knockdown.

White matter is highly vulnerable to ischemia owing to its limited blood supply. Poststroke white matter damage has been associated with poor neurological outcome, small-vessel disease, and higher risk of recurrent stroke, cardiac complications, and mortality [35]. White matter remodeling such as increased myelin and axon density facilitates restoration of conduction of nerve signals and interneuronal communications [5]. Axon outgrowth enables rewiring in the ischemic boundary region, forming long-distance connections that can, over time, restore significant sensorimotor functions. DM-BMSC treatment significantly increased white matter remodeling, including enhanced axon, myelin, and neurofilament densities, compared with Nor-BMSC- and PBS-treated T1DM stroke rats; this may have contributed to the observed improvement in poststroke functional outcome in T1DM rats.

miR-145 acts as a communication molecule between smooth muscle cells and endothelial cells [36], and reduces endothelial cell proliferation and capillary tube formation in response to growth factors [32]. miR-145 can thereby modulate endothelial cell function in angiogenesis and vessel stabilization [36]. Although it has been reported that miR-145 is selectively expressed in smooth muscle cells and inhibits their proliferation [37], anti-miR-145 inhibition has been reported to promote angiogenesis [38]. Oligodendrocytes impact the development of axons and neurons; for instance, they facilitate the growth and maturation of axons via ensheathment with myelin. miR-145 inhibits

oligodendrocyte precursor cell differentiation [39] and neurite outgrowth [40]. We have shown that DM-BMSCs exhibit significantly lower levels of miR-145 compared with Nor-BMSCs, and DM-BMSC-conditioned medium, and treatment of oligodendrocytes with DM-BMSCs increases cell survival, as well as increases axonal outgrowth in vitro compared with Nor-BMSC-conditioned medium. DM-BMSC treatment of stroke in T1DM rats significantly decreased brain miR-145 expression compared with that seen in Nor-BMSC- or PBS-treated T1DM stroke rats. Our results suggest that miR-145 affects functional improvement and neurovascular remodeling because treatment of stroke in T1DM rats with DM-BMSCs promoted vascular and white matter remodeling as well as functional improvement; however, miR-145 in DM-BMSCs attenuated these beneficial effects.

We found elevated levels of ABCA1 and IGFR1 proteins in the ischemic brain in DM-BMSC-treatment rats. Inhibition of miR-145 promotes ABCA1 [41] and IGFR1 [11] expression. ABCA1 plays an important role in promoting white matter/axonal remodeling as well as vascular remodeling after stroke [42]. ABCA1 increase also exerts beneficial effects in diabetes; it can alleviate pancreatic cell islet defects and regulate cellular cholesterol levels [43]. In diabetic stroke, ABCA1 deficiency induces a reduction of IGF1 expression and has been implicated in worse poststroke neurological outcome, enhanced BBB leakage, increased neuroinflammation, and axonal and white matter injury [20]. IGF1 promotes neurogenesis, oligodendrogenesis, and angiogenesis [44]. IGF1 and IGFR1 are key players in several neuroprotective mechanisms after stroke, such as maintaining neuron viability, improving function outcome, and enhancing neovascularization and neurogenesis [45]. IGF1 and IGF1R may also mediate reduction of BBB leakage and apoptosis, and repair of excitotoxic damage, and may promote cell differentiation and migration [46]. In the present study, DM-BMSC treatment of stroke decreased miR-145 expression and increased ABCA1 and IGFR1 expression in the ischemic boundary zone compared with Nor-BMSC treatment. However, treatment with miR-145 +/+DM-BMSCs decreased ABCA1 and IGFR1 expression in the ischemic boundary zone and attenuated functional benefit effects induced by DM-BMSCs. Thus, the miR-145/ABCA1/IGFR1 pathway may lead to neurological recovery induced by DM-BMSC treatment in T1DM stroke. This has been summarized in supplemental online Figure 1.

There are a few limitations to this study, including that DM-BMSCs may regulate other miRs. How DM-BMSCs decreases miR-145 has not been investigated and future studies are warranted.

## CONCLUSION

Treatment of stroke in T1DM rats initiated 24 hours after ischemic insult via intravenous administration of BMSCs derived from T1DM rats significantly improved functional benefit by inducing neurorestorative effects such as increasing vascular and white matter remodeling and decreasing miR-145 expression. The beneficial effects of DM-BMSCs may be mediated by downregulation of miR-145 and upregulation of the miR-145 target genes *ABCA1* and *IGFR1* signaling pathways.

## AUTHOR CONTRIBUTIONS

C.C. and P.V.: collection and/or assembly of data, data analysis and interpretation, manuscript writing; X.Y., A.Z., T.Y., and R.N.: collection and/or assembly of data, data analysis and interpretation; M.C.: conception and design, final approval of manuscript; P.Y.: collection and/or assembly of data; G.C.: conception and design; J.C.: conception and design, data analysis and interpretation, manuscript writing, final approval of manuscript.

## DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST

M.C. has compensated research funding. The other authors indicated no potential conflicts of interest.

## REFERENCES

**1** Yong M, Kaste M. Dynamic of hyperglycemia as a predictor of stroke outcome in the ECASS-II trial. Stroke 2008;39:2749–2755.

**2** Chen J, Cui X, Zacharek A et al. White matter damage and the effect of matrix metalloproteinases in type 2 diabetic mice after stroke. Stroke 2011;42:445–452.

**3** Chen J, Li Y, Wang L et al. Therapeutic benefit of intravenous administration of bone marrow stromal cells after cerebral ischemia in rats. Stroke 2001;32:1005–1011.

**4** Larsson E, Fredlund Fuchs P, Heldin J et al. Discovery of microvascular miRNAs using public gene expression data: miR-145 is expressed in pericytes and is a regulator of Fli1. Genome Med 2009;1:108.

**5** Chen J, Venkat P, Zacharek A et al. Neurorestorative therapy for stroke. Front Hum Neurosci 2014;8:382.

**6** Chen J, Ye X, Yan T et al. Adverse effects of bone marrow stromal cell treatment of stroke in diabetic rats. Stroke 2011;42:3551–3558.

**7** Ning R, Chopp M, Yan T et al. Tissue plasminogen activator treatment of stroke in type-1 diabetes rats. Neuroscience 2012;222:326–332.

**8** Jia L, Hao F, Wang W et al. Circulating miR-145 is associated with plasma high-sensitivity C-reactive protein in acute ischemic stroke patients. Cell Biochem Funct 2015;33:314–319.

**9** Dharap A, Bowen K, Place R et al. Transient focal ischemia induces extensive temporal changes in rat cerebral microRNAome. J Cereb Blood Flow Metab 2009;29:675–687.

**10** Karasinska JM, Rinninger F, Lütjohann D et al. Specific loss of brain ABCA1 increases brain cholesterol uptake and influences neuronal structure and function. J Neurosci 2009;29:3579–3589.

**11** Su J, Liang H, Yao W et al. MiR-143 and MiR-145 regulate IGF1R to suppress cell proliferation in colorectal cancer. PLoS One 2014;9:e114420.

**12** Oram JF. Molecular basis of cholesterol homeostasis: Lessons from Tangier disease and ABCA1. Trends Mol Med 2002;8:168–173.

**13** Anlar B, Sullivan KA, Feldman EL. Insulin-like growth factor-I and central nervous system development. Horm Metab Res 1999;31:120–125.

**14** Fernandez AM, Torres-Alemán I. The many faces of insulin-like peptide signalling in the brain. Nat Rev Neurosci 2012;13:225–239.

**15** Zacharek A, Chen J, Cui X et al. Simvastatin increases notch signaling activity and promotes arteriogenesis after stroke. Stroke 2009;40:254–260.

**16** Ye X, Chopp M, Liu X et al. Niaspan reduces high-mobility group box 1/receptor for advanced glycation endproducts after stroke in type-1 diabetic rats. Neuroscience 2011;190:339–345.

**17** Yan T, Venkat P, Ye X et al. HUCBCs increase angiopoietin 1 and induce neurorestorative effects after stroke in T1DM rats. CNS Neurosci Ther 2014;20:935–944.

**18** Chen H, Chopp M, Zhang ZG et al. The effect of hypothermia on transient middle cerebral artery occlusion in the rat. J Cereb Blood Flow Metab 1992;12:621–628.

**19** Rogers DC, Campbell CA, Stretton JL et al. Correlation between motor impairment and infarct volume after permanent and transient middle cerebral artery occlusion in the rat. Stroke 1997;28:2060–2065; discussion 2066.

**20** Cui X, Chopp M, Zacharek A et al. Deficiency of brain ATP-binding cassette transporter A-1 exacerbates blood-brain barrier and white matter damage after stroke. Stroke 2015;46:827–834.

**21** Zacharek A, Chen J, Cui X et al. Angiopoietin1/Tie2 and VEGF/Flk1 induced by MSC treatment amplifies angiogenesis and vascular stabilization after stroke. J Cereb Blood Flow Metab 2007;27:1684–1691.

**22** Zacharek A, Shehadah A, Chen J et al. Comparison of bone marrow stromal cells derived from stroke and normal rats for stroke treatment. Stroke 2010;41:524–530.

**23** Ding J, Cheng Y, Gao S et al. Effects of nerve growth factor and Noggin-modified bone marrow stromal cells on stroke in rats. J Neurosci Res 2011;89:222–230.

**24** Wong C, Chabot N, Kok MA et al. Modified areal cartography in auditory cortex following early- and late-onset deafness. Cereb Cortex 2014;24:1778–1792.

**25** Zhang Y, Ueno Y, Liu XS et al. The MicroRNA-17-92 cluster enhances axonal outgrowth in embryonic cortical neurons. J Neurosci 2013;33:6885–6894.

**26** Taylor AM, Blurton-Jones M, Rhee SW et al. A microfluidic culture platform for CNS axonal injury, regeneration and transport. Nat Methods 2005;2:599–605.

**27** Cho WC, Chow AS, Au JS. MiR-145 inhibits cell proliferation of human lung adenocarcinoma by targeting EGFR and NUDT1. RNA Biol 2011;8:125–131.

**28** Lykov AP, Nikonorova YV, Bondarenko NA et al. Proliferation, migration, and production of nitric oxide by bone marrow multipotent mesenchymal stromal cells from Wistar rats in hypoxia and hyperglycemia. Bull Exp Biol Med 2015;159:443–445.

**29** Zhang P, Kumar A, Katz LS et al. Induction of the ChREBPβ isoform is essential for glucose-stimulated β-cell proliferation. Diabetes 2015; 64:4158–4170.

**30** Wang Z, Fang BO, Tan Z et al. Hypoxic preconditioning increases the protective effect of bone marrow mesenchymal stem cells on spinal cord ischemia/reperfusion injury. Mol Med Rep 2016;13:1953–1960.

**31** Fan L, Zhang C, Yu Z et al. Transplantation of hypoxia preconditioned bone marrow mesenchymal stem cells enhances angiogenesis and osteogenesis in rabbit femoral head osteonecrosis. Bone 2015;81:544–553.

**32** Reese TS, Karnovsky MJ. Fine structural localization of a blood-brain barrier to exogenous peroxidase. J Cell Biol 1967;34:207–217.

**33** Nagy Z, Peters H, Hüttner I. Fracture faces of cell junctions in cerebral endothelium during normal and hyperosmotic conditions. Lab Invest 1984;50:313–322.

**34** Belayev L, Busto R, Zhao W et al. Quantitative evaluation of blood-brain barrier permeability following middle cerebral artery occlusion in rats. Brain Res 1996;739:88–96.

**35** Yan T, Venkat P, Chopp M et al. Neurorestorative therapy of stroke in type 2 diabetes mellitus rats treated with human umbilical cord blood cells. Stroke 2015;46:2599–2606.

**36** Krupinski J, Kaluza J, Kumar P et al. Role of angiogenesis in patients with cerebral ischemic stroke. Stroke 1994;25:1794–1798.

**37** Xiong Y, Mahmood A, Chopp M. Angiogenesis, neurogenesis and brain recovery of function following injury. Curr Opin Investig Drugs 2010;11:298–308.

**38** Wei L, Erinjeri JP, Rovainen CM et al. Collateral growth and angiogenesis around cortical stroke. Stroke 2001;32:2179–2184.

**39** Leys D, Englund E, Del Ser T et al. White matter changes in stroke patients. Relationship with stroke subtype and outcome. Eur Neurol 1999;42:67–75.

**40** Climent M, Quintavalle M, Miragoli M et al. TGFβ triggers miR-143/145 transfer from smooth muscle cells to endothelial cells, thereby modulating vessel stabilization. Circ Res 2015; 116:1753–1764.

**41** Cordes KR, Sheehy NT, White MP et al. miR-145 and miR-143 regulate smooth muscle cell fate and plasticity. Nature 2009;460: 705–710.

**42** Zhang H, Pu J, Qi T et al. MicroRNA-145 inhibits the growth, invasion, metastasis and angiogenesis of neuroblastoma cells through targeting hypoxia-inducible factor 2 alpha. Oncogene 2014;33:387–397.

**43** Hoffmann SA, Hos D, Küspert M et al. Stem cell factor Sox2 and its close relative Sox3 have differentiation functions in oligodendrocytes. Development 2014;141:39–50.

**44** Zhang HY, Zheng SJ, Zhao JH et al. MicroRNAs 144, 145, and 214 are down-regulated in primary neurons responding to sciatic nerve transection. Brain Res 2011;1383:62–70.

**45** Kang MH, Zhang LH, Wijesekara N et al. Regulation of ABCA1 protein expression and function in hepatic and pancreatic islet cells by miR-145. Arterioscler Thromb Vasc Biol 2013; 33:2724–2732.

**46** Karasinska JM, de Haan W, Franciosi S et al. ABCA1 influences neuroinflammation and neuronal death. Neurobiol Dis 2013;54: 445–455.

**47** Wijesekara N, Zhang LH, Kang MH et al. miR-33a modulates ABCA1 expression, cholesterol accumulation, and insulin secretion in pancreatic islets. Diabetes 2012;61:653–658.

**48** Aberg MA, Aberg ND, Hedbäcker H et al. Peripheral infusion of IGF-I selectively induces neurogenesis in the adult rat hippocampus. J Neurosci 2000;20:2896–2903.

**49** Zhu W, Fan Y, Frenzel T et al. Insulin growth factor-1 gene transfer enhances neurovascular remodeling and improves long-term stroke outcome in mice. Stroke 2008;39: 1254–1261.

**50** Carro E, Trejo JL, Núñez A et al. Brain repair and neuroprotection by serum insulin-like growth factor I. Mol Neurobiol 2003;27: 153–162.

See www.StemCellsTM.com for supporting information available online.