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Eye Opener in Stroke

Mitochondrial Dysfunction and Stem Cell Repair in Retinal Ischemia

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Background and Purpose—Retinal ischemia is a major cause of visual impairment in stroke patients, but our incomplete understanding of its pathology may contribute to a lack of effective treatment. Here, we investigated the role of mitochondrial dysfunction in retinal ischemia and probed the potential of mesenchymal stem cells (MSCs) in mitochondrial repair under such pathological condition.

Methods—In vivo, rats were subjected to middle cerebral artery occlusion then randomly treated with intravenous MSCs or vehicle. Laser Doppler was used to evaluate the blood flow in the brain and the eye, while immunohistochemical staining assessed cellular degeneration at days 3 and 14 poststroke. In vitro, retinal pigmented epithelium cells were exposed to either oxygen-glucose deprivation or oxygen-glucose deprivation and coculture with MSCs, and subsequently, cell death and mitochondrial function were examined immunocytochemically and with Seahorse analyzer, respectively.

Results—Middle cerebral artery occlusion significantly reduced blood flow in the brain and the eye accompanied by mitochondrial dysfunction and ganglion cell death at days 3 and 14 poststroke. Intravenous MSCs elicited mitochondrial repair and improved ganglion cell survival at day 14 poststroke. Oxygen-glucose deprivation similarly induced mitochondrial dysfunction and cell death in retinal pigmented epithelium cells; coculture with MSCs restored mitochondrial respiration, mitochondrial network morphology, and mitochondrial dynamics, which likely attenuated oxygen-glucose deprivation-mediated retinal pigmented epithelium cell death.

Conclusions—Retinal ischemia is closely associated with mitochondrial dysfunction, which can be remedied by stem cell-mediated mitochondrial repair.

Visual Overview—An online visual overview is available for this article. (***Stroke*. 2019;50:2197-2206. DOI: 10.1161/STROKEAHA.119.025249.)**

Key Words: cell survival ■ endothelial cells ■ glucose ■ mitochondrial dynamics ■ oxygen

Stroke is the fifth cause of death and the leading cause of disability affecting ≈800 000 people and costing $34.3 billion annually in the United States.[1] In spite of the severity and prevalence of stroke, the therapeutic options are limited to tPA (tissue-type plasminogen activator) and endovascular interventions.[1] Moreover, the therapeutic window for tPA administration is limited to 4.5 hours from onset, and the criteria for mechanical thrombectomy are stringent with high risk of hemorrhagic transformation.[2–6]

Visual impairment is a prevalent stroke consequence that negatively affects rehabilitation, functional recovery, and quality of life.[7,8] Visual impairments occur in 92% of stroke patients[7]; and 20.5% of stroke patients display persistence visual impairment at 90 days.[9] Furthermore, patients with monocular vision loss have a higher risk of concurrent ischemic stroke and vice versa.[9–14] Retinal ischemia is the major cause of visual impairment in ≈16% of the stroke patients and shares a pathology with other common ocular vascular diseases, such as diabetic retinopathy, glaucoma, retinal vein occlusion, and central retinal artery occlusion.[15–20] Despite many similarities between retinal ischemia and cerebral ischemia, the underlying mechanisms between them remain unclear which may contribute to limited effective treatments for retinal ischemia and stroke as a whole.[21,22] Therefore, there is a need for a better understanding of stroke pathology that incorporates retinal ischemia.

The multifaceted important functions of mitochondria in cell survival and death have been implicated in stroke and in various neurological diseases, for example, fragile x-associated tremor/ataxia syndrome, Alzheimer disease, Parkinson disease, and Huntington disease,[23–28] and retinal ischemia and optic neuropathy.[29–32] During cerebral/retinal ischemia,

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mitochondria, the powerhouse of the cells, cannot maintain energy production among other metabolic activities, triggering a cascade of cell death events.[33–37] Probing the role of mitochondrial dysfunction in retinal ischemia pathology may provide mechanistic and translational insights into developing more effective treatments for stroke and other disorders with retinal ischemia pathology. Indeed, mitochondrial transfer from either astrocytes or stem cells to ischemic neurons is deemed a novel stroke therapy.[38,39]

Here, we used a combination of in vitro cell culture and in vivo rat models to examine the role of mitochondria dysfunction in stroke-related retinal ischemia and whether stem cells could repair the mitochondria and rescue the ischemic retinal cells. Our results demonstrated that both middle cerebral artery occlusion (MCAO) and oxygen-glucose deprivation (OGD) stroke models produced consistent retinal ischemia accompanied by massive alterations in retinal cells' mitochondrial respiration, network morphology, and dynamics and treatment, which were reversed by stem cell treatment.

Methods

Ethics Statement

All experiments were conducted in accordance with the National Institute of Health Guide and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee of the University of South Florida, Morsani College of Medicine. The article adheres to the Transparency and Openness Promotion Guidelines, and all data supporting the findings of this study are available from the corresponding authors on reasonable request.

MCAO Model

Adult male Sprague-Dawley rats (≈250 g) were subjected to transient intraluminal MCAO procedure (n=24) or sham surgery (n=6); see in the online-only Data Supplement.

Laser Doppler Blood Flow Measurement

Brain and eye blood flow measurements were measured using laser Doppler (Perimed, Periflux System 5000) at baseline, during MCAO, and 5 minutes after reperfusion; see in the online-only Data Supplement.

Mesenchymal Stem Cells Transplantation

At day 1 post-MCAO, animals were anesthetized and transplanted intravenously via the jugular vein with mesenchymal stem cells (MSCs; 4×10^6 cells/500 µL of sterile PBS) or with PBS only; see in the online-only Data Supplement.

Optic Nerve Measurement and Immunohistochemistry

At days 3 and 14 post-MCAO, the animals were euthanized by CO_2 and perfused with 0.9% saline. The animals' eyes and optic nerves were quickly harvested and fixed. Optic nerve images were obtained on a bright field Olympus microscope. Optic nerve widths were measured using the CellSens program. The retinas were stained with NeuN (neuronal nuclei) antibody (1:500; ab104225, Abcam), a marker for neuronal cells including the ganglion cells; see in the online-only Data Supplement.

Retinal Pigmented Epithelium Cells and MSC Culture

Retinal pigmented epithelium (RPE, CRL-4000;ATCC) cells and MSCs (T4835;abm) were cultured according to manufactures' protocols and were passaged at 90% confluency. All cells for experiments were from passage 7 to10; see in the online-only Data Supplement.

OGD and Coculture

The OGD was slightly modified from previously described method.[40] After OGD, the RPE cells were cocultured with MSCs by placing the inserts into the wells of the 6-well plate for 24 hours; see in the online-only Data Supplement.

Mitochondrial Respiration Assay

To determine cellular oxygen consumption rate, the Seahorse extracellular flux analyzer XFe96 (102416;Agilent) was used in combination with sequential injection of various compounds. Oxygen consumption rate measurements were performed following the manufacturer's protocol; see in the online-only Data Supplement.

Mitochondrial Network Analysis

The RPE cells were stained with MitoTracker (M22426;Invitrogen). Images were captured using an Olympus FV1200 Spectral Inverted Laser Scanning Confocal Microscope and analyzed using ImageJ (National Institutes of Health) with mitochondrial network analysis plugin. The mitochondrial network analysis's method and measured parameters are described in a recent study.[41] The source code for mitochondrial network analysis plugin is available at https://github.com/ScienceToolkit/MiNA; see in the online-only Data Supplement.

Cell Viability Assay

The RPE cells were incubated with calcein AM (1 µmol/L; 4892010K;Trevigen) for 30 minutes in an incubator (37°C humidified, with 5% CO_2, 95% air). Bright green fluorescence was retained within living cells. The number of cells were counted using ImageJ (National Institutes of Health) and averaged per field of view.

Mitochondria Live Cell Imaging

The mitochondria of RPE cells were incubated with either mitochondrial membrane potential probe JC-1 (tetraethylbenzimidazolylcarbocyanine iodide, T3168; Invitrogen) or with MitoTracker (M22426;Invitrogen). Live images were captured at a 5-minute interval over 25 minutes using an Olympus FV1200 Spectral Inverted Laser Scanning Confocal Microscope; see in the online-only Data Supplement.

Immunocytochemistry

The RPE cells were stained for Ki67 (NCL-Ki67P;LeicaBiosystems), Drp1 (dynamin-related protein 1, 70278;Life Technologies), or Mfn2 (mitofusin-2, 711803;eBioscience); see in the online-only Data Supplement.

Statistical Analysis

The data were evaluated using ANOVA followed by post hoc Bonferroni tests except for laser Doppler data, which were analyzed using unpaired *t* test. Statistical significance was preset at $P<0.05$. Data are presented as mean±SD.

Results

MCAO Reduces Blood Flow to Brain and Eye and Induces Retinal Ganglion Cell Loss: Therapeutic Target for MSCs

We initially investigated whether MCAO caused a reduction in blood flow to the brain, as well as to the eye. Laser Doppler was used to measure blood flow to brain and eye at baseline, during MCAO and 5-minute after reperfusion (Figure 1A). At baseline, there were no significant differences between the control group

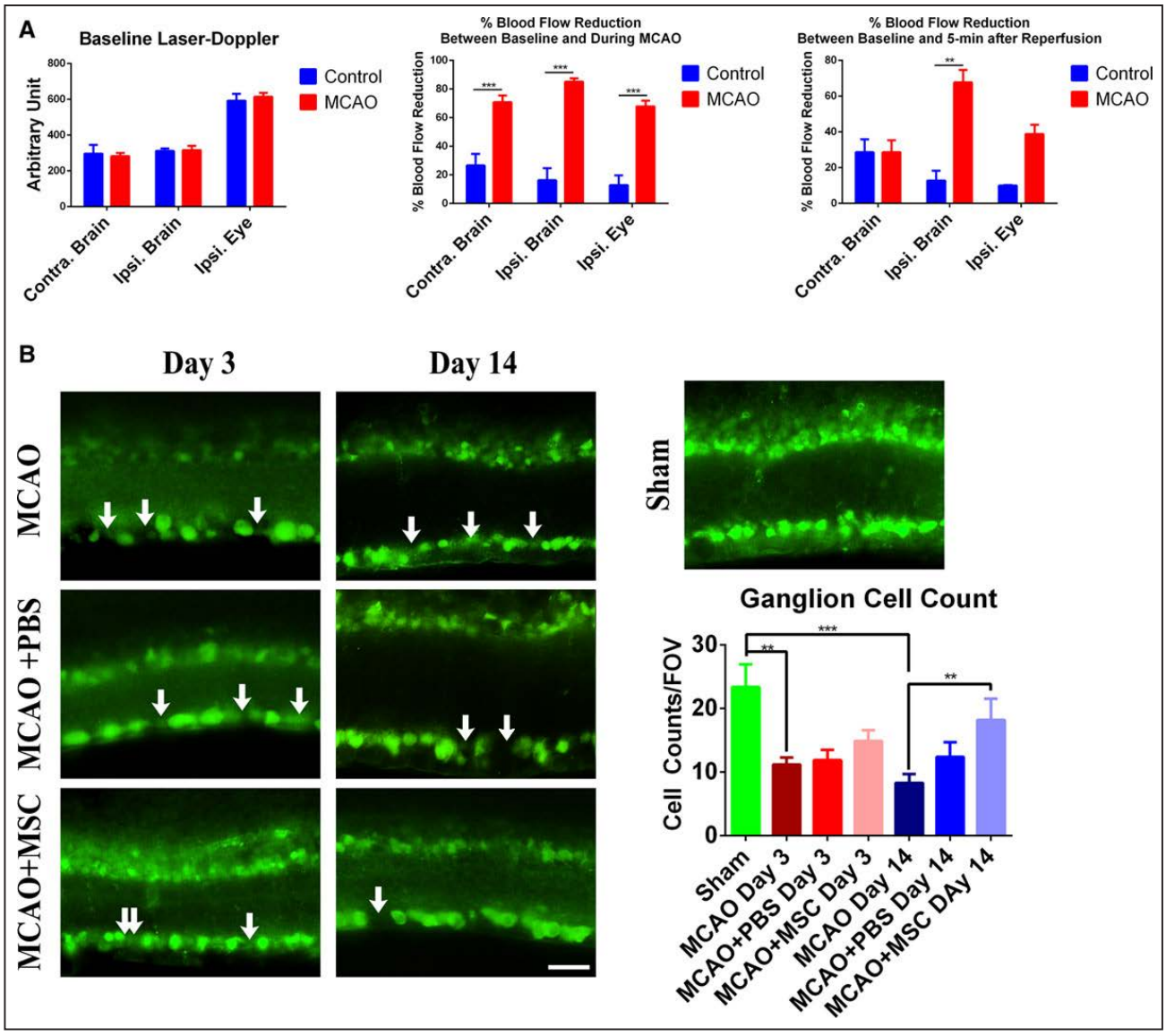

Figure 1. Middle cerebral artery occlusion (MCAO) reduces blood flow to brain and eye and induced ganglion cell loss in the retina and transplantation of mesenchymal stem cells (MSCs) rescued ganglion cell death at day 14 poststroke. **A**, Laser Doppler was used to measure blood flow to brain and eye at baseline, during MCAO, and 5-minute after reperfusion. MCAO caused a significant reduction in blood flow to the contralateral (Contra) hemisphere, ipsilateral (Ipsi) hemisphere, and Ipsi eye compared with control. **B**, Representative images and quantification of immunohistochemical staining of NeuN. Transplantation of MSC rescued ganglion cell loss at day 14 poststroke. ANOVA with Bonferroni post hoc test **P*<0.05; ***P*<0.01; and ****P*<0.001. Scale bar 50 μm. FOV indicates field of view.

and MCAO group in the laser Doppler measurements of ipsilateral hemisphere, contralateral hemisphere, or ipsilateral eye (311±23 and 316±87; 296±49 and 282±18; and 592±67 and 614±81, respectively, unpaired *t* tests *P*>0.05). During MCAO, there were significant differences between the control group and MCAO group in the percentage of blood flow reduction of contralateral hemisphere, ipsilateral hemisphere, and ipsilateral eye compared with the baseline (16±14 and 85±9; 26±14 and 70±17; 12±11 and 67±15, respectively, unpaired *t* tests *P*<0.05). After reperfusion, there was a significant difference between the control group and MCAO in the percentage of blood flow reduction only in the ipsilateral hemisphere compared with the baseline (12±9 and 67±26, unpaired *t* tests *P*<0.05). Altogether, these results indicate that MCAO caused a significant reduction in blood flow to the eye which mirrored the reduction in the brain.

We next examined whether the reduction in blood flow to the eye during MCAO caused significant ganglion cell loss and optic nerve degeneration in stroke animals. At days 3 and 14 poststroke, there was a significant reduction in the ipsilateral optic nerve width of stroke animals compared with sham animals (*P*<0.001; Figure I in the online-only Data Supplement). There was a significant reduction in ganglion cell death at days 3 and 14 in the ipsilateral eye compared with sham group (*P*=0.0003 and *P*<0.0001, respectively; Figure 1B).

Next, we hypothesized that MSCs could rescue the ganglion cell death caused by MCAO. Animals received either MSCs or PBS via intravenously transplantation using the jugular vein at 24 hours after surgery. Interestingly, transplantation of MSCs showed a trend toward a reduction in ganglion cell death at day 3 and a significant reduction in the ganglion

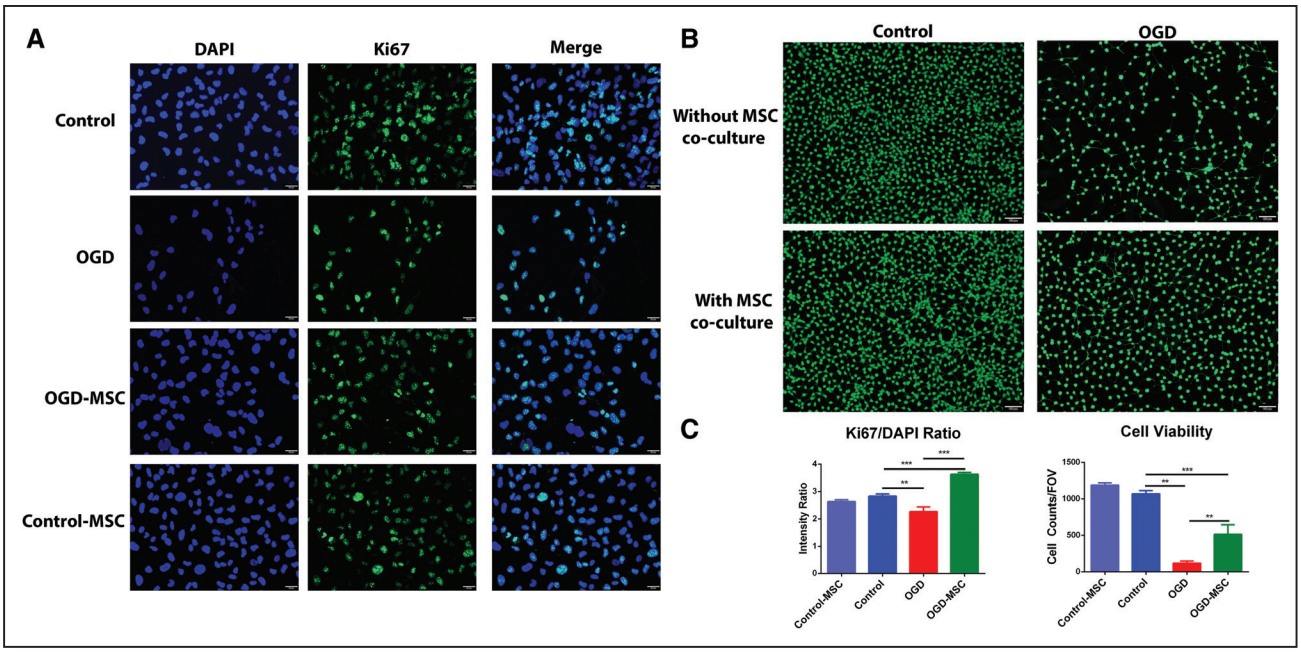

Figure 2. Mesenchymal stem cells (MSCs) rescue against retinal pigmented epithelium (RPE) cells loss caused by oxygen-glucose deprivation (OGD) by promoting cell proliferation. **A**, Representative images of immunocytochemical staining of Ki67 (marker for cell proliferation). OGD produced a significant decrease in Ki67 expression. Coculture with MSCs restored cell Ki67 expression after OGD. **B**, Representative images of Calcein AM cell viability test. OGD induced a significant decrease in cell viability. Coculture with MSCs rescued RPE cell death after OGD. **C**, Quantification graphs of Ki67 intensity and cell viability. ANOVA with Bonferroni post hoc test **P*<0.05; ***P*<0.01; and ****P*<0.001. Scale bar 50 μm.

cell loss at day 14 (*P*>0.05 and *P*=0.0026, respectively) compared with respective MCAO groups. There were no significant differences between MCAO group and MCAO+PBS group at days 3 and 14 poststroke (*P*>0.05; Figure 1B). Overall, these results demonstrate that MCAO caused a reduction in blood flow to the brain and the eye which led to significant ganglion cell loss and optic nerve degeneration; and intravenous transplantation of MSCs rescued the ganglion cell death at day 14. Statistical results are summarized in Table I in the online-only Data Supplement.

MSCs Ameliorate OGD-Induced RPE Cells Loss by Promoting Cell Proliferation

We further investigated the observed therapeutic effect of MSCs under in vitro settings using OGD model. Cell viability and cell proliferation were assessed using calcein and Ki67 staining, respectively. ANOVA revealed significant differences in the Ki67 intensity between groups (F(3, 76)=9.795, *P*<0.0001) with OGD-RPE cells displaying a significant decrease in Ki67 intensity compared with the control (237.9±84.3 and 333.3±60.0, respectively, *P*<0.001; Figure 2A). Coculture with MSCs after OGD increased the Ki67 intensity compared with OGD group (350.8±77.9 and 237.9±84.3, respectively, *P*<0.001; Figure 2A). Additionally, ANOVA revealed significant differences in cell viability between groups (F(3, 20)=45.75, *P*<0.0001), with OGD-RPE cells showing a significant decrease in cell viability compared with the control (119±70 and 1068±110, respectively, *P*<0.001; Figure 2B). In contrast, coculture with MSCs after OGD rescued the RPE cells' viability compared with OGD group (512±327 and 119±70, respectively, *P*<0.01; Figure 2B). Overall, the results demonstrate that MSCs prevented cell loss after OGD by promoting cell proliferation.

MSCs Attenuate RPE Cells' Mitochondrial Respiration Deficits Caused by OGD

Next, we examined the effect of MSCs on the RPE cells' mitochondrial dysfunction caused by OGD. RPE cells' mitochondrial respiration were analyzed using Seahorse XFe96 extracellular flux analyzer (Figure 3). OGD caused significant reduction in the overall RPE cells' mitochondrial respiration compared with control characterized by decreased in basal respiration, decreased in spare respiratory capacity, and decreased in ATP (adenosine triphosphate) production (*P*<0.0001). Coculture with MSCs significantly rescued the overall mitochondrial respiration across all indices compared with OGD group as revealed by increased in basal respiration, increased in spare respiratory capacity, and increased in ATP production (*P*<0.0001). Interestingly, we observed also a decreased in proton leak in the OGD group compared with the control or the OGD-MSCs (*P*<0.0001). In summary, these results revealed that MSCs restored the mitochondrial respiration deficits caused by OGD. Statistical results are summarized in Table II in the online-only Data Supplement.

MSCs Restore RPE Cells' Mitochondrial Networks That Were Altered by OGD

We also investigated the effect of OGD on RPE cells' mitochondrial network morphology and whether MSCs could reverse such impairment. RPE cells' mitochondrial network was analyzed using immunocytochemistry and ImageJ with mitochondrial network analysis plugin (Figure 4). The measured parameters were previously described.[41] Compared with control group, OGD produced a significant reduction in total individual mitochondria (post hoc test *P*<0.0001), decreased in number of network (*P*=0.0087), and decreased in average

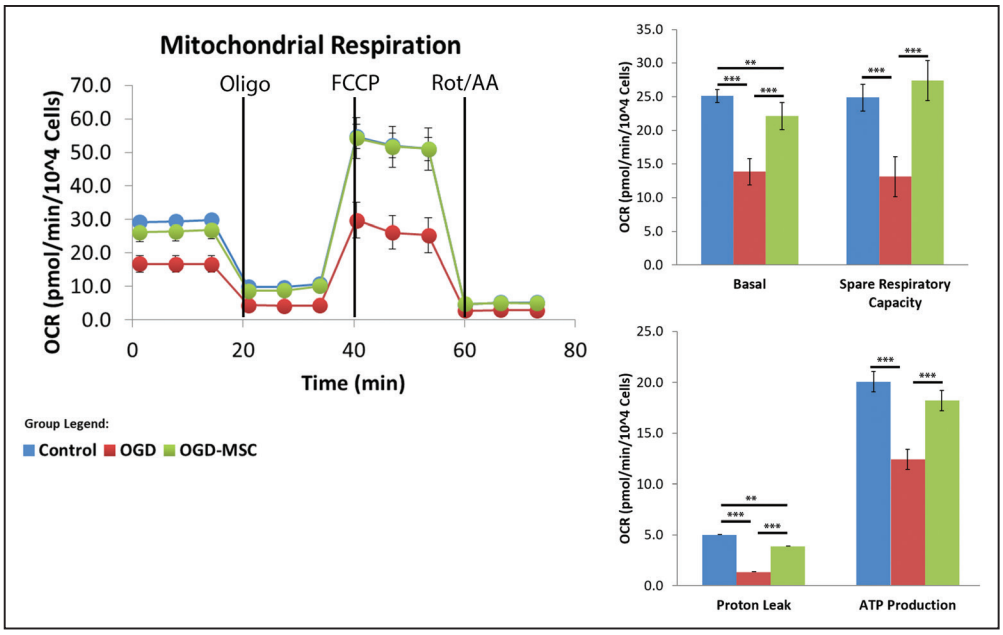

Figure 3. Mesenchymal stem cells (MSCs) ameliorate retinal pigmented epithelium (RPE) cells' mitochondrial respiration deficits caused by oxygen-glucose deprivation (OGD). RPE cells' mitochondrial respiration were analyzed using Seahorse XFe96 extracellular flux analyzer with sequential injection of various compounds (1 μmol/L oligomycin [Oligo], 1 μmol/L carbonyl cyanide 4-(trifluoromethoxy) phenylhydrazone [FCCP], 0.5 μmol/L rotenone and Antimycin A [Rot/AA]). Coculture with MSCs restored RPE cells' mitochondrial basal respiration, spare respiratory capacity, proton leak, and ATP production compared with OGD. ANOVA with Bonferroni post hoc test *P<0.05; **P<0.01; ***P<0.001.

branch length (post hoc test P<0.0001) while increased significantly the circularity of the mitochondria (post hoc test P<0.0001). Compared with OGD group, coculture with MSCs significantly increased the total individuals of mitochondria (post hoc test P=0.0046), increased the number of network (post hoc test P=0.0180), and decreased circularity (post hoc

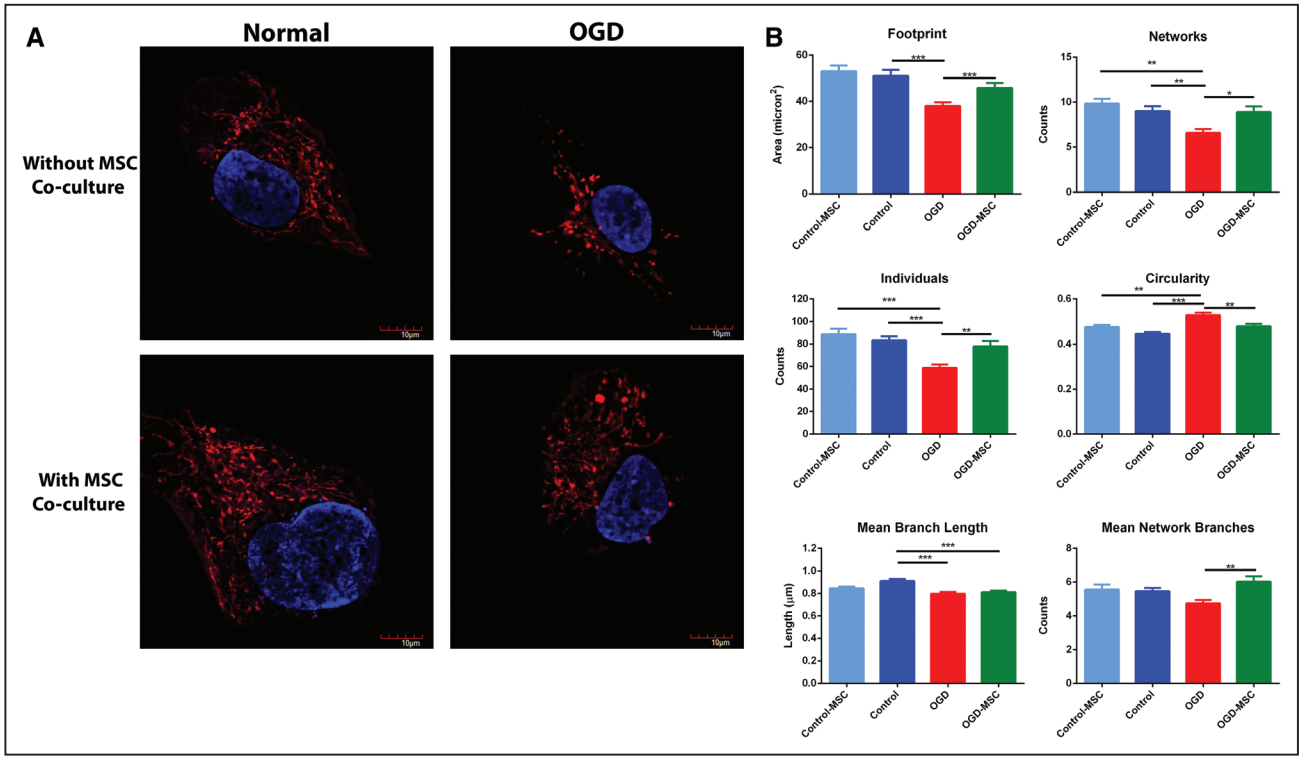

Figure 4. Mesenchymal stem cells (MSCs) restore retinal pigmented epithelium (RPE) cells' mitochondrial networks altered by oxygen-glucose deprivation (OGD). **A**, Representative images of RPE cells stained with MitoTracker. **B**, Analysis and quantification of RPE cells' mitochondrial network morphology. Coculture with MSCs increased RPE cells' number of mitochondrial networks, number of individual mitochondria, and number of branches but not average length of the branches compared with OGD. In addition, coculture with MSCs decreased the circularity of RPE cells' mitochondria compared with OGD. ANOVA with Bonferroni post hoc test *P<0.05; **P<0.01; and ***P<0.001. Mean±SEM. Scale bar 10 μm.

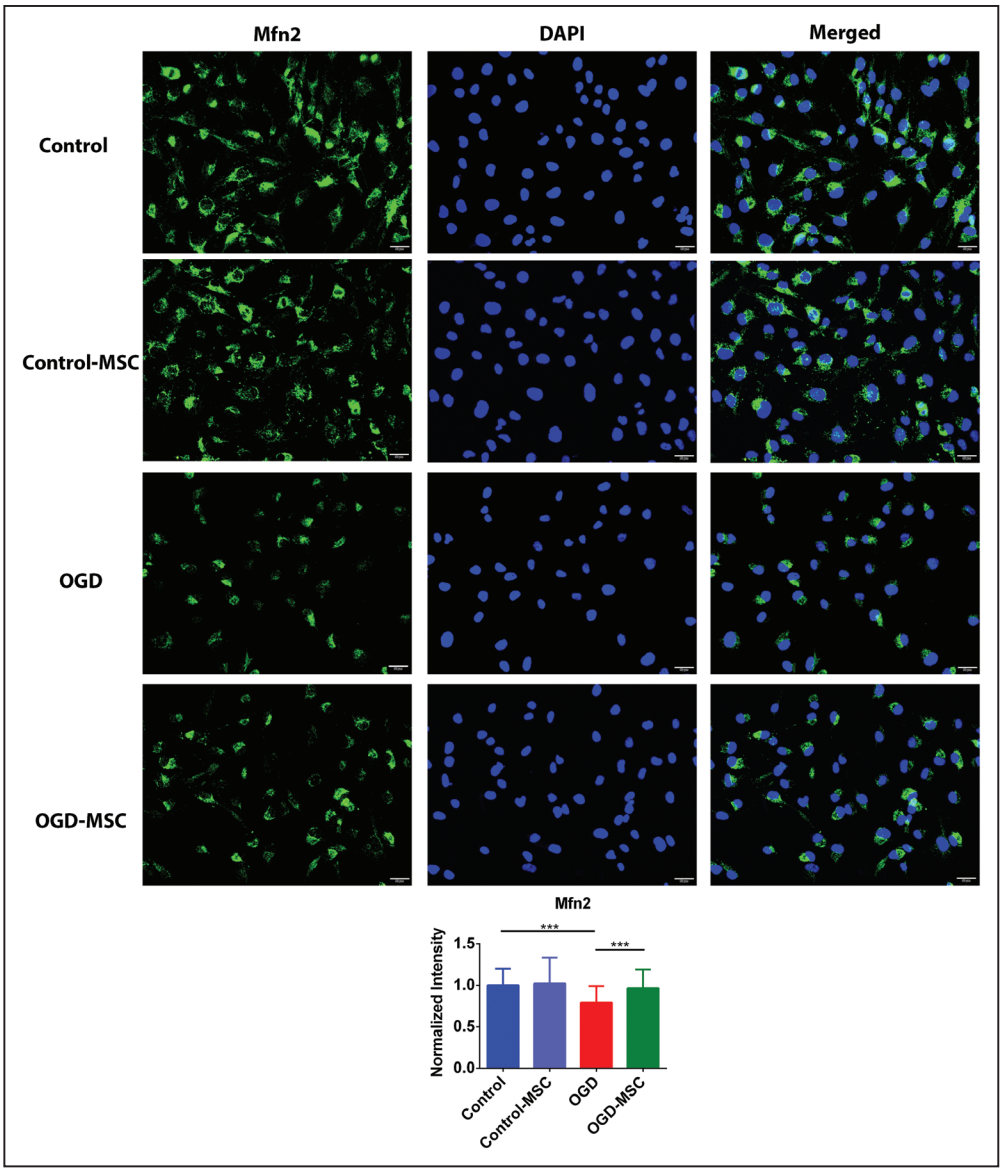

Figure 5. Mesenchymal stem cells (MSCs) normalize retinal pigmented epithelium (RPE) cells' mitochondrial dynamics via Mfn2 (mitofusin-2) after oxygen-glucose deprivation (OGD). Representative images of Mfn2 expression (**left** columns), DAPI (**middle** columns), and merged (**right** columns). OGD caused a significant decrease in Mfn2 expression. Coculture with MSC significantly increased the Mfn2 expression compared to OGD. ANOVA with Bonferroni post hoc test *P<0.05; **P<0.01; ***P<0.001. Scale bar 50 μm.

test P=0.0028) but not the average branch length (post hoc test P>0.5). In addition, live imaging of RPE cells' mitochondria confirmed the immunocytochemical results in that OGD induced visible disorganization of mitochondrial network, but coculture with MSCs robustly improved the mitochondrial network of RPE cells (Movies in the online-only Data Supplement). It is worth noting that MSCs' mitochondria were observed in both OGD-MSC and control-MSC groups. This mitochondrial transfer phenomenon was confirmed with immunocytochemical staining as evidenced by deposition of MSCs' mitochondria inside RPE cells (Figure II in the online-only Data Supplement). Altogether these results demonstrate that OGD significantly altered the mitochondrial network morphology towards an impaired state, that is, fragmented circular mitochondria, whereas coculture with MSCs restored the mitochondrial network morphology. Statistical results are summarized in Table II in the online-only Data Supplement.

MSCs Repair RPE Cells' Mitochondrial Dynamics via Mfn2 After OGD

We further investigated the deleterious effect of OGD and therapeutic effect of MSCs on mitochondrial dynamic proteins Mfn2 and Drp1. Immunocytochemical assay of Mfn2 revealed that there were significant differences between groups ([F(3, 307)=15.65, P<0.0001; Figure 5). OGD significantly reduced the expression of Mfn2 compared with the control (post hoc test P<0.0001). Coculture with MSCs significantly restored the expression of Mfn2 compared with the OGD-RPE group (post hoc test P<0.0001). However, OGD significantly increased the expression of Drp1 compared with the control, but coculture with MSCs did not significantly restore the

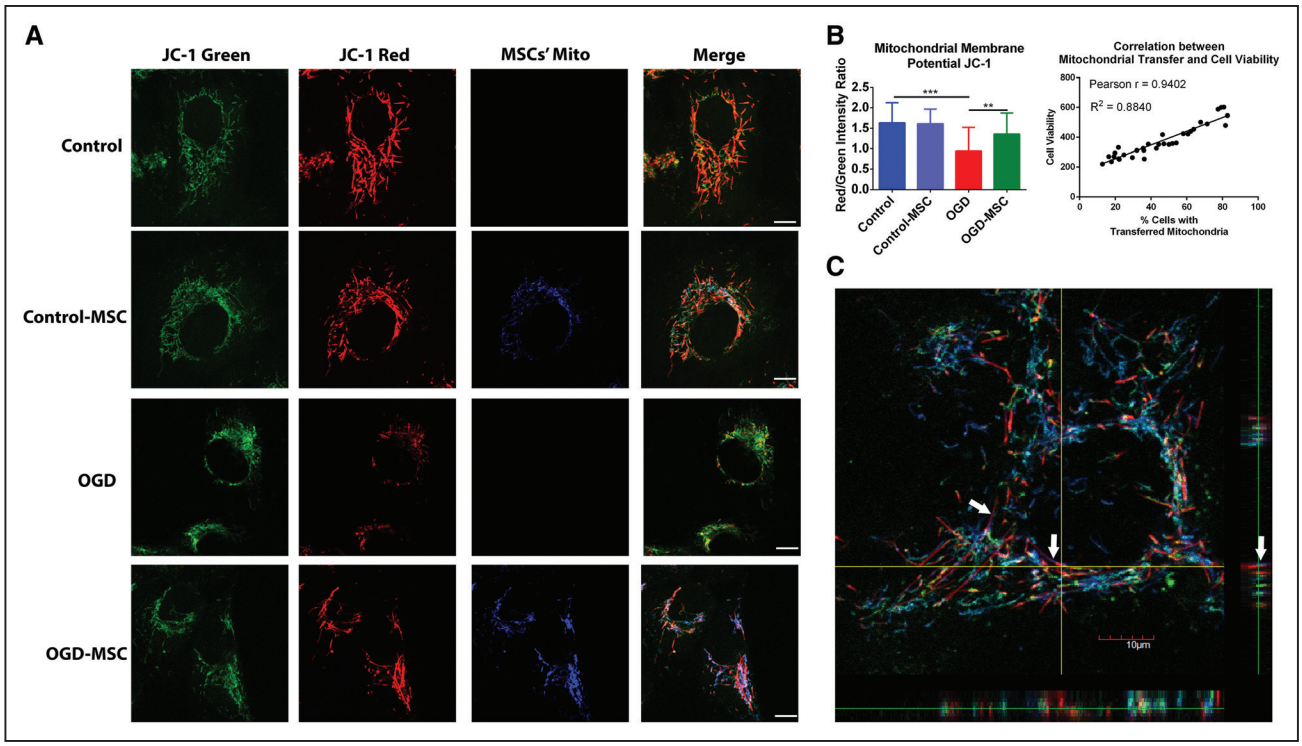

Figure 6. Mesenchymal stem cells (MSCs) reduce retinal pigmented epithelium (RPE) cells' mitochondrial membrane depolarization caused by oxygen-glucose deprivation (OGD). RPE cells' mitochondrial membrane potential was analyzed using JC-1 staining. **A**, Representative images of JC-1 dye and transferred MSC's mitochondria. **B**, Bar graph represents red/green (healthy/unhealthy) intensity ratio of JC-1 staining and correlational analysis between mitochondrial transfer and cell viability. OGD-RPE cells displayed a significant decrease in the JC-1 red/green intensity ratio compared with the control RPE cells. Coculture with MSCs significantly increased JC-1 red/green intensity ratio compared to OGD. **C**, Confocal imaging revealed colocalization between MSCs mitochondria (blue) and JC-1 (red, arrows) indicating the transfer of functional mitochondria from MSCs to RPE cells. ANOVA with Bonferroni post hoc test **P*<0.05; ***P*<0.01; ****P*<0.001. Scale bar 10 µm.

expression of Drp1 to normal level (Figure III in the online-only Data Supplement). These results show that OGD altered the mitochondrial dynamic proteins Mfn2 and Drp1, while coculture with MSCs normalized the expression of Mfn2 but not Drp1.

MSCs Reduce RPE Cells' Mitochondrial Membrane Depolarization Induced by OGD

RPE cells' mitochondrial membrane potential was analyzed using JC-1 staining (Figure 6). ANOVA revealed significant differences between groups in the JC-1 red/green intensity ratio ($F(3, 119)=13.50$, $P<0.0001$). Bonferroni post hoc tests showed that OGD-RPE cells had significant decrease in the JC-1 red/green intensity ratio compared with the control RPE cells (0.94±0.59 and 1.63±0.49, respectively, $P<0.0001$; Figure 6A and 6B). Coculture with MSCs significantly increased JC-1 red/green intensity ratio compared with OGD group (1.35±0.51 and 1.63±0.49, respectively, post hoc test $P<0.005$; Figure 6A and 6B). Confocal imaging revealed colocalization between MSCs' mitochondria (blue) and JC-1 (red) indicating the transfer of functional mitochondria from MSCs to RPE cells (Figure 6C). Furthermore, a positive correlation was detected between the percentage of cells with transferred mitochondria and the cell viability ($r=0.9402$, n=35, $P<0.0001$ with an $R^2=0.8840$; Figure 6B). In summary, these results indicate that MSCs reduced RPE cells' mitochondrial membrane depolarization caused by OGD possibly via transfer of MSCs' functional mitochondria.

Discussion

We demonstrated that MCAO and OGD induced retinal ischemia, associated with mitochondrial dysfunction. Treatment with MSCs rescued against retinal cell loss, likely through stem cell transfer of healthy mitochondria and subsequent restoration of mitochondrial function, network morphology, and dynamics.

Blood flow was reduced by 80% in ipsilateral hemisphere and ipsilateral eye in our stroke animals as previously reported.[42,43] The retinal blood flow recovered after reperfusion about 5 minutes faster than hemispheric blood flow, reflecting discrepant brain and retina reperfusion profiles because of increased vasculatures in the retina.[44,45] However, the lack of collaterals in the retina likely equalized the reperfusion profiles, allowing retinal blood flow to mirror the hemispheric blood flow up to 3 days poststroke.[46–48] This reduction in blood flow coincided with ganglion cell loss to the ipsilateral eye and decreased optic nerve width at days 3 and 14 poststroke. Intravenous transplantation of MSCs showed a trend towards rescue at day 3 and significantly attenuated both cellular and optic nerve deficits at day 14. In addition, OGD produced similar retinal cell loss, which was ameliorated by MSC coculture. The retinal cell deaths in vivo and in vitro were accompanied by mitochondrial dysfunction,

which was reversed by MSCs characterized by restored mitochondrial respiration and normalized mitochondrial network morphology. Mitochondrial network protects mitochondrial DNA integrity, improves respiratory capacity, and response to energy demand or cellular stress. The overall morphology of mitochondrial network may depend on a balanced ratio between mitochondrial fusion and fission, which is necessary to maintain tubular shape and form interconnected network in healthy mitochondria. Conversely, a low ratio of fusion to fission creates fragmented spherical mitochondria. Coculture with MSCs increased the numbers of mitochondrial network compared with OGD. MSCs also rescued the overall mitochondria that exist outside of network (individuals) with less spherical shape (lower circularity). Our results concur with previous observations that OGD altered the mitochondrial dynamics by upregulating fission protein Drp1 and downregulating fusion protein Mfn2.[49–52] We observed that MSCs significantly restored Mfn2 but not Drp1 expression level. Finally, using JC-1 mitochondrial membrane dye and live cell imaging, we are the first to show that MSCs transferred functional mitochondria to retinal cells and attenuated mitochondrial membrane depolarization caused by OGD.

Visual impairment is a common and significant symptom in stroke patients.[7–9] Because of the anatomic juxtaposition of the ophthalmic artery to the MCA, blood flow to the ophthalmic artery is easily hindered in the event of MCAO, causing retinal ischemia,[53] which is a major predisposing factor of visual impairment and shares a pathology with other common ocular vascular diseases.[15–21] Time is of the essence for cerebral and retinal ischemia with early detection and intervention likely to improve outcomes.[54–57] Because of the unique dosing of vasculatures and collaterals in the brain and the retina, their discordant reperfusion profiles may affect the stem cell distribution or mitochondrial transfer in these tissues. Despite the lack of collaterals, retinal cells exhibit resistance to ischemic insults.[18,20] Indeed, clinical studies suggest that the effective time window for central retinal artery occlusion with intravenous tPA is 6 to 6.5 hours.[20,58,59]

Here, we provided evidence that stem cell transplantation afforded functional benefits against cerebral[60–63] and retinal ischemia[53,64–67] by abrogating mitochondrial dysfunction, in part, by stem cell-mediated mitochondrial transfer. However, other well-known mechanisms mediating stem cell therapy, such as the bystander effects[68–70] stand as equally potent cell survival pathways. Mitochondrial transfer may occur via tunneling nanotubes, extracellular vesicles, gap junctions, and cell fusion.[37,71–75] Ischemic cells release help me signals which could be used to guide the migration of stem cells and their mitochondria to reach ischemic regions.[37,76] Optimizing the routes of delivery and the timing of transplantation as stand alone or in combination with tPA may improve functional outcomes of mitochondria-based stem cell therapy for retinal ischemia. Diagnosis of stroke warrants examination of retinal ischemia, with ample consideration for treating visual impairments.

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