# Original Research

# SIRT3 deficiency increases mitochondrial oxidative stress and promotes migration of retinal pigment epithelial cells

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**Impact statement**

Sirtuin-3 (SIRT3) deficiency contributes to epithelial–mesenchymal transition and migration of retinal pigment epithelial cells (RPE) caused by high glucose and platelet-derived growth factor (PDGF) and increases mitochondrial reactive oxygen species (ROS) levels in RPE cells. Increasing SIRT3 expression or decreasing the mitochondrial ROS level improves RPE cell function in response to challenge with high glucose or PDGF. These findings indicate the important role of SIRT3 in regulating RPE cell function and provide further informative evidence for diabetes-related RPE cell dysfunction therapies by potentially targeting at SIRT3 deficiency or mitochondrial ROS production.

## Abstract

Retinal pigment epithelial cells are closely associated with the pathogenesis of diabetic retinopathy. The mechanism by which diabetes impacts retinal pigment epithelial cell function is of significant interest. Sirtuins are an important class of proteins that primarily possess nicotinamide adenine dinucleotide-dependent deacetylases activity and involved in various cellular physiological and pathological processes. Here, we aimed to examine the role of sirtuins in the induction of diabetes-associated retinal pigment epithelial cell dysfunction. High glucose and platelet-derived growth factor (PDGF) treatment induced epithelial–mesenchymal transition and the migration of retinal pigment epithelial cells, and decreased sirtuin-3 expression. Sirtuin-3 knockdown using siRNA increased epithelial–mesenchymal transition and migration of retinal pigment epithelial cells. In contrast, sirtuin-3 overexpression attenuated the effects caused by high glucose and PDGF on epithelial–mesenchymal transition and migration of retinal pigment epithelial cells, suggesting that sirtuin-3 deficiency contributed to retinal pigment epithelial cell dysfunction induced by high glucose and PDGF. Mechanistically, sirtuin-3 deficiency induced retinal pigment epithelial cell dysfunction by the overproduction of mitochondrial reactive oxygen species. These results suggest that sirtuin-3 deficiency mediates the migration of retinal pigment epithelial cells, at least partially by increasing mitochondrial oxidative stress, and shed light on the importance of sirtuin-3 and mitochondrial reactive oxygen species as potential targets in diabetic retinopathy therapy.

**Keywords:** Retinal pigment epithelial cells, sirtuin-3, migration, high glucose, mitochondrial reactive oxygen species

***Experimental Biology and Medicine* 2021; 246: 877–887. DOI: 10.1177/1535370220976073**

## Introduction

Among the microvascular complications of diabetes, diabetic retinopathy (DR) is one of the most common and a primary contributor to vision loss. A monolayer of pigmented cells is formed between the choroid and neuroretina by retinal pigment epithelial (RPE) cells, which are reported to closely involve in DR pathogenesis.[1,2] RPE cells play critical roles in multiple processes, including the defense mechanism of the macula immunity, photoreceptor outer segments phagocytosis, ocular angiogenic balance maintenance, antioxidative response, photoreceptors renewal, and protection of photoreceptor phototransduction.[3–5] Dysfunction of RPE cell is linked to major ocular clinical changes such as the degeneration of retinal and unalterable vision damage.[4,6]

Recently, the effects of diabetes on RPE cell function have received much attention. Investigations have explored the effect of high glucose, a key underlying factor in initiating and progressing the major complications of diabetes, including DR, on RPE cell function.[7–11] As a significant energy source for retinal metabolism, glucose is transported largely via RPE cells,[12–14] which provide 60%–80% of retinal glucose through their high-capacity distribution system.[15] Meanwhile, RPE cells are highly susceptible to high concentrations of glucose, and how hyperglycemia

impacts RPE cell function remains a question of great interest. High glucose induces epithelial–mesenchymal transition (EMT) and migration of, oxidative stress in, and expression of fibrogenic factors by RPE cells.[7,8] However, the mechanism underlying high glucose-induced RPE cell dysfunction remains largely unknown.

Sirtuins, a class of proteins that predominantly possess nicotinamide adenine dinucleotide ($NAD^+$)-dependent deacetylases activity, are involved in multiple cellular physiological and pathological processes such as cell cycle, apoptosis, metabolism, redox balance, and mitochondrial function.[16] Recently, sirtuins such as SIRT1 and SIRT3 were reported to affect the regulation of RPE cell function.[17,18] In the present study, we attempted to investigate the function of sirtuins in the regulation of RPE cell migration and found that SIRT3 deficiency increased mitochondrial oxidative stress and mediated the migration of RPE cells.

## Materials and methods

### Culture of human RPE cells

The human RPE cell line ARPE-19 (American Type Culture Collection, Manassas, VA, USA) was obtained and maintained as depicted previously.[8] Briefly, cells were cultured using Dulbecco's modified Eagle's medium with 10% fetal bovine serum and 100 U/mL penicillin/streptomycin (Invitrogen, Carlsbad, CA, USA) in an incubator (37°C with 5% $CO_2$). On attaining 80%–90% confluency, ARPE-19 cells were detached, passaged, and seeded in six-well plates (Falcon) with a plating density of $3 \times 10^5$ cells per well.

### Treatment of RPE cells

For treatment with glucose, ARPE-19 cells were exposed to 5 mM (mormal), and 15 or 25 mM (high) of D-glucose for 24 h. Mannitol (TargetMol, Boston, MA, USA) was used as an osmotic control compound. For the treatment with platelet-derived growth factor (PDGF; SignalChem, Richmond, BC, Canada), ARPE-19 cells were pretreated with 20 ng/mL PDGF-BB (Selleck Chemicals, Houston, TX, USA) for 1 h. RPE cells were treated with SS31 (50 $\mu$M) (GenePharma, Shanghai, China) for 24 h for high-glucose treatment or MitoTEMPO (1 $\mu$M) (Sigma-Aldrich, St. Louis, MO, USA) for 1 h for PDGF treatment. Because high glucose and PDGF-BB showed maximum effects on the promotion of EMT at 24 h and 1 h, respectively (Figure S1), these time points were selected for subsequent experiments.

### Transwell assay

Modified Corning chambers (Corning Inc., Corning, NY, USA) were used to conduct a transwell assay. After 48 h incubation at 37°C, RPE cells in the upper chamber were allowed to migrate, as reported previously,[1,7,8] and the migrated RPE cells in the lower chamber were quantified under a microscope.

### Scratch wound assay

Cells ($10^6$) were seeded in 60-mm culture dishes and reached confluency after two to three days. A wound was introduced into the monolayer with a 1000 $\mu$L micropipette tip. The cells were rinsed twice with growth medium to remove detached cells and incubated with growth medium containing 1 $\mu$M 5-fluorouracil (Sigma) to avoid cell proliferation. Wound closure was examined by phase-contrast microscopy at various time points (0 and 48 h), as reported previously.[1,7,8] Quantitative data were obtained from the measurements of migrated distance as a percentage of total distance.

### Detection of mitochondrial reactive oxygen species

Mitochondrial ROS were measured using an assay kit (Cayman Chemical, Ann Arbor, MI, USA) according to the manufacturer's instructions. A mitochondrial ROS detection reagent (MitoSOX, a mitochondria-targeted ROS dye) was used to measure the level of mitochondrial ROS. A fluorescent plate reader (FLUOstar Omega) was used to measure the fluorescence intensity (excitation, 480 nm; emission, 560–600 nm). A higher fluorescence intensity represents a higher level of mitochondrial ROS.

### Western blot analysis

RPE cells were harvested, washed with phosphate buffer solution, and lysed with ice-cold RIPA buffer containing a protease inhibitor cocktail. Total proteins were obtained and separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis, followed by transferring to polyvinylidene difluoride membranes. Immunoblots were probed with anti-E-cadherin (1:500, ab40772), anti-vimentin (1:500, ab8978), anti-matrix metalloproteinase-2 (MMP2, 1:1000; ab92536), anti-MMP9 (1:1000; ab137867), anti-SIRT1 (ab32441), anti-SIRT2 (ab51023), anti-SIRT3 (ab86671), anti-SIRT5 (ab105040), anti-superoxide dismutase 2 (SOD2, 1:2000, ab13533), anti-Ac-SOD2 (1:1000, ab137037), anti-p38 (ab31828), anti-p-p38 (ab47363), anti-extracellular-related kinase (ERK) (ab32537), anti-p-ERK (ab131438), or anti-$\beta$-actin (ab8227) overnight at 4°C and followed by 1 h incubation with the corresponding secondary antibodies at room temperature. All antibodies were purchased from Abcam (Cambridge, UK). The blots were visualized using ECL-plus reagent. Densitometry analysis was done by using ImageJ software (NIH, Bethesda, MD, USA).

### siRNA transfection and infection

Transfections were performed with Lipofectamine 2000 (Invitrogen) according to the manufacturers' protocol, where two candidate small interfering RNAs (siRNAs) that specifically target at SIRT3 mRNA and their scrambled RNA (GenePharma) selected. The siRNA sequences were as follows: human SIRT3: 1. sense 5′-CCAGCAUGAAA UACAUUUATT-3′, anti-sense 5′-UAAAUGUAUUUCAU GCUGGTT-3′; 2. sense 5′-CCAGUGGCAUUCCAGAC UUTT-3′, anti-sense 5′-AAGUCUGGAAUGCCACUGG TT-3′; scrambled siRNA: sense 5′-UUCUCCGAACGUGU

CACGUTT-3′, anti-sense 5′-ACGUGACACGUUCGGAGA ATT-3′. The transfected cells were used in subsequent testing after 48 h culture. SIRT3 adenovirus was used to overexpress SIRT3 in RPE cells.

### Statistical analysis

Data generated in this study are presented as means ± standard error of the means. The Kolmogorov–Smirnov normality test confirmed that all datasets were normally distributed. Data were compared using one-way analysis of variance (ANOVA) or two-way ANOVA, followed by an unpaired *t*-test, as appropriate. Bonferroni's correction for multiple comparisons was applied. Differences were considered statistically significant when *P*-values are less than 0.05.

## Results

### SIRT3 deficiency was associated with increased migration of RPE cells

Tight junctions with normal E-cadherin expression on undifferentiated RPE cells are shown in Figure S2. High concentrations of glucose can induce migration of RPE cells, contributing to DR pathogenesis and macular edema.[7,8] In this study, treatment with high glucose promoted the migration of RPE cells with dose-dependent response, as evidenced by scratch and Transwell assays (Figure 1(a)). Treatment with high glucose also suppressed the expression of E-cadherin and enhanced the expression of vimentin, which is an epithelial and mesenchymal marker in RPE cells, respectively (Figure 1(b)). Expression of MMP2 and MMP9, which support cells to degrade extracellular matrix and migrate,[19] was also increased after treatment with high glucose (Figure 1(b)). These effects were further confirmed by changes in cell morphology, i.e. cell–cell contacts and cobblestone morphology disappearance and presence of elongated mesenchymal features (Figure 1(c)).

PDGF exhibits a chemotactic effect on RPE cell migration and has been used to induce RPE cell migration.[1] Among the isoforms of PDGF, PDGF-B shows significant effects on RPE cell migration.[20] Similarly, in this study, treatment of RPE cells with 20 ng/mL PDGF-BB, a homodimer of PDGF-B, enhanced the migration of RPE cells, as indicated by scratch and Transwell assays (Figure 1(d)). Meanwhile, PDGF-BB treatment decreased E-cadherin levels; increased expression of vimentin, MMP2, and MMP9 levels; and changed the cell morphology from cobblestone to elongated mesenchymal features (Figure 1(e) and (f)). These results indicated that treatment with high glucose and PDEF-BB increased EMT and migration of RPE cells.

To investigate the function of sirtuins in the induction of migration, we probed RPE cells for the major isoforms of the sirtuin family members, including SIRT1, SIRT2, SIRT3, and SIRT5. As shown in Figure 1(g), treatment with high glucose decreased SIRT1 and SIRT3 expression in a dose-dependent manner, whereas SIRT2 and SIRT5 expression were unchanged. Treatment with PDGF-BB only decreased SIRT3 expression in RPE cells and had no significant effect on the expressions of SIRT1, SIRT2, and SIRT5 (Figure 1(h)). SIRT3, a mitochondrial $NAD^+$-dependent deacetylase that regulates mitochondrial metabolism and ROS homeostasis, is actively associated with the regulation of mitochondrial function.[21,22] These results indicated that SIRT3 deficiency could be linked to the migration of RPE cells.

### SIRT3 knockdown increased the migration of RPE cells

To examine whether SIRT3 deficiency promotes to the migration of RPE cells, we knocked down SIRT3 in these cells using siRNA. SIRT3 knockdown decreased SIRT3 expression in RPE cells (Figure 2(a)) and increased the migration of these cells, as indicated by scratch and Transwell assays (Figure 2(b) and (c)). Meanwhile, SIRT3 knockdown decreased E-cadherin and increased vimentin, MMP2, and MMP9 expressions (Figure 2(d)). Furthermore, SIRT3 knockdown changed the cell morphology from cobblestone to elongated mesenchymal features (Figure 2(e)). Thus, SIRT3 deficiency increased EMT and migration of RPE cells.

### SIRT3 overexpression attenuated the effects of high glucose- and PDGF-induced migration

To test whether treatment with high glucose and PDGF-BB increased EMT and migration of RPE cells following SIRT3 deficiency, SIRT3 was overexpressed using an adenovirus vector. SIRT3 overexpression increased SIRT3 expression in RPE cells (Figure 3(a)). SIRT3 overexpression did not significantly affect EMT and migration of RPE cells in control group but attenuated the effects of high glucose and PDGF-BB on EMT and migration of RPE cells (Figure 3(b) and (c)). These results indicate that SIRT3 deficiency contributes to the pro-migration effects of high glucose and PDGF.

### SIRT3 deficiency induced mitochondrial ROS generation

With the mitochondrial $NAD^+$-dependent deacetylase activity that regulates the metabolism of mitochondrial and ROS balance, SIRT3 is reported to mediate in the regulation of mitochondrial function in metabolic disorders.[21,22] Thus, we determined whether SIRT3 deficiency enhanced the migration of RPE cells via the increase of mitochondrial ROS levels. As expected, treatment with both high glucose and PDGF-BB increased mitochondrial ROS levels as assessed by MitoSOX, an indicator for mitochondrial ROS (Figure 4(a)). SIRT3 overexpression decreased mitochondrial ROS in RPE cells in response to treatment with both high glucose and PDGF-BB (Figure 4 (a)). In addition, SIRT3 knockdown increased mitochondrial ROS in RPE cells (Figure 4(b)).

Increased mitochondrial ROS induced by SIRT3 deficiency has been associated with acetylated SOD2, the primary mitochondrial superoxide scavenging enzyme. Thus, we also detected SOD2 acetylation in RPE cells. Treatment with high glucose and PDGF-BB increased SOD2 acetylation in RPE cells (Figure 4(c) and (d)). In addition, SIRT3 knockdown increased SOD2 acetylation in RPE cells (Figure 4(e)). These results indicate that SIRT3 deficiency

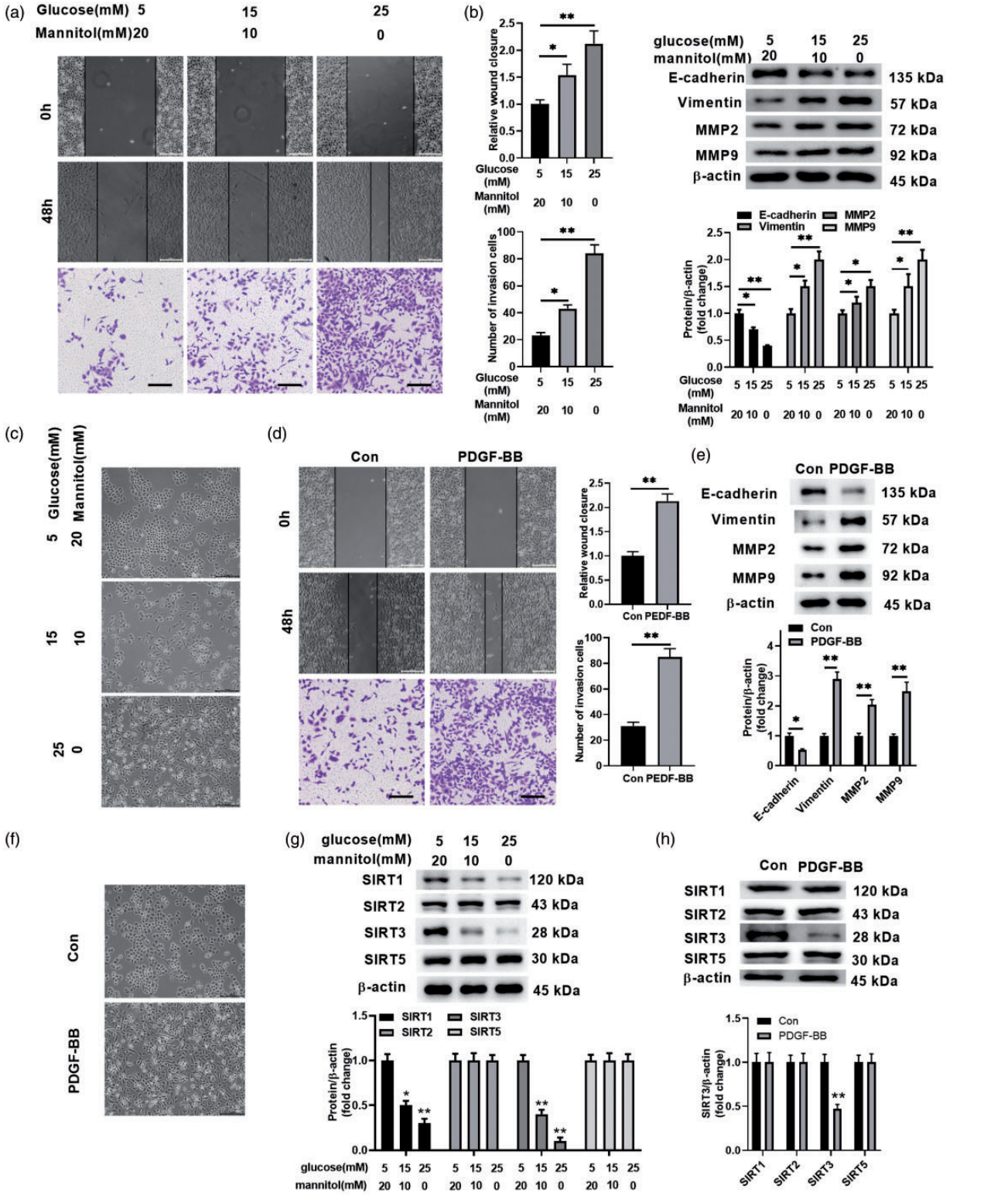


**Figure 1.** SIRT3 deficiency was associated with increased migration of retinal pigment epithelial cells. (a) High glucose (15 and 25 mM) treatments (24 h) increased migration of RPE cells in a dose-dependent manner, as evidenced by scratch and Transwell assays (left panel). Quantified results are shown in the right panel. (b) Treatment with high glucose increased EMT of RPE cells, as detected by the expression of four proteins by WB (upper panel); quantified results are shown (lower panel). (c) Morphology of RPE cells. (d) Treatment with PDGF-BB (20 ng/mL for 1 h) enhanced the migration of RPE cells, as indicated by scratch and Transwell assays (left panel). Quantified results are shown in the right panel. (e) Treatment with PDGF-BB increased EMT of RPE cells. WB results are shown in the upper panel, and the quantified results are shown in the lower panel. (f) Morphology of RPE cells. (g) Treatment with high glucose decreased SIRT1 and SIRT3 expression in RPE cells in a dose-dependent manner. WB results are shown in the upper panel, and the quantified results are shown in the lower panel. (h) Treatment with PDGF-BB decreased SIRT3 expression in RPE cells. WB results are shown in the upper panel, and the quantified results are shown in the lower panel. Each experiment was repeated six times within one month. $^{*}P < 0.05$; $^{**}P < 0.01$. (A color version of this figure is available in the online journal.)

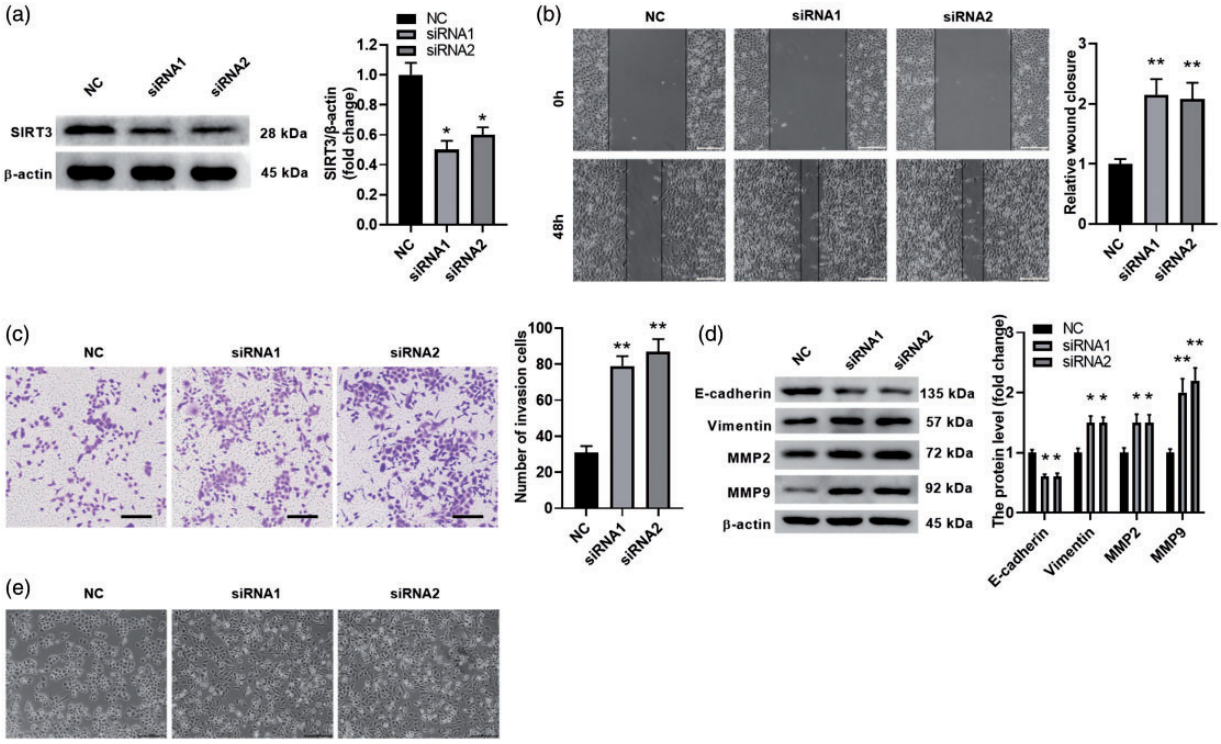


**Figure 2.** SIRT3 knockdown increased the migration of retinal pigment epithelial cells. (a) SIRT3 knockdown decreased SIRT3 expression in RPE cells. WB results are shown in the upper panel, and the quantified results are shown in the lower panel. (b–c) SIRT3 knockdown increased the migration of RPE cells, as indicated by scratch (b) and Transwell (c) assays (left panel), and the quantified results are shown in the right panel. (d) SIRT3 knockdown decreased E-cadherin and increased vimentin, MMP2, and MMP9 expression. WB results are shown in the upper panel, and the quantified results are shown in the lower panel. (e) Morphology of RPE cells. Each experiment was repeated six times within one month. $^{*}P < 0.05$. $^{**}P < 0.01$. (A color version of this figure is available in the online journal.)

may induce migration of RPE cells via the enhancement of mitochondrial oxidative stress.

### Mitochondrial ROS scavenging attenuated SIRT3 deficiency-induced migration

To examine the effects of mitochondrial ROS on RPE cell migration, mitochondrial ROS was scavenged by either SS31 or MitoTEMPO, mitochondrial-targeted ROS scavengers. Indeed, treatment with SS31 or MitoTEMPO attenuated mitochondrial ROS levels (Figure 5(a)) and impaired the effects of high glucose and PDGF-BB on EMT and migration of RPE cells (Figure 5(b) and (c)). However, they had little effect on SIRT3 expression and SOD2 acetylation (Figure 6(a)). Treatment with SS31 and MitoTEMPO also attenuated the effects of SIRT3 knockdown on RPE cell migration (Figure 6(b)), increased the expression of E-cadherin, and decreased the expression of vimentin, MMP2, and MMP9 in RPE cells with SIRT3 knockdown (Figure 6 (c)). These results suggest that SIRT3 deficiency promoted migration of RPE cells by increasing mitochondrial ROS levels.

### SIRT3 deficiency activated mitogen-activated protein kinase signaling

MAPK signaling, an upstream signal regulator of the migration of RPE cells, can be activated by mitochondrial ROS. Thus, we next tested whether SIRT3 deficiency-induced RPE cell migration is associated with MAPK signaling. High glucose, PDGF-BB, and SIRT3 knockdown activated MAPK signaling, as evidenced by increased phosphorylation of p38 and ERK1/2 (Figure 7(a) and (b)). Moreover, the scavenging of mitochondrial ROS by SS31 or MitoTEMPO attenuated the activation of MAPK signaling, and it was confirmed by the decreased phosphorylation of p38 and ERK1/2 (Figure 7(a) and (b)). These results suggest that SIRT3 deficiency activated MAPK signaling via the enhancement of mitochondrial ROS levels, thereby contributing to EMT and migration of RPE cells.

## Discussion

RPE cells are closely involved in the pathogenesis of DR; however, the effects of diabetes on RPE cell function and its underlying mechanism are yet to be unveiled. Here, we found that treatment with both high glucose and PDGF induced EMT and migration of RPE cells and decreased SIRT3 expression. SIRT3 knockdown further increased EMT and migration of RPE cells, whereas SIRT3 overexpression attenuated the effects of EMT and migration of RPE cells induced by high glucose and PDGF, suggesting that SIRT3 deficiency contributes to high glucose- and PDGF-induced RPE cell dysfunction. These results indicate that SIRT3 can be a potential target in the treatment of DR.

As a major complication of diabetes, macular edema is induced by a compromised outer retinal barrier, which is constructed by RPE cells by forming specialized junctions. This barrier regulates the crosstalk of materials between the outer retina and choriocapillaris. How diabetes contributes

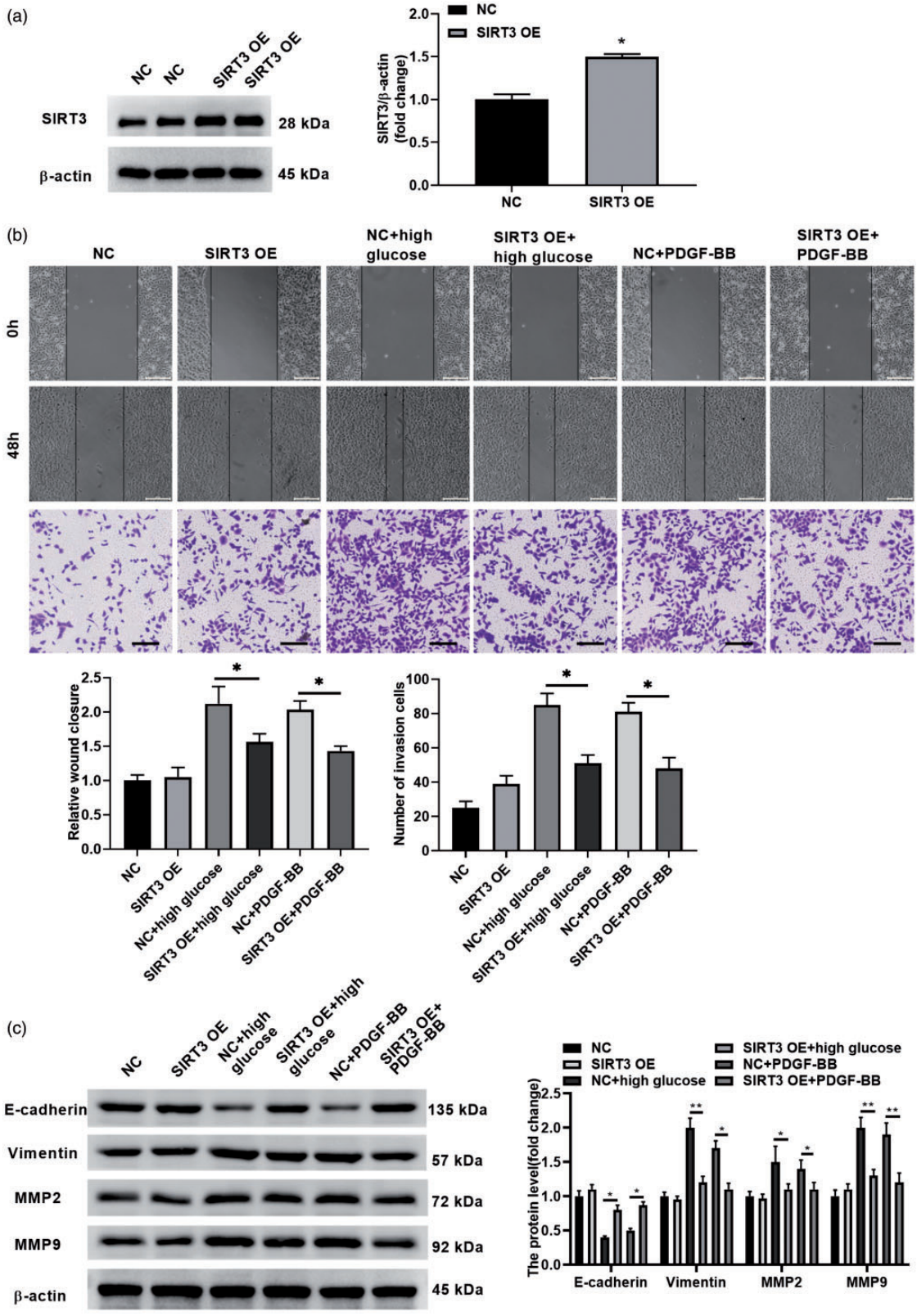


**Figure 3.** SIRT3 overexpression attenuated the effects of RPE migration induced by high glucose and PDGF treatment. (a) SIRT3 overexpression increased SIRT3 expression in RPE cells. WB results are shown in the upper panel, and the quantified results are shown in the lower panel. (b) SIRT3 overexpression attenuated the effects of high glucose and PDGF-BB on the results of scratch and Transwell assays of RPE cells. Representative images of scratch and Transwell results are shown in the upper panel, and the quantified results are shown in the lower panel (b). (c) WB results of EMT-related proteins. The quantified results are shown in the right panel. Each experiment was repeated six times within one month. $^{*}P < 0.05$; $^{**}P < 0.01$. (A color version of this figure is available in the online journal.)

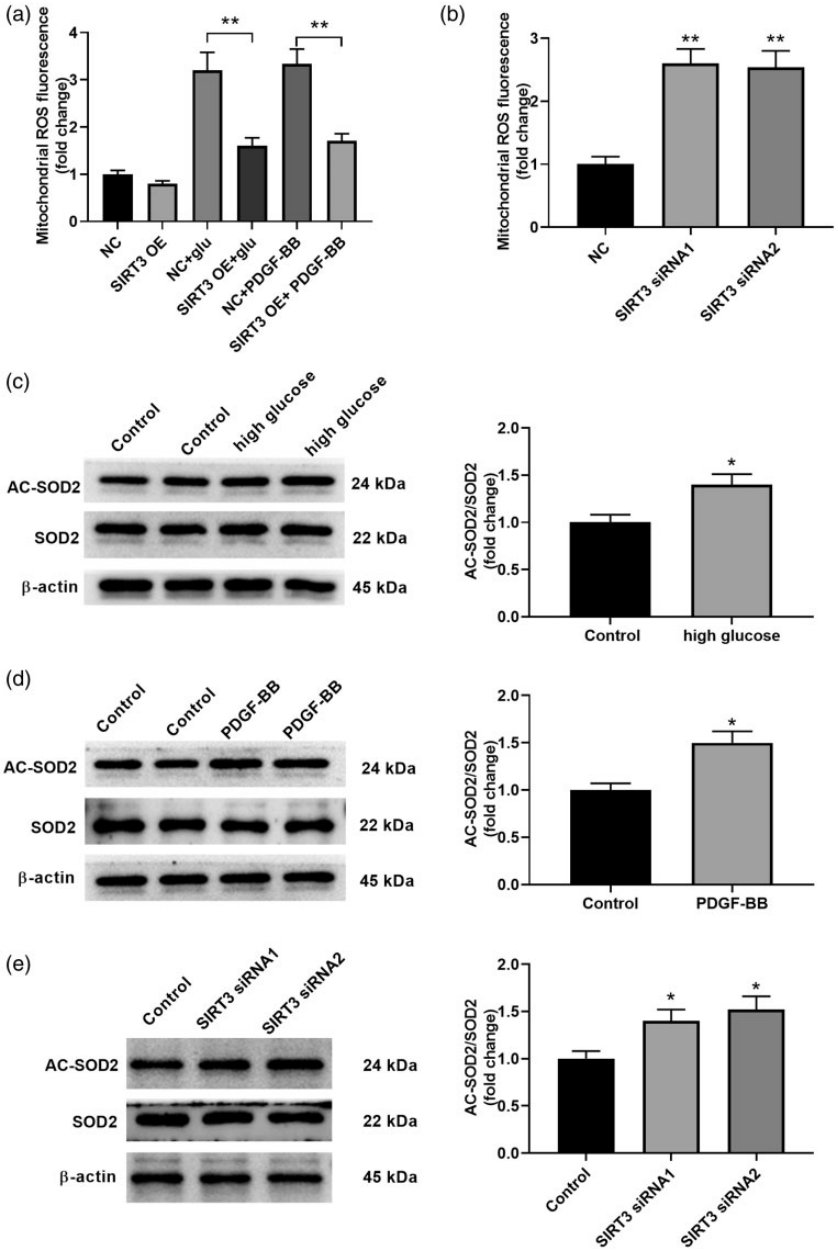


**Figure 4.** Deficiency of SIRT3 induced mitochondrial generation of ROS. (a) SIRT3 overexpression decreased mitochondrial ROS in RPE cells in response to treatment with high glucose and PDGF-BB. (b) SIRT3 knockdown decreased mitochondrial ROS in RPE cells. (c–d) High glucose (c) and PDGF-BB (d) treatments increased SOD2 acetylation in RPE cells. WB results are shown in the left panel, and the quantified results are shown in the right panel. (e) SIRT3 knockdown increased SOD2 acetylation in RPE cells. WB results are shown in the left panel, and the quantified results are shown in the right panel. Each experiment was repeated six times within one month. $^{*}P < 0.05$; $^{**}P < 0.01$.

to the barrier dysfunction and to be exact, how RPE cells contribute to DR, requires further elucidation. High glucose induces EMT, migration, oxidative stress, and apoptosis of RPE cells.[7,8,23–25] Additionally, PDGF is a key factor in angiogenesis, which stimulates the migration of various types of cells, including RPE cells.[26–28] PDGF signaling has been reported to be enhanced in diabetes.[29,30] Here we used both high glucose and PDGF to induce RPE cell dysfunction and found that both induced EMT and migration of RPE cells following SIRT3 knockdown. These results suggest a common mechanism for both high glucose- and PDGF-induced RPE cell dysfunction.

Sirtuins are associated with multiple cellular physiological and pathological processes.[16,31,32] SIRT3 is one of the most extensively studied sirtuin, and SIRT3 deficiency has been reported to involve in various diseases occurrence, including metabolic syndrome, pulmonary arterial hypertension, and aging.[33–35] Several studies have indicated that sirtuins, including SIRT1 and SIRT3, may affect the induction of RPE cell dysfunction.[36,37] However, the role of sirtuins in RPE cells has not been well investigated. We identified the major isoforms of sirtuins in RPE cells, including SIRT1, SIRT2, SIRT3, and SIRT5. Treatment with high glucose in RPE cells decreased SIRT1 and SIRT3

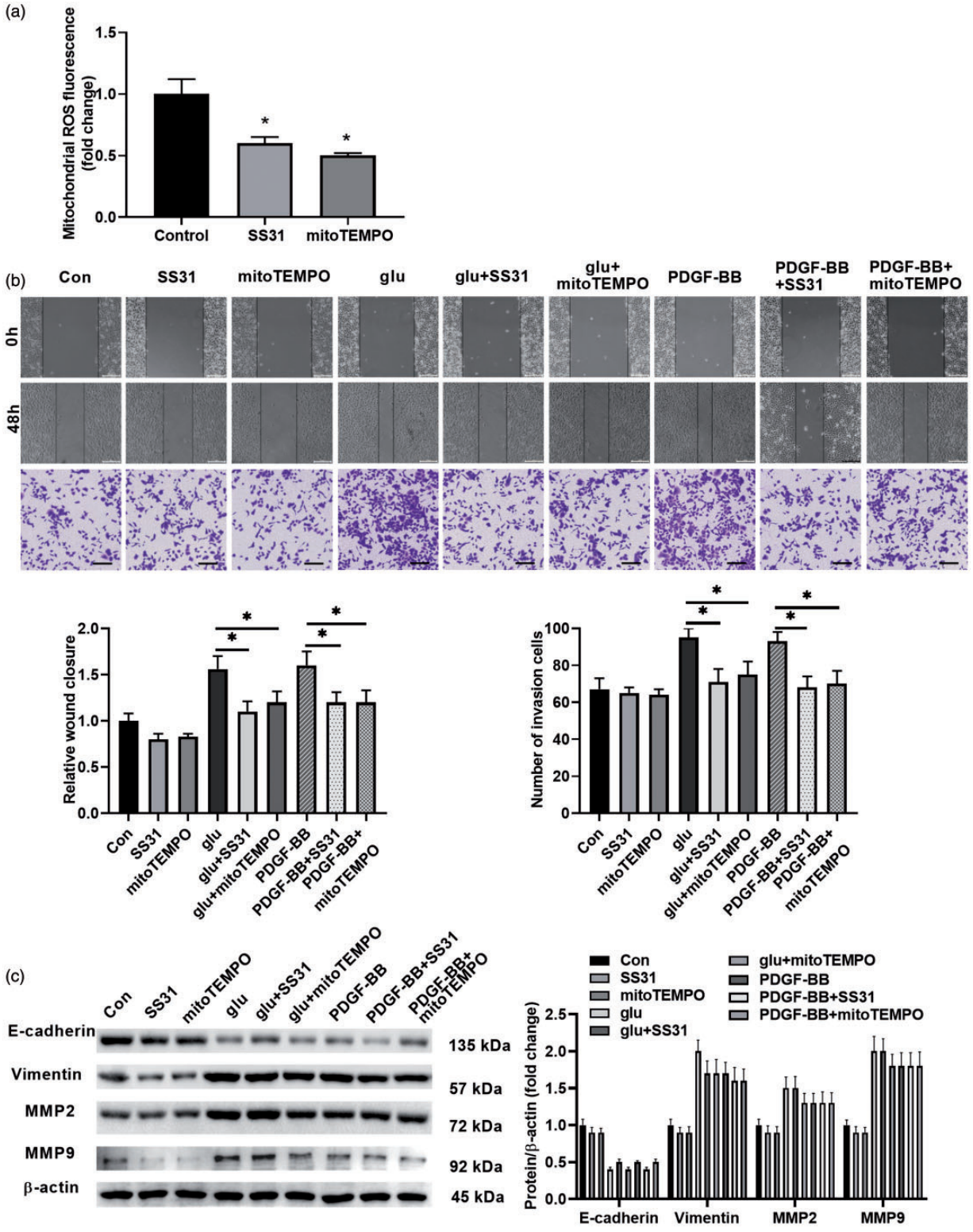


**Figure 5.** ROS scavenging by SS31 and MitoTEMPO attenuated RPE cell migration and EMT induced by high glucose and PDGF-BB. (a) Treatment of RPE cells with SS31 (50 $\mu$M) or MitoTEMPO (1 $\mu$M) attenuated mitochondrial ROS levels. (b) SS31 and MitoTEMPO treatments impaired the effects of high glucose and PDGF-BB treatments on scratch and Transwell of RPE cells. Scratch and Transwell assay results are shown in the upper panel, and the quantified results are shown in the lower panel. (c) WB results are shown in left, and the quantified results are shown in right. (A color version of this figure is available in the online journal.)

expression, whereas treatment with PDGF-BB decreased only SIRT3 expression. Importantly, SIRT3 knockdown mimicked the effects of high glucose or PDGF in RPE cells, whereas SIRT3 overexpression attenuated the effects of both high glucose and PDGF-induced EMT and migration of RPE cells. Thus, SIRT3 deficiency may be a common causal factor in the induction of EMT and migration of RPE cells.

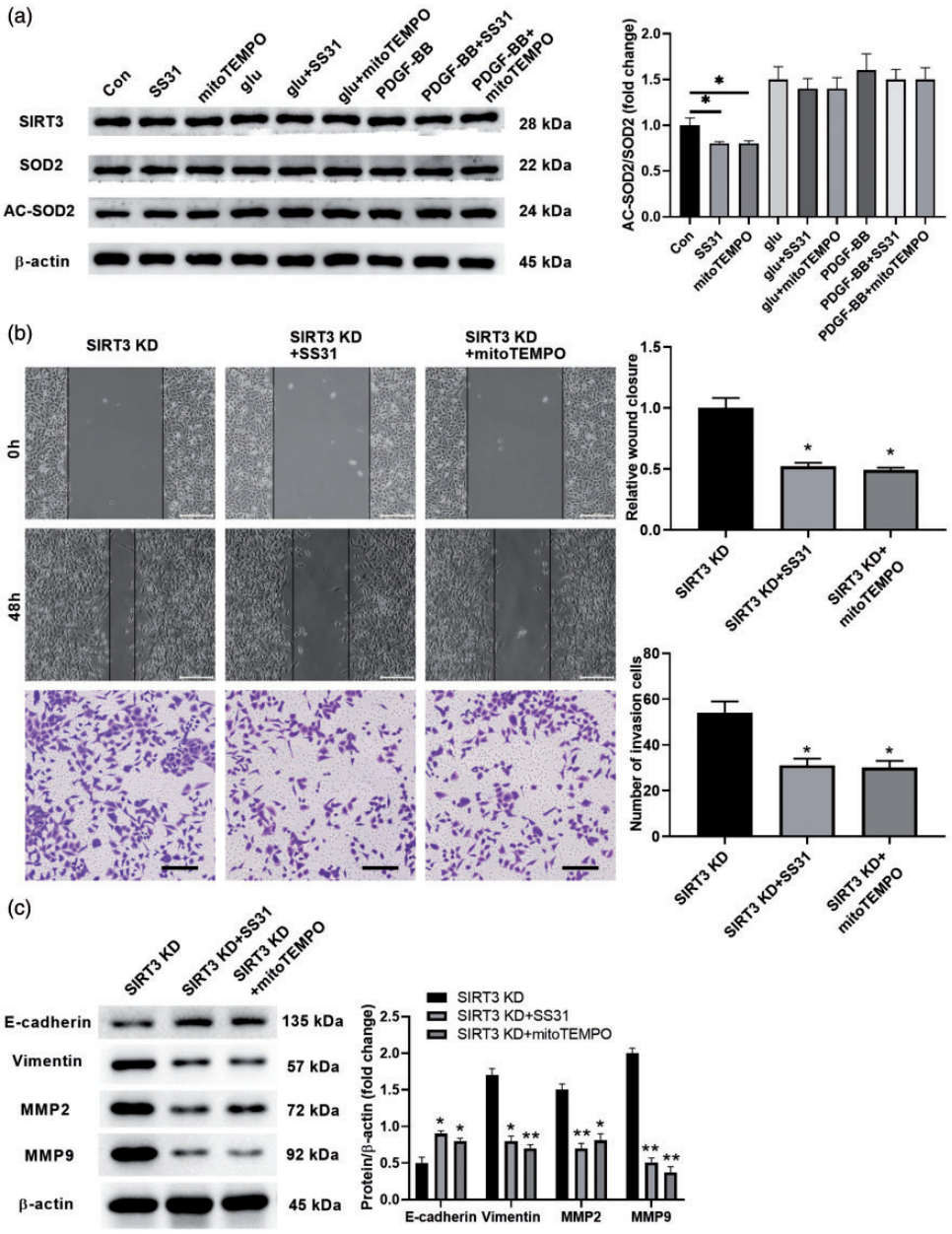


**Figure 6.** SS31 and MitoTEMPO attenuated SIRT3 knockdown on migration and EMT of RPE cells. (a) Treatment with SS31 and MitoTEMPO showed little effects on SIRT3 expression and SOD2 acetylation. WB results are shown in left, and the quantified results are shown in right. (b) SS31 and MitoTEMPO also attenuated the effects of SIRT3 knockdown on scratch and Transwell assays of RPE cells. Typical results (left) and their quantifications (right) are shown for each panel. (c) WB results are shown in left, and the quantified results are shown in right. Each experiment was repeated six times within one month. $^{*}P < 0.05$, $^{**}P < 0.01$. (A color version of this figure is available in the online journal.)

SIRT3 is a mitochondrial $NAD^{+}$-dependent deacetylase that regulates mitochondrial metabolism and ROS homeostasis, indicating that mitochondrial function may be related to the regulation of RPE cell function corresponding to treatment with high glucose and PDGF. Increasing evidence shows that oxidative stress affects initiating and progressing the cell dysfunction, which further contributes to diseases such as hyperlipidemia, diabetes mellitus, and hypertension and that SIRT3 deficiency is associated with excessive mitochondrial ROS levels.[38,39] In the present study, SIRT3 deficiency was linked to an increase in mitochondrial ROS levels. Inhibition of mitochondrial ROS using mitochondria-targeted ROS scavengers SS31 and MitoTEMPO decreased mitochondrial ROS levels and ameliorated RPE cell dysfunction induced by high glucose and PDGF. These results suggest that SIRT3 deficiency induces RPE cell dysfunction by increasing mitochondrial ROS levels. In addition, SIRT3 deficiency-increased mitochondrial ROS levels were associated with an increase in SOD2 acetylation in RPE cells. Thus, mitochondrial ROS may activate MAPK signaling, which induces EMT and migration of RPE cells.

Collectively, we found that SIRT3 deficiency contributes to high glucose- and PDGF-induced EMT and migration of RPE cells and increases mitochondrial ROS levels in

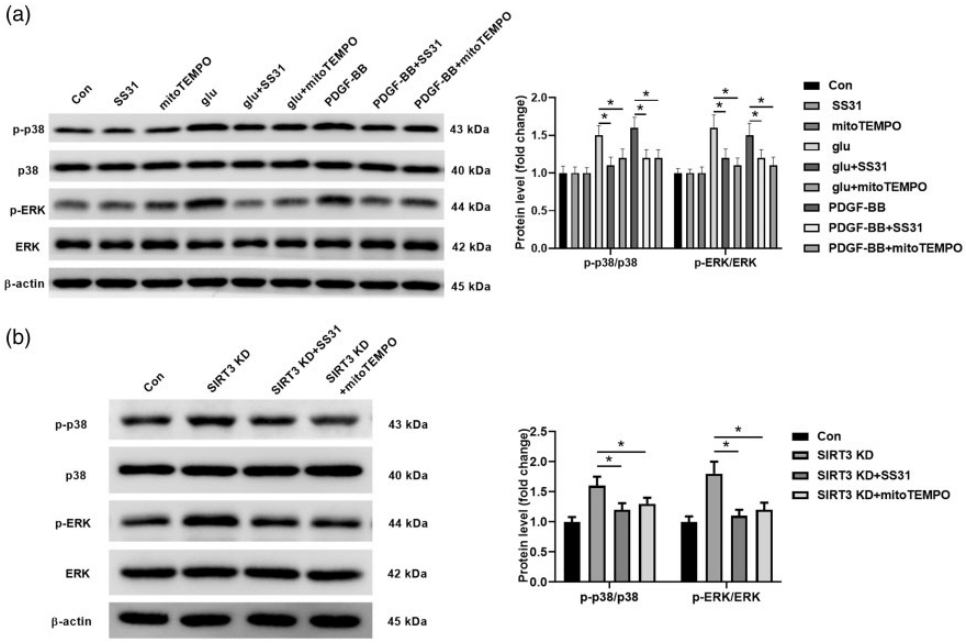


**Figure 7.** SIRT3 deficiency activated MAPK signaling. WB detects the activation of key proteins in MAPK signaling under high glucose or PDGF-BB with SS31 treatment (a) or SIRT3 knockdown (b). WB results are shown in left, and the quantified results are shown in right. Each experiment was repeated six times within one month. $^{*}P < 0.05$.

RPE cells. Increasing SIRT3 expression or decreasing mitochondrial ROS levels ameliorates RPE cell function in response to challenge with high glucose or PDGF. These results indicate an innovative role of SIRT3 in regulating RPE cell function and provide further informative evidence for diabetes-related RPE cell dysfunction therapies by potentially targeting at SIRT3 deficiency or mitochondrial ROS.

## AUTHORS' CONTRIBUTIONS

WJX conceived and designed the experiments; YY performed the experiments; LWY contributed reagents/materials/analysis tools. WJX wrote the manuscript. All authors read and approved the final manuscript.

## DECLARATION OF CONFLICTING INTERESTS

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

## FUNDING

The author(s) received no financial support for the research, authorship, and/or publication of this article.

## DATA AVAILABILITY

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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*(Received November 10, 2019, Accepted November 3, 2020)*