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Intravenous delivery of mesenchymal stromal cells reverses Müller cell endoplasmic reticulum stress in diabetic retinopathy

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Abstract

Background Mesenchymal stromal cells (MSCs) have emerged as a promising disease-modifying therapy for the complications of diabetes mellitus (DM), including diabetic retinopathy (DR). However, the optimal treatment regimen remains unclear, and challenges persist regarding the timing, route of delivery and the mechanisms underlying the therapeutic effects. This study focused on human umbilical cord-derived mesenchymal stromal cells (hUC-MSCs), to elucidate their retinal protective effects, and investigate the underlying mechanisms by which a single intravenous injection might ameliorate the pathological alterations of DR.

Methods Two time points after the development of DM were chosen for the *in vivo* experiments to study the effects of the intervention after different times of exposure to hyperglycemia. hUC-MSCs were injected via the tail vein at 8 and 16 weeks after STZ injection. Retinal samples were collected 2 weeks post-treatment to analyze the therapeutic effect of MSCs on DR. *In vitro* experiments were conducted using a Müller cell line and a retinal microvascular endothelial cell line cultured under high-glucose conditions, with treatment by hUC-MSCs conditioned media (MSC-CM), to explore the underlying mechanisms.

Results After a single intravenous injection of hUC-MSCs at week 16 and not 8 weeks post-STZ injection, retinal tissue showed improved thickness of the inner nuclear layer. There was also an increase in the number of acellular capillaries observed in retinal flat mounts of diabetic animals which was improved in the DM and MSC treatment group. MSC treatment reduced high glucose induced activation markers (GFAP and Vimentin) of Müller cells and alleviated endoplasmic reticulum (ER) stress. VEGF expression was also reduced in the retina. MSC-conditioned media also reversed high glucose-induced expression of VEGF in Müller cells. Finally, in a retinal microvascular endothelial cell line, high glucose concentrations, demonstrated increased ER stress which was reduced by MSC conditioned media.

Conclusions Single Intravenous injection of hUC-MSC to DM animals could alleviate DR via reducing Müller cell and endothelial cell activation and ER stress, and thus might represent a promising therapy for DR.

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Keywords Diabetic retinopathy, Human umbilical cord mesenchymal stromal cells, Müller cells, Endoplasmic reticulum stress

Background

According to the “Global Diabetes Map (10th edition)” released by the International Diabetes Federation (IDF) in 2021, the prevalence of DM is increasing globally. Presently, approximately 1 in 10 people have DM, with projections indicating that this figure will rise to 1 in 8 adults (around 783 million individuals) by the year 2045 [1, 2]. Patients with DM are at risk of microvascular complications of retinopathy, neuropathy and nephropathy.

In terms of diabetes-related microvascular complications, long-term hyperglycemia results in more than one third of diabetic patients developing diabetic retinopathy (DR) with the attendant risk of visual impairment [3–5]. The increasing prevalence of DM and DR necessitates the development of novel treatments for this complication. While tight glycemic control reduces the risk of DR, this can be difficult to achieve over the lifetime of the patient. Current treatments for DR are available to combat the advanced vascular proliferative and inflammatory aspects of the disease, including laser therapy and intravitreal administration of anti-VEGF drugs to destroy hypoxic retinal cells producing VEGF [6, 7], and anti-inflammatory treatments target VEGF, chemokines, angiopoietin-2, and proteases [8–10]. Despite these treatments however, significant numbers of patients develop visual impairment. Furthermore, the effects of these therapies on the early pathological changes of DR and on glial cells are very limited, and further studies are needed.

The early morphological signs of DR include basement membrane thickening, tight junction damage, and blood-retinal barrier (BRB) breakdown. In addition, there is pericyte loss and Müller cell activation. In DM and DR, it has been established that Müller cells undergo activation which is reflective of reactive gliosis [11–13]. While glial fibrillary acidic protein (GFAP) is a widely used marker of reactive gliosis, one of the most important indicators of Müller cell activation is the up-regulation of GFAP [14]. Recent studies have shown that Müller cells activate ER stress-related pathways under stress conditions, including up-regulation of Ddit3 (CHOP) and Atf4 [15, 16]. In vivo and in vitro investigations have revealed that endoplasmic reticulum stress (ERS) plays a crucial role in various stages of diabetic retinopathy (DR), encompassing Müller cells [17], microglia [18], photoreceptor cells [19], and retinal pigment epithelium cells [20]. The presence of persistent hyperglycemia induces ER dysfunction within the retina, resulting in Müller cell activation and subsequent retinal inflammation [21].

Recent studies have found that the transplantation of MSCs and their exosomes can alleviate ER stress [22, 23]

and oxidative stress caused by diseases [24, 25]. Therefore, targeting the restoration of ER (ER) stress in the diabetic retina may hold significant therapeutic potential for DR. However, the effect of intravenous MSC administration on ER stress in DR has not previously been examined. In this study, we administered a single intravenous injection of human umbilical cord-derived MSCs to mouse models of diabetes mellitus at 2 different timepoints after development of DR. We have previously studied the effect of MSC injection on renal and cardiac pathologies in these animals [26, 27].

In the present study, we aimed to investigate the effect of MSC treatment delivered by an intravenous route on DR in vivo and used in vitro experiments using a Müller cell and retinal microvascular endothelial cell line to explore potential mechanisms with an emphasis on endoplasmic reticulum stress.

Methods

Differentiation potential and identification of UC-MSC

The cells were obtained from Qilu Cell Therapy Technology (Shandong, China) and were cultured according to the supplier’s recommended protocol. The medium was replaced every 2 days. The used medium was collected and stored in a negative 80 degree freezer as hUC-MSCs conditioned medium for subsequent cell experiments. When the hUC-MSCs reached 80–90% confluence, the medium was discarded, the cell culture flask was washed three times with PBS, and the cells were lifted with MSC digestion solution (Jing Meng, Beijing, China). The cells were shown to have tri-differentiation potential and the appropriate flow cytometry characterization as previously described and shown in supplementary Fig. 1 [26, 27].

Animal model

The project and all procedures were approved by the Ethics Committee of Hebei Medical University (No. IACUC-Hebmu-2021-035) before starting the experiments. Our entire experimental protocol complied with international standards for biomedical research involving animals.

We employed a diabetic model induced by STZ in male C57BL/6J mice to carry out these experiments with intravenous injections of MSCs or vehicle control (saline) administered at earlier (8 weeks) and later (16 weeks) time-points after induction of DM and with termination of the experiments 2 weeks later (10 and 18 weeks after DM induction) (see Fig. 1 for illustrations of the experimental protocols). The model and experimental design has been described in detail in two recent publications in

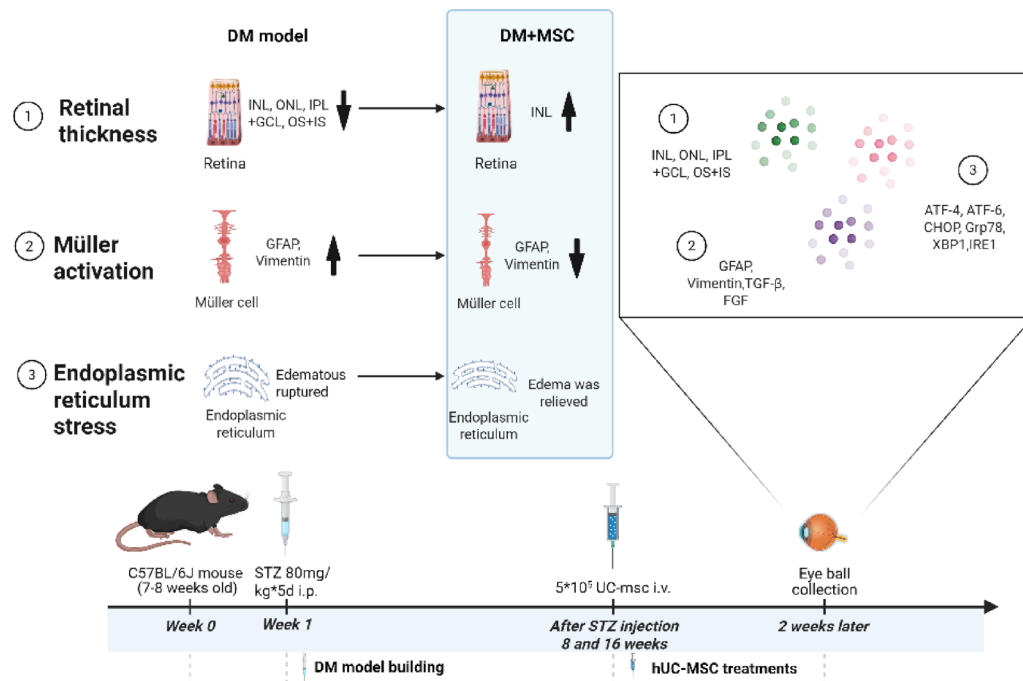


Fig. 1 Schematic of in vivo experimental design summarizing the design of two experiments to investigate the effects of hUC-MSCs on retinal disease in streptozotocin (STZ)-induced diabetic C57BL/6 mice model at two time points after induction of DM

which we reported on the effect of MSC administration on diabetic nephropathy and cardiomyopathy [26, 27]. In brief, the mice, aged 6–7 weeks and weighing an average of (20 ± 3) g, were sourced from Beijing HFK Biotechnology Co., Ltd. (China). To create the diabetic model, STZ was administered via intraperitoneal injections at a dosage of 80 mg/kg body weight for five days consecutively, using a citrate buffer solution with a pH of 4.5. A week after the injections, the blood glucose levels of the mice rose above 16.7 mmol/L, confirming the onset of diabetes mellitus. Following confirmation of DM, the mice were observed for either 8 weeks (earlier time-point) or 16 weeks (later time-points), following which they were randomly divided into groups of 6 that received intravenous injections by tail vein of either 5×10^5 freshly cultured hUC-MSC in 0.2 mL of sterile saline or 0.2 mL of saline alone. The mice were humanely euthanized 2 weeks after the hUC-MSCs injection. An additional group of non-diabetic animals were euthanized as additional controls. Throughout the experiments, General distress score (GDS) and Animal General Distress Scoring sheets (AGS) were constructed weekly by two researchers to monitor the health status of mice. After the mice were anesthetized with 2–3% isoflurane (Orbipharma) inhalation, their cervical vertebrae were dislocated to euthanize the mice, and their eyeballs were taken for subsequent experiments. All efforts were made to minimize animal suffering with the pre-defined humane endpoints. For the current study, eyes were dissected at euthanasia. Six left

eyes were fixed with 4% paraformaldehyde and embedded in paraffin, and paraffin sections were stained for subsequent experiments. After fixing the six right eyes, the retinas were digested and flattened. The rest of the retinas were collected on ice and stored in a negative 80 degree freezer for subsequent RT-qPCR experiments.

Hematoxylin-eosin staining

Fixed eyeballs were longitudinally sectioned along the optic tract after paraffin embedding. The sections were deparaffinized and stained with hematoxylin staining solution for 3–5 min. After washing with tap water, the sections were rinsed with hematoxylin differentiation solution for 2–5 s and thoroughly rinsed with running water. The hematoxylin-blue solution was returned to blue for 2–5 s, and the sections were dehydrated by gradient ethanol after thorough rinsing with running water. The cells were then stained with eosin staining solution (alcohol-soluble) for 5 min, and dehydrated twice with absolute ethanol for 5 min each. The slides were dehydrated with fresh absolute ethanol for 5 min, xylene for 5 min, replaced with fresh xylene and transparent for 5 min, then neutral gum was added to seal the slices for histological evaluation.

Retina digestion and periodic acid schiff staining

The eyeballs were fixed in 4% paraformaldehyde for 24 h, then stored in PBS. Under a dissecting microscope, the sclera and choroid were carefully removed to preserve

the retinal vasculature. The retinas were digested in 1% trypsin at 37 degree for 45 min, with periodic shaking. After PBS washes, the neuroretinal layer was gently pipetted away. The vascular networks were treated with 1% periodate (5 min), stained with Schiff reagent (15 min, dark), and counterstained with hematoxylin (1 min). Samples were dehydrated in alcohol, cleared in xylene, and mounted with neutral gum for microscopy. The number of acellular capillaries in the retina flat mounts were quantified in retinas obtained from 6 mice in 4 randomly selected 100 μm^2 areas and averaged.

Immunofluorescence staining

Fresh eyeballs were initially placed in 4% paraformaldehyde and fixed overnight. Following the completion of the paraffin embedding process, discontinuous sections were obtained from the region adjacent to the optic nerve, with a thickness set to 5 μm . After dewaxing the sections, antigen retrieval was conducted using sodium citrate buffer. Subsequently, the sections were maintained at room temperature and blocked with a solution containing 0.01% Triton and 10% donkey serum for 1 h. Next, either Chicken anti-GFAP polyclonal antibody (1:50, Abcam, Cambridge, UK, catalog no.ad4674) or anti Vimentin Mouse Monoclonal Antibody (1:100, HUABIO, China, catalog no.EM0401) was applied as the primary antibody and incubated overnight at 4 degree. After 2 h of incubation with the corresponding fluorescent secondary antibody (Goat anti-chicken IgG (H+L) Secondary Antibody 488 Conjugated, Repotbiotech, Repotbiotech, S6006) (594 goat anti-mouse, Servicebio, China, catalog no.70073), DAPI dye was utilized to stain the cell nucleus. Finally, after sealing with an anti-fluorescence quenching agent, the samples were imaged using a confocal microscope (Olympus FV1200). During the fluorescence intensity analysis, since the position of the Muller cells runs throughout the retina, the fluorescence of the inner and outer segment areas of the GFAP antibody was not calculated.

Immunohistochemical staining

Following deparaffinization, and antigen retrieval, endogenous peroxidase was blocked with 3% H_2O_2 (25 min, dark), followed PBS washes. Sections were blocked with 3% goat serum (30 min, RT) before overnight incubation (4 degree) with primary antibodies against CHOP (1:50, HUABIO), GRP78 (1:50, Santa), and VEGF (1:50, Santa). After PBS washes, HRP-conjugated secondary antibody was applied (50 min, RT). DAB development was microscopically monitored, followed by alcohol dehydration (75%–100%), xylene clearing, and resinous mounting for light microscopy.

mRNA extraction and RT-qPCR

Total RNA was purified from retinal tissue or cultured cells stored at negative 80 degree freezer using the Rapid Total RNA Extraction Kit (NCM, M5105), following the manufacturer's instructions. The isolated RNA was utilized for cDNA synthesis by reverse transcription reaction, employing 2 μg of RNA and the cDNA Synthesis Kit. Quantitative PCR was conducted using the SYBR Green PCR Master Mix (ZS-M13002, Zhongshi TON-TAU, Tianjin)(ROCHE, Lightcycler@480). The quantitative method used was $\Delta\Delta\text{CT}$, and the relative expression content of the target gene was calculated for normalized comparison with β -actin for housekeeping genes. The primer sequences for the amplification of TGF- β , FGF, GFAP, ATF-4, ATF-6, CHOP, Grp78, and XBP1 are detailed in Appendix Table 2.

Transmission electron microscopy (TEM)

TEM was employed to investigate the morphological changes of the ER in Müller cells within the retinas of various groups of mice. In this experiment, the optic cup was initially fixed in ice-cold 2.5% glutaraldehyde solution in 0.1 M cacodylate buffer for a duration of 12 h. Following fixation, representative areas were selected for ultrathin sectioning after embedding processing. Subsequently, a 150-mesh Fanghua film copper mesh was utilized to extract the sections, which were then stained in light-proof conditions according to standard procedures. Finally, the samples were examined using TEM at a magnification of 5000x.

Müller cell culture and reagents

The Müller cell line was purchased from Bluef Cell (Shanghai, China) and were cultured according to the supplier's recommended protocol [28]. The cells were maintained in DMEM medium (Sigma, D6046) with 10% fetal bovine serum (FBS, Gibco) at 37 degree, 5% CO_2 incubator. The medium was replaced every 1 or 2 days. When the cells had reached 80–90% confluence rate, they were washed 2–3 times with PBS and digested with Trypsin-EDTA containing 0.25%. Digested cells were cultured at 10^5 per well in six-well plates. The QMMuC-1 cell line is an immortalized cell line derived from mouse Müller cells (BLUEFBIO, Shanghai). According to the company's recommendations, the culture medium utilized for this cell line is DMEM supplemented with a glucose concentration of 25 mmol/L. Consequently, the glucose concentration for the normal control group in the experiment was established at 25 mmol/L. When the cells grew to 70% confluence rate, cells were divided into different groups:

Control: DMEM medium with 25 mmol/L of D-glucose; High glucose: DMEM medium with 65 mmol/L of D-glucose; Mannitol: DMEM with 25 mmol/L of

D-glucose and 40 mmol/L of mannitol; HG+hUC-MSC medium: 75%DMEM medium with 65 mmol/L of D-glucose + 25% hUC-MSC medium (Jing-Meng, Beijing, China); HG+hUC-MSC CM: 75%DMEM medium with 65 mmol/L of D-glucose + 25% hUC-MSC conditioned medium (Jing-Meng, Beijing, China).

Retinal microvascular endothelial cell culture and reagents

The retinal microvascular endothelial cells were purchased from Procell Life Science & Technology (Hubei, China). Based on the published literature on the same type of cells, the glucose concentration in the normal group was 5 mmol/L, while that in the high glucose group was 25 mmol/L [29]. The other group conditions were consistent with those of the Muller cells mentioned above.

Cell counting kit-8 (CCK-8)

Cells were seeded in 96-well plates at a density of 1×10^4 cells per milliliter and cultured under these conditions for 24 h. Once the confluence of the cells reached 70% to 80%, 100 μ L of serum-free medium with varying glucose concentrations was added to each well (5 replicate wells per group), and the culture was continued for an additional 12 to 36 h. To determine cell density, 10 μ L of CCK-8 solution was added, followed by incubation at 37 degree for 1.5 h. Finally, absorbance was measured using a microplate reader at a wavelength of 450 nm. The QMMuC-1 cell line is an immortalized cell line derived from mouse Müller cells (BLUEFBI0, Shanghai). According to the company's recommendations, the culture medium utilized for this cell line is DMEM supplemented with a glucose concentration of 25 mmol/L. Consequently, the glucose concentration for the normal control group in the experiment was established at 25 mmol/L. The CCK8 assay was employed to evaluate the cell survival rate of QMMuC-1 cells after 12, 24, 48, and 72 h of culture at varying glucose concentrations ranging from 25 mmol/L to 65 mmol/L.

Western blot analysis

Cell lysis involved the addition of 60 μ L of ice-cold RIPA lysis buffer, which contained 1 mM PMSF (Servicebio, G2008), to each well of approximately six-well plates. The protein concentration was evaluated using a BCA assay. For the detection of proteins, 20 μ g of lysates were introduced into each well and subsequently subjected to electrophoresis on a 10% SDS-PAGE gel. After electrophoresis was completed, proteins were transferred to PVDF membranes. The membranes were blocked for 15 min in a rapid blocking solution and then incubated overnight at 4 degree with specific antibodies against CHOP (1:1000, Cell Signaling Technologies, L63F7), ATF6 (1:1000, SantaCruz, sc-166659), XBP1s (1:3000,

Proteintech, USA), β -actin (1:1000, Proteintech, 20986-1-AP), IRE1 (1:1000, AFFINITY, DF7709) and VEGF (1:500, SantaCruz, sc-7269). Following this step, the PVDF membrane was treated with the relevant secondary antibody (1:10000, Servicebio, China) for 2 h at room temperature. The specific protein bands were then visualized using an enhanced chemiluminescence kit, while the detection process employed the ChemiDoc™ detection XRS+ system (Bio-Rad, USA). Each band's quantification was performed with Image J software, determining the optical density of the target band, and the results were expressed as the mean values for each band.

Data analysis and statistics

The experimental data for each group were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test. All results are presented as mean $\pm$ SD, with the analyses conducted using GraphPad Prism 6.0 software. The findings indicate that a p-value of less than 0.05, along with a 95% confidence interval that does not include zero, suggests a statistically significant difference between the groups. The work has been reported in line with the ARRIVE guidelines 2.0.

Results

Effects of hUC-MSC treatment on retinal microstructure

In order to study and compare the differences in retinal abnormalities and the therapeutic effects of intravenous mesenchymal stem cell (IV MSC) injection in mice after exposure to high blood glucose for different periods of time, we chose to administer MSC treatment at 10 and 18 weeks after the injection of STZ for observation. We acknowledge that these are not different stages of disease but rather represent different durations of exposure to hyperglycemia in vivo. The baseline characteristics and results of fasting blood glucose and body weight monitoring of the groups of DM and Non-DM mice used for this study were as previously described [26, 27]. Retinal thickness was quantified in H&E-stained sections, and the number of nuclei in the inner nuclear layer (INL) and outer nuclear layer (ONL) was quantified in eyes of Non-DM mice and of DM mice at earlier (10 weeks) and later (18 weeks) time-points after induction of diabetes, with, in both cases, either saline alone (DM group) or MSC (DM + MSC group) administered intravenously 2 weeks earlier. At 10 weeks, the results demonstrated that each retinal layer exhibited significantly reduced thickness in the DM group compared to the Non-DM group. There was no significant difference in retinal layer thickness between the DM group and DM + MSC group. At 18 weeks, each retinal layer showed decreased thickness in the DM group compared to the Non-DM group. At this timepoint however, there was a statistically significant increase in INL thickness within the DM + MSC group

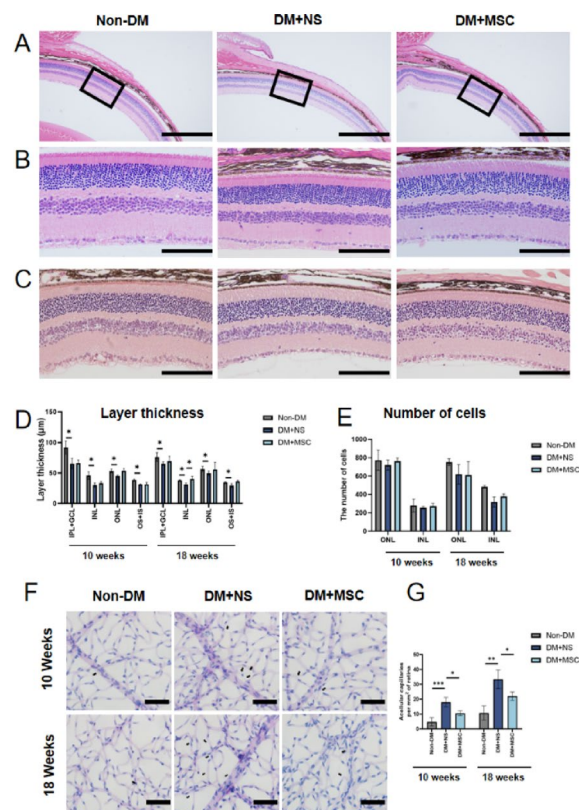


Fig. 2 Individual layer thickness of the retina in three groups at timepoint 1 (A, B) and timepoint 2 (C). Error bars represent SD. **D** Retinal layer thickness statistics at two timepoints. **E** Number of cells at two timepoints. **F-G** Effect of MSC treatment on the number of acellular capillaries in the retina of mice at two timepoints. Error bars represent SD. *N*=6; **p*<0.05, ***p*<0.01, ****p*<0.001. Scale bars: 500 μm in A; 100 μm in B and C; 50 μm in F. INL, inner nuclear layer; ONL, outer nuclear layer

when compared to the DM group (Fig. 2A-D), indicating a beneficial effect of the MSC injection on maintaining the integrity of this layer. No significant differences were observed in cell numbers within both ONL and INL among all groups at both time points (Fig. 2E). Based on these findings, it was concluded that hUC-MSC injection does not have a substantial impact on most STZ-induced retinal thinning but does attenuate reduction of INL

thickness specifically 18 weeks after induction of DM. The specific data are in Table 1.

Quantification of acellular capillaries in the retina was assessed by Periodic Acid Schiff (PAS) staining at the two time points for each of the three groups of mice. The results showed that the number of acellular capillaries in the DM group was significantly higher than that in the Non-DM group, while this was alleviated in the MSC-treated group with diabetes mellitus (Fig. 2F, G).

Effects of MSC treatment on Müller cell activation and ER stress in DM mice

The expression of GFAP and Vimentin in the retina was assessed by immunofluorescence at the two time points for each of the three groups of mice (Fig. 3). At 10 weeks, the expression of GFAP in the DM group was significantly higher than that in the Non-DM group. There were no significant differences between the Non-DM group and DM + MSC group (*P*>0.05). No significant differences were observed in Vimentin expression among the three groups at this timepoint (Fig. 3A, C).

At 18 weeks, there were statistically significant differences in GFAP expression levels among all three groups, with higher levels observed in the DM group compared to both Non-DM and DM + MSC groups. Similarly, Vimentin expression level was significantly higher in DM group compared to Non-DM and DM + MSC groups, following a similar trend as GFAP expression at corresponding time points (Fig. 3B, D).

To assess the extent of ER stress in Müller cells, immunofluorescence staining for CHOP and GRP78 was examined. VEGF expression was also assessed. The expression of CHOP, GRP78 and VEGF was higher in the diabetic group than in the control group, and this observation was reduced in the DM MSC group (Fig. 4A-C). Electron microscopy was utilized to examine morphology within the three groups at the later timepoint. The severity of ER swelling observed in Müller cells from the DM group was greater than that seen in the Non-DM group, an effect which was reduced in the DM + MSC group (Fig. 5A, B).

Table 1 The thickness of each retinal layer and the number of cells in the outer nuclear layer of the inner nuclear layer were measured at two time points.

	Non-DM group		DM group		DM + MSC group	
	10 weeks	18 weeks	10 weeks	18 weeks	10 weeks	18 weeks
Thickness (um)						
IPL + GCL	76.35 ± 7.24	91.76 ± 11.80	65.36 ± 2.78	65.61 ± 9.49	66.05 ± 5.30	69.21 ± 9.16
INL	37.91 ± 0.91	45.77 ± 6.94	31.48 ± 2.11	30.28 ± 3.85	33.36 ± 3.13	40.23 ± 4.80
ONL	56.62 ± 4.17	53.39 ± 3.47	49.77 ± 3.86	43.25 ± 1.43	53.61 ± 3.84	55.83 ± 12.94
OS + IS	34.61 ± 1.03	38.36 ± 1.84	29.62 ± 3.09	31.25 ± 0.56	31.54 ± 2.83	35.97 ± 2.23
Cell counts						
ONL	773 ± 110	751 ± 41	719 ± 54	620 ± 120	762 ± 37	610 ± 165
INL	279 ± 110	483 ± 16	255 ± 14	317 ± 62	271 ± 31	379 ± 31

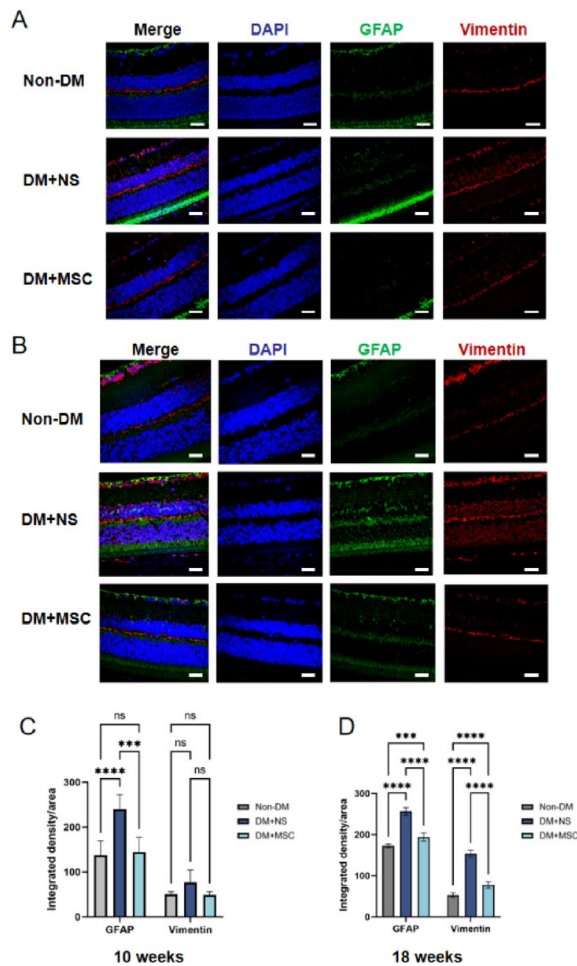


Fig. 3 Quantification of GFAP and Vimentin intensity expressed as mean integrated intensity at timepoint 1 (**A**, **C**) and timepoint 2 (**B**, **D**) measured by ImageJ. (Data represent combined mean $\pm$ SD from $N=4$ animals/group, Scale bars: 20 μ m in **A** and **B**, *** $p < 0.001$, **** $p < 0.0001$)

Activated Müller cells upregulate the production of pro-fibrogenic factors, including TGF- β and FGF. RT-qPCR was employed to quantify the mRNA levels of TGF- β and FGF in the entire retina across three groups at two time points. The results revealed significantly higher mRNA expression of TGF- β and FGF in the DM group compared to both Non-DM and DM + MSC groups, indicating increased expression of pro-fibrotic factors in diabetic mouse retinas that was mitigated two weeks after a single i.v. injection of MSCs (Fig. 5C, D) when administered at 8 or 16 weeks.

RT-qPCR was used to detect the expression of ER stress-related genes ATF4, ATF6, CHOP and GRP78 in retinal tissue of all groups. The mRNA expression of ATF6, CHOP and GRP78 was significantly higher in retinas of the DM group but not the DM+MSC group compared to the Non-DM group at 10 weeks. At 18 weeks, ATF4, ATF6, CHOP and GRP78 mRNA expression was significantly higher in retinas of the DM group

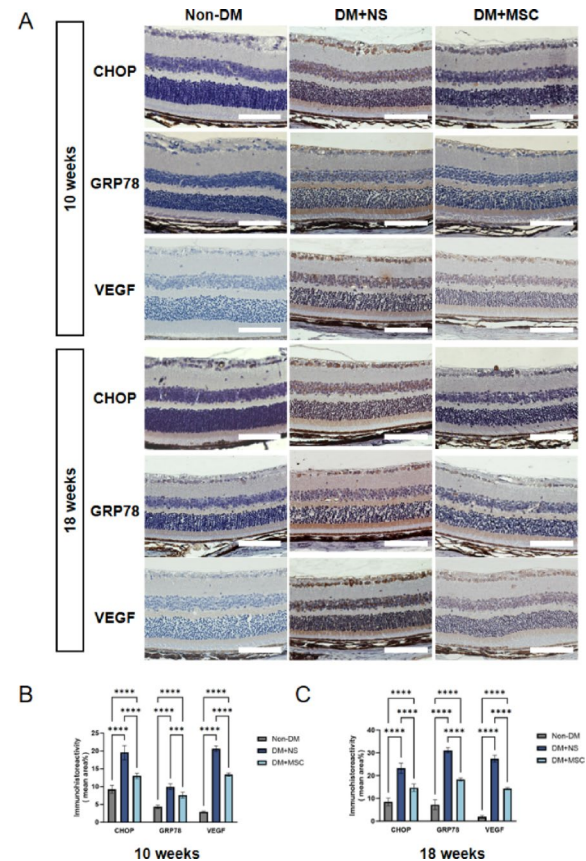


Fig. 4 The expression of CHOP, GRP78 and VEGF in retinal tissue at two timepoints after induction of diabetes mellitus. **A** Immunohistochemical staining of CHOP, GRP78 and VEGF in the retina tissues in three groups at two timepoints. **B** Quantitative immunohistochemical staining of retinal sections at timepoint 1. **C** Quantitative immunohistochemical staining of retinal sections at timepoint 2. Data represent as mean $\pm$ SD, $N=6$ /group, Scale bars: 100 μ m in **A**, *** $p < 0.001$, **** $p < 0.0001$

but not the DM+MSC group compared to the Non-DM group (Fig. 5E, F). Diabetic mice at both time points showed retinal Müller cell activation and ER stress, which were reduced after hUC-MSC injection.

Effect of MSC conditioned media on high glucose-induced ER stress in Müller cells and retinal microvascular endothelial cells

The QMMuC-1 cell experimental results indicated that, after culturing QMMuC-1 cells at a glucose concentration of 65 mmol/L for 48 h, cell viability decreased significantly to approximately 50% (Fig. 6A). Therefore, in subsequent experiments, the CCK8 assay was employed as the condition involving culture at a glucose concentration of 65 mmol/L for 48 h. Different proportions of MSC-CM were added to QMMuC-1 cells cultured in high glucose for microscopic observation of the growth state of the cells. The results show that when the MSC culture medium accounts for 25% of the total culture medium volume, the cell condition is optimal. Based on

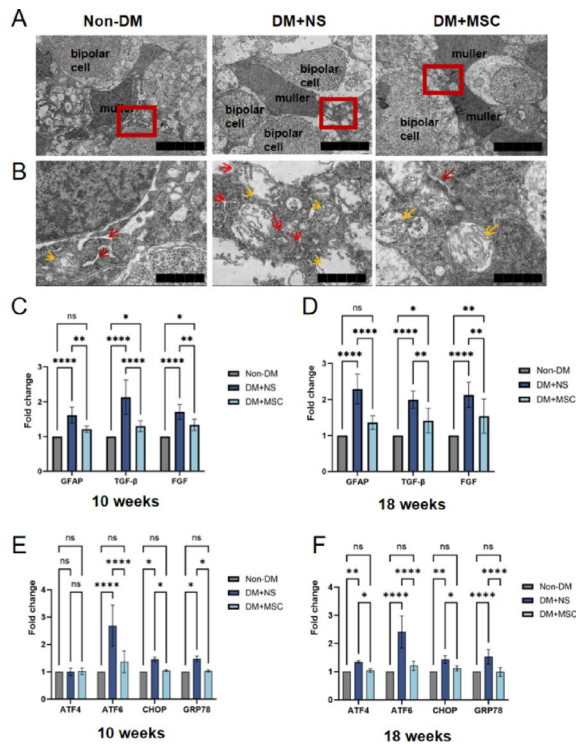


Fig. 5 The three animal groups at time point 2 were examined using electron microscopy to observe the morphology of ER and mitochondria in Müller cells located in the inner nuclear layer (**A** and **B**). mRNA expression of GFAP, TGF- β and FGF in three groups at Timepoint 1 (**C**) and Timepoint 2 (**D**). mRNA expression of ATF4, ATF6, CHOP and GRP78 in three groups at Timepoint 1 (**E**) and Timepoint 2 (**F**). The red arrow indicates enlarged, swollen ER, while the yellow arrow indicates swollen mitochondria with disrupted internal structures. $N=6$, Scale bars: 2 μm in **A**; 10 μm in **B**. Error bars represent SD. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.0001$

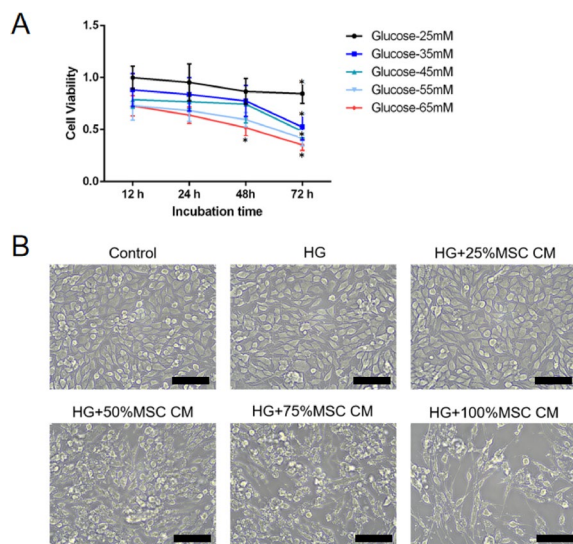


Fig. 6 CCK-8 results of Müller cells treated with different time and glucose concentration gradients (**A**). Müller cell status after treatment with different stem cell conditioned media (**B**). Scale bars: 100 μm

the experimental results and literature review, the usage ratio of the MSC culture medium in the experiment was determined to be 25% (Fig. 6B) [30].

Analyses of ER stress-related mRNAs and proteins in QMMuC-1 cells under 5 different culture conditions were performed by qRT-PCR and Western blotting respectively as described in Methods. The results of qRT-PCR analysis demonstrated that, in comparison to the Control medium, mRNA expression levels of ATF4, ATF6, CHOP, and GRP78 were increased following culture in high glucose medium and high glucose medium containing 25% unconditioned MSC medium but not following culture in high glucose medium containing 25% MSC conditioned medium (MSC-CM). Expression of XBP1s mRNA was significantly higher in cells exposed to high glucose medium than in cells exposed to Control medium but was not significantly reduced by exposure to high glucose containing MSC-CM (Fig. 7A). Exposure to culture media containing the alternative osmolyte, mannitol, did not result in increased expression of ER stress-related mRNAs. It was also observed that MSC CM could alleviate the increase in endoplasmic reticulum-related factors in retinal microvascular endothelial cells caused by high glucose, while the MSC culture medium did not have this effect (Fig. 7B).

At the protein level, compared with the control group, the expressions of ATF6, CHOP, XBP1s, IRE1 α and VEGF significantly increased under high glucose (HG) conditions ($p < 0.05$) and in the high glucose plus mesenchymal stem cell culture medium conditions (see Fig. 7C-J). Mesenchymal stem cell conditioned medium treatment improved the upregulation of these four endoplasmic reticulum stress-related genes induced by high glucose.

Overall, these results were interpreted as providing evidence that products released by hUC-MSC into culture medium mitigate Müller cell ER stress and VEGF expression induced by high glucose.

Discussion

In this manuscript, we report novel in vivo experimental evidence for beneficial therapeutic effects on DR with reduced Müller cell activation and ER stress observed 2 weeks after a single tail vein injection of UC-derived MSCs at two timepoints after the induction of DM in mice. We also show that this animal model of insulin deficient diabetes mellitus has an increase in the number of acellular capillaries and that this is improved by the MSC treatment. Additionally, we provide complementary results from in vitro experiments examining the impact of MSC conditioned media on high glucose-induced ER stress in cultured Müller cells and retinal microvascular endothelial cells, which suggest that the effects are mediated through a paracrine mechanism. We have previously

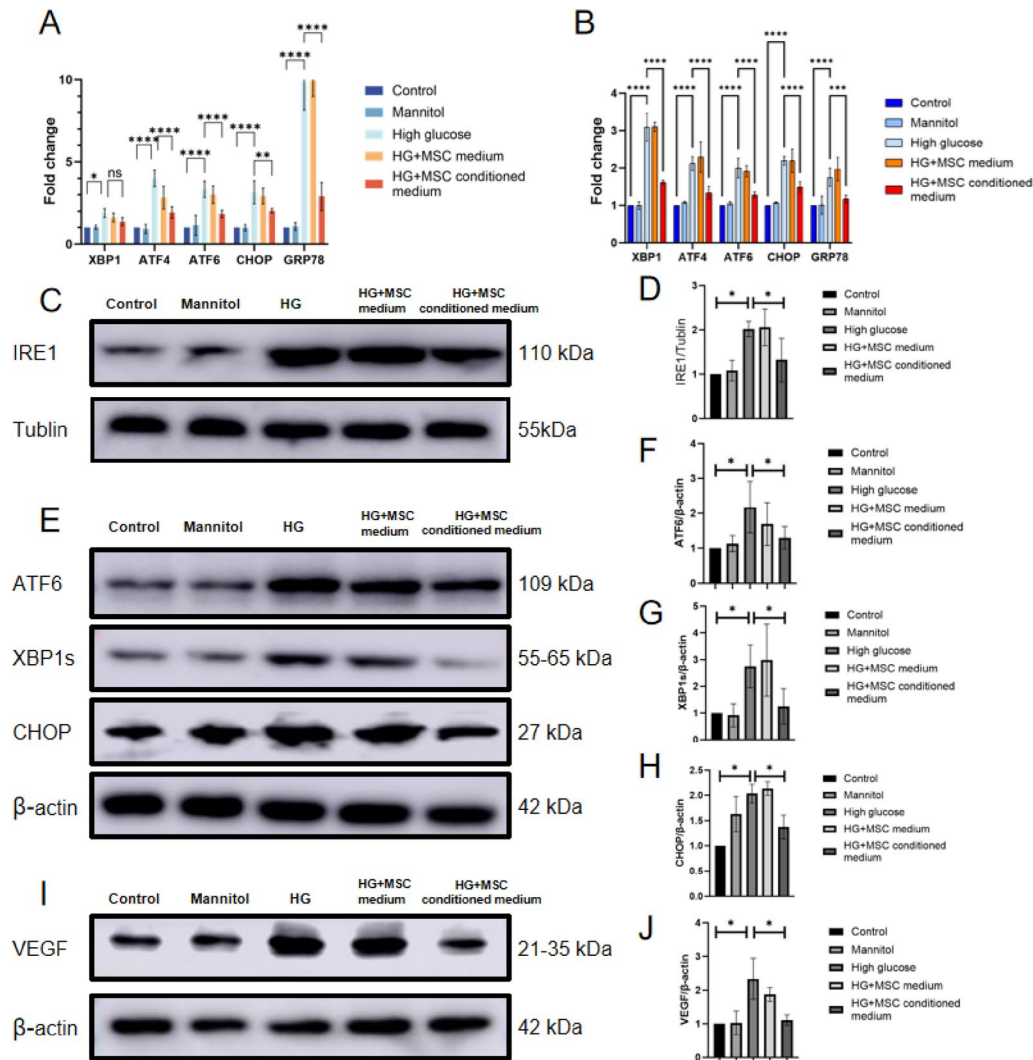


Fig. 7 **A** mRNA expression of XBP1, ATF4, ATF6, CHOP and GRP78 in five groups in Müller cells. **B** mRNA expression of XBP1, ATF4, ATF6, CHOP and GRP78 in five groups in Mouse retinal microvascular endothelial cells. **C–J** Western blots of ER stress related proteins (IRE1, ATF6, XBP1, CHOP) and housekeeping proteins (β-actin, Tubulin) from Western blots of Müller cells from groups of control, mannitol, high glucose, high glucose+MSC medium and high glucose+MSC medium, $N=3$. Western blots of VEGF proteins in Müller cells, $N=4$

reported, using the same animals as in the current publication, that there are beneficial effects of MSC injection on diabetic nephropathy and diabetic cardiomyopathy [26, 27]. Thus, we have demonstrated beneficial therapeutic effects on multiple organs in DM after a single intravenous injection of hUC-MSCs.

Diabetic retinopathy is a leading cause of blindness among working-age adults worldwide. Understanding the prevalence of DR and its association with major modifiable risk factors, especially for vision-threatening DR (VTDR), is crucial for guiding public health education and the optimal clinical management of DM [31]. About a third of people with DM will also have DR, and the longer they have DM, the greater the chance of developing DR [32]. In its earliest stages, DR is mainly

manifested as non-proliferative retinopathy, which will become proliferative with the increase of disease duration, resulting in abnormal formation of new blood vessels, which are prone to rupture and bleed, increasing the risk of retinal detachment, and even causing blindness in patients. Another hazard of DR is macular edema, which can lead to blurred and significantly reduced vision, seriously affecting the patients quality of life [33]. Therefore, new interventions are needed to prevent or reverse disease progression in the early stages of DR. A number of recent articles have investigated the therapeutic role of MSC as a relatively safe treatment with the potential to also treat other DM complications. It has been reported that in the early stage of DR, the main pathological features are the proliferation of glial cells and the expression

of inflammatory factors. Secretory factors produced by proliferation and persistent inflammation will cause retinal photoreceptor cell degeneration and eventually make the inner nuclear layer and outer nuclear layer thinner [34–37]. Recent studies have shown that INL thickness appears to be determined by tangential retinal displacement in the epiretinal membrane [38]. This plays an important role in the structural distortion of Müller cells. Therefore, in this study, *in vitro* and *in vivo* experiments were performed to investigate the therapeutic effect of MSC transplantation on DR, with experiments focusing on Müller cells and retinal microvascular endothelial cells.

In the retina, Müller cells are an important type of retinal glial cell that are known to undergo reactive gliosis upon injury – a response that can be detected by their expression of GFAP and vimentin [39, 40]. Indeed, previous studies have suggested that the early features of DR in mice include microglial activation and increased GFAP expression in Müller cells [41, 42]. In addition, it has been reported that intravitreal injection of adipose-derived stem cells and bone marrow MSC conditioned media can improve the increased expression of GFAP and vimentin in the retina of animals with DR [43, 44]. In keeping with this, but using a different route of delivery and umbilical cord-derived MSCs, in our experiments a single intravenous injection of hUC-MSCs reduced retinal GFAP expression in diabetic mice following administration at 8 and 16 weeks after induction of diabetes mellitus. A similar effect was also observed for vimentin expression, but the difference was statistically significant only following administration at the later timepoint; suggesting that intravenous hUC-MSC therapy may reverse reactive gliosis within the retina even after a more prolonged duration of hyperglycemia.

Stem cell therapy is a novel approach to treating DR which is currently at an investigational stage [45]. Stem cells have multidirectional differentiation potential and tissue repair ability, and it is generally believed that they are promising agents to repair damaged cells and microvessels within the retina through modulating the local microenvironment [46]. Compared to conventional surgical and pharmacological interventions, MSC-based therapy holds significant promise if it can effectively exert paracrine effects via intravenous administration without inducing significant adverse reactions. The simplicity of this delivery route could greatly enhance its clinical feasibility. Furthermore, given its broad therapeutic potential, MSC-based treatment may have beneficial effects on other organs, paving the way for use in patients with multiple complications of DM. Current studies have focused on MSCs, embryonic stem cells and induced pluripotent stem cells, with MSCs being the most commonly studied due to their lower immune properties and higher safety

profile. Stem cells can secrete growth factors, reduce inflammation and improve angiogenesis, properties that may slow or even reverse the progression of retinopathy [47]. However, MSCs are unlikely to migrate to the retina after intravenous administration, mainly due to the presence of the blood-retinal barrier. The effects of MSCs in therapeutic applications in many settings suggest that the effect may be mediated via paracrine mechanisms. MSCs can regulate immune responses and repair injured tissue by releasing extracellular vesicles, growth factors, chemokines and cytokines [48, 49]. For example, MSCs secrete a large amount of neurotrophic substances (bFGF, NGF, NT3, NT4 and GDNF) which enhance neuroprotection, growth factors (bFGF, IGF-1, HGF, VEGF, etc.) to stimulate cell proliferation and tissue reorganization, anti-inflammatory factors (IL-10 and IL1-RA) that inhibit local inflammation, and exosomes and extracellular RNA to trigger intercellular communication and regulation [50–52]. Studies have shown that MSCs release prostaglandin E2 receptor through paracrine means, which can mediate platelet-derived growth factor and nerve growth factor effects, thus protecting the function of rat retinal ganglion cells and promoting neurite formation [53]. Furthermore, in a diabetes-induced rat model, transplanted MSCs showed selective protection against retinal gliosis, as well as restoration of retinal vascular integrity and function [54]. These studies have shown that the paracrine mechanism of MSCs can protect the structure and function of the retina, demonstrating its significant potential in the neuroprotection and treatment of retinal diseases. However, there are a limited number of studies investigating the effect of IV delivery of MSCs on DR. In these experiments the focus has mainly been on improving retinal microvascular dysfunction and inflammation [54–56]. The functions of Müller cells and changes in ER stress have not yet been examined after IV delivery. The specific mechanism of action of MSCs on Müller cells also remains to be elucidated. Recent studies have shown that intravitreal injection of MSCs can improve the pathological changes of DR, such as reducing retinal inflammation [57–61], increasing the expression of neurotrophic factors [62–64], and improving retinal vascular permeability [65, 66]. However, frequent intravitreal injections may lead to the risk of endophthalmitis, retinal detachment, and hemorrhage. Moreover, diabetes is a systemic disease, which is often accompanied by a variety of complications, while intravitreal injection of stem cells is mostly confined to the retina. Therefore, we chose intravenous delivery of MSCs as the method of administration. Furthermore, when the results of this study are taken together with our prior reports of improvements in nephropathy and cardiomyopathy in the same animals, our current results demonstrating amelioration of DR severity following hUC-MSC administration highlight

the potential advantage of intravenous delivery as these microvascular complications frequently co-exist [67, 68].

From a mechanistic perspective, we have explored the potential for MSC delivery to modulate ER stress as this and UPR pathways have recently attracted great interest due to their critical roles in the pathogenesis of a variety of diseases including metabolic disorders [69], neurodegenerative diseases [70], and cancer [71, 72]. Excess nutrients, viruses, environmental toxins, and chronic inflammation alter calcium levels, protein glycosylation, and REDOX status in the ER, which leads to abnormal protein folding and secretion. The accumulation of unfolded and misfolded proteins leads to ER stress, and cells respond to this stress condition by activating the UPR pathway [73]. In diabetes, ER stress also plays an important role in various complications such as DR, DN [74], DCM [29, 75] and diabetes-related cognitive impairment (CI) [76]. In diabetic animals, elevated expression of ER stress markers is partially localized to Müller cells in the retina, and high glucose has been reported to induce ER stress in cultured Müller cells [77], which is consistent with our experimental results. Moreover, in our study, MSC conditioned media alleviated high glucose-induced ER stress in vitro and the intravenous delivery of MSCs in vivo also reduced Müller cell ER stress. We acknowledge that the mechanisms of MSC therapeutic benefit are pleiotropic and the current manuscript describes just one of the potential mechanisms of action. In addition, it has been found that induction of ER stress leads to up-regulation of VEGF expression by regulating the c-Jun N-terminal kinase pathway [77]. In our results, VEGF expression in Müller cells was increased by high glucose exposure, which was alleviated by the addition of hUC-MSC CM. These results indicate for the first time that hUC-MSC CM can ameliorate the high glucose-induced increase of VEGF expression in Müller cells together with modulating ER stress. It should be noted that in vitro experiments cannot demonstrate in vivo distribution or pathways of IV administered MSCs and also that diabetes mellitus is a multisystem disorder. In relation to the former, the importance of the in vitro experiments is to demonstrate that the mechanism does not require cell to cell contact and thus secretome-mediated effects are plausible. In relation to the latter point, many patients have clinical evidence of triopathy (retinopathy, neuropathy and nephropathy) in addition to cardiomyopathy and newly recognized and appreciated complications such as osteopathy. For this reason in this manuscript, we explored a systemic approach with IV delivery. We believe it is important to develop a systemic approach which would have beneficial effects on multiple organs affected by the complication of diabetes mellitus. We have also previously reported in a human study of patients with diabetic nephropathy that a single IV injection of MSCs has long

lasting effects on the immune system [78] which supports our premise that the observed therapeutic benefits may be due to paracrine mechanisms rather than requiring cell retention in the affected organs. Meanwhile, it is worth noting that, given the retina's highly intricate architecture, our current single-cell studies using both endothelial cells and Müller cells represent only an initial step. To comprehensively evaluate the therapeutic potential and mechanistic actions of MSC-conditioned medium, retinal organoid models may be more suitable in subsequent investigations. This advanced model system will better recapitulate the retinal microenvironment and enable more physiologically relevant functional analyses.

In our previous 2 publications using this animal group we have shown that intravenous delivery of MSCs was associated with restoration of autophagy and reversal of microRNA dysregulation in the kidney and heart respectively [26, 27]. This suggests that MSCs have pleiotropic mechanisms of action in restoring diabetic tissues to health. It should also be noted that we did not examine functional changes and vascular permeability in DR in the current study, and so the clinical relevance cannot be proven from our data. Future experiments should be conducted to address this issue. Also, in this manuscript we have only used 1 model of diabetes, that of STZ-induced insulin deficiency. Other models would need to be used to broaden the interpretation of the results to other types of diabetes mellitus. It has previously been reported that IV delivery of MSCs can plug pulmonary arteries [55]. Notably, no instances of pulmonary embolism were observed following intravenous administration in this study. This absence of vascular complications may be attributable to the controlled cell dosage administered in our animal model.

Prior to this study, the therapeutic effect of intravenous injection of MSC for ER stress in DR had not been evaluated, and so in this initial study we used a single dose of a single injection for the study which was administered 2 weeks before the tissues were harvested. The effect of increasing the number of injections and the dose administered on the treatment outcome will need evaluation in future studies. The durability of the effect will also need to be studied to assess if the therapeutic benefit is prolonged at later timepoints other than the 2 week period evaluated in this study. While we are hypothesizing a paracrine/systemic effect of the IV delivered MSCs, the underlying mechanism remains unclear and future studies should examine proteomics of the conditioned media to better identify the bioactive factors and signaling pathways that mediate these effects. In future experiments, single cell analysis could be used with a view to studying multiple cell types simultaneously [79].

Table 2 List of primers used in quantitative RT-PCR

Gene	Primers sequence
GFAP-R	TGGTTTCATCTTGGAGCTTCTG
GFAP-F	TGGAGGTGGAGAGGGACAAC
Atf4-F	CCCCCTTCGACCAGTCGGGT
Atf4-R	CCGCCCTGCTGCTGGAGAACC
Atf6-F	CAGACTCGTGTCTTCAAC
Atf6-R	GGCTTCTCTTCTTCAGT
Chop-F	GTCCCTAGCTTGGCTGACAGA
Chop-R	TGGAGAGCGAGGGCTTTG
Grp78-F	TCATCGGACGCACTTGGAA
Grp78-R	CAACCACCTTGAATGGCAAGA
XPB1-F	TCACCCCTCCAGAATCATCTC
XPB1-R	AAAGGGAGGCTGTAAGGAA

The research described in the current manuscript shows that a single intravenous injection of hUC-MSCs has the capacity to reduce Müller cell activation and attenuate retinal ER stress in an STZ-induced mouse model of DM whether administered after 8 or 16 weeks of hyperglycemia. The beneficial effect is therefore seen after earlier and later durations of DR. We would accept that these are not 2 different stages of the disease but rather allowed a comparison of the effects relative to the duration of DM. As we have previously reported, MSC injections at these time-points did not result in reduced blood glucose levels compared to control-treated mice [26, 27]. Importantly, in mice with a longer duration of diabetes, the administration of MSCs led to morphological improvement of the retina as shown by an enhancement in the thickness of the inner nuclear layer of the retina. Administration of MSCs by the intravenous route also improved the number of acellular capillaries found in this model of diabetes mellitus. These results further strengthen the suggestion that hUC-MSCs may be a promising therapeutic avenue for human DR. The results in DR presented in the current manuscript, in addition to the results in cardiomyopathy and nephropathy which we have previously reported, demonstrate that this approach using a single i.v. injection of MSCs has multi-organ protective effects in DM and should be explored in clinical trials.

Conclusion

A single intravenous injection of hUC-MSC has beneficial effects on acellular capillary number, Müller cell activation and ER stress in DM animals. MSC-conditioned medium reversed HG-induced ER stress and reduced expression of VEGF in Müller cells. hUC-MSC exerts protective effects by reducing Müller cell activation, ER stress and VEGF expression, which may provide a new therapeutic target for DR treatment. Beneficial effects on ER stress in retinal microvascular endothelial cells were also observed in this study.

Appendix

See Table 2.

Abbreviations

ATF6	Activating transcription factor 6
DM	Diabetes mellitus
DR	Diabetic retinopathy
ERAD	ER-associated degradation
ER stress	Endoplasmic reticulum stress
EVs	Extracellular vesicles
GFAP	Glial fibrillary acidic protein
H&E	Hematoxylin and eosin
hUC-MSCs	Human umbilical cord mesenchymal stromal cells
IRE1	Inositol requiring enzyme 1
MSC-CM	Mesenchymal stromal cell conditioned medium
PCR	Polymerase chain reaction
STZ	Streptozotocin
UPR	Unfolded protein response

Supplementary Information

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Supplementary Material 1

Supplementary Material 2

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Graphical abstract was created with BioRender.com. The authors declare that they have not used AI-generated work in this manuscript.

Author contributions

YW participated in conception and design, collection and assembly of data, data analysis and interpretation, and manuscript writing. GRY, LT and LYK participated in conception and design, data analysis and interpretation. PR, LBX and HJJ participated in data analysis and interpretation. MDG, SOH, SS and YL participated in conception and design, reviewed the manuscript. TOB and JM participated in conception and design, reviewed the manuscript, and gave final approval for manuscript submission.

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Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate

The experiments were conducted in accordance with the National Institutes of Health guidelines for the care of animals. The animal studies came from the project of "Effect and Mechanism of Mesenchymal Stem Cell in the Treatment of Diabetic Complications" and was approved by the Laboratory Animal Ethical and Welfare Committee of Hebei Medical University (Approval No. IACUCHebmu-2021035, Date: 8 December 2021). UC-MSC cells were obtained from Qilu Cell Therapy Technology (Shandong, China) and the company obtained the cells with ethical approval and patient consent (Approval No. CXSL2300854).

Consent for publication

Not applicable.

Competing interests

TOB is global co-editor in chief of Stem Cell Research and Therapy. He will have no involvement in the peer review process or decisions on this article. He is also a Director and equity holder in Orbsen Therapeutics, a stem cell company. MD Griffin reports honoraria from the American Society of Nephrology; Théa Pharma Ltd., Ireland; and Novo Nordisk; research funding from Orbsen Therapeutics Ltd.; and advisory roles as an Editorial Board member for the journals Transplantation and Frontiers in Pharmacology and an Associate Editor for Mayo Clinic Proceedings. The other authors declare that they have no competing interests.

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References

1. Sun H, Saeedi P, Karuranga S, Pinkepank M, Ogurtsova K, Duncan BB, et al. IDF diabetes atlas: global, regional and country-level diabetes prevalence estimates for 2021 and projections for 2045. *Diabetes Res Clin Pract*. 2022;183:109119.
2. Teo ZL, Tham YC, Yu M, Chee ML, Rim TH, Cheung N, et al. Global prevalence of diabetic retinopathy and projection of burden through 2045: systematic review and meta-analysis. *Ophthalmology*. 2021;128(11):1580–91.
3. Cheung N, Mitchell P, Wong TY. Diabetic retinopathy. *Lancet*. 2010;376(9735):124–36.
4. Leasher JL, Bourne RR, Flaxman SR, Jonas JB, Keeffe J, Naidoo K, et al. Global estimates on the number of people blind or visually impaired by diabetic retinopathy: a meta-analysis from 1990 to 2010. *Diabetes Care*. 2016;39(9):1643–9.
5. Zoungas S, Arima H, Gerstein HC, Holman RR, Woodward M, Reaven P, et al. Effects of intensive glucose control on microvascular outcomes in patients with type 2 diabetes: a meta-analysis of individual participant data from randomised controlled trials. *Lancet Diabetes Endocrinol*. 2017;5(6):431–7.
6. Salam A, Mathew R, Sivaprasad S. Treatment of proliferative diabetic retinopathy with anti-VEGF agents. *Acta Ophthalmol*. 2011;89(5):405–11.
7. Tremolada G, Del Turco C, Lattanzio R, Maestroni S, Maestroni A, Bandello F, et al. The role of angiogenesis in the development of proliferative diabetic retinopathy: impact of intravitreal anti-VEGF treatment. *Exp Diabetes Res*. 2012;2012:728325.
8. Agrawal NK, Kant S. Targeting inflammation in diabetes: newer therapeutic options. *World J Diabetes*. 2014;5(5):697–710.
9. Kode JA, Mukherjee S, Joglekar MV, Hardikar AA. Mesenchymal stem cells: immunobiology and role in immunomodulation and tissue regeneration. *Cytotherapy*. 2009;11(4):377–91.
10. Arrigo A, Aragona E, Bandello F. VEGF-targeting drugs for the treatment of retinal neovascularization in diabetic retinopathy. *Ann Med*. 2022;54(1):1089–111.
11. Gerhardinger C, Costa MB, Coulombe MC, Toth I, Hoehn T, Grosu P. Expression of acute-phase response proteins in retinal Müller cells in diabetes. *Invest Ophthalmol Vis Sci*. 2005;46(1):349–57.
12. Kusner LL, Sarthy VP, Mohr S. Nuclear translocation of glyceraldehyde-3-phosphate dehydrogenase: a role in high glucose-induced apoptosis in retinal Müller cells. *Invest Ophthalmol Vis Sci*. 2004;45(5):1553–61.
13. Puro DG. Diabetes-induced dysfunction of retinal Müller cells. *Trans Am Ophthalmol Soc*. 2002;100:339–52.
14. Yego EC, Vincent JA, Sarthy V, Busik JV, Mohr S. Differential regulation of high glucose-induced glyceraldehyde-3-phosphate dehydrogenase nuclear accumulation in Müller cells by IL-1 β and IL-6. *Invest Ophthalmol Vis Sci*. 2009;50(4):1920–8.
15. Chucair-Elliott AJ, Ocañas SR, Pham K, Van Der Veldt M, Cheyney A, Stanford D, et al. Translational response of retinal Müller glia to acute and chronic stress. *Neurobiol Dis*. 2022;175:105931.
16. Palko SI, Benoit MR, Yao AY, Mohan R, Yan R. ER-stress response in retinal Müller glia occurs significantly earlier than amyloid pathology in the Alzheimer's mouse brain and retina. *Glia*. 2024;72(6):1067–81.
17. Yang J, Chen C, McLaughlin T, Wang Y, Le YZ, Wang JJ, et al. Loss of X-box binding protein 1 in Müller cells augments retinal inflammation in a mouse model of diabetes. *Diabetologia*. 2019;62(3):531–43.
18. Zeng HY, Green WR, Tso MO. Microglial activation in human diabetic retinopathy. *Arch Ophthalmol*. 2008;126(2):227–32.
19. McLaughlin T, Siddiqi M, Wang JJ, Zhang SX. Loss of XBP1 leads to Early-Onset retinal neurodegeneration in a mouse model of type I diabetes. *J Clin Med*. 2019;8(6).
20. Roybal CN, Yang S, Sun CW, Hurtado D, Vander Jagt DL, Townes TM, et al. Homocysteine increases the expression of vascular endothelial growth factor by a mechanism involving endoplasmic reticulum stress and transcription factor ATF4. *J Biol Chem*. 2004;279(15):14844–52.
21. Elshaer SL, Evans W, Pentecost M, Lenin R, Periasamy R, Jha KA, et al. Adipose stem cells and their paracrine factors are therapeutic for early retinal complications of diabetes in the Ins2Akita mouse. *Stem Cell Res Ther*. 2018;9(1):322.
22. Lu X, Lv C, Zhao Y, Wang Y, Li Y, Ji C, et al. TSG-6 released from adipose stem cells-derived small extracellular vesicle protects against spinal cord ischemia reperfusion injury by inhibiting endoplasmic reticulum stress. *Stem Cell Res Ther*. 2022;13(1):291.
23. Luo R, Li L, Liu X, Yuan Y, Zhu W, Li L, et al. Mesenchymal stem cells alleviate palmitic acid-induced endothelial-to-mesenchymal transition by suppressing endoplasmic reticulum stress. *Am J Physiol Endocrinol Metab*. 2020;319(6):E961–80.
24. Xiao X, Xu M, Yu H, Wang L, Li X, Rak J, et al. Mesenchymal stem cell-derived small extracellular vesicles mitigate oxidative stress-induced senescence in endothelial cells via regulation of miR-146a/Src. *Signal Transduct Target Ther*. 2021;6(1):354.
25. Wang T, Jian Z, Baskys A, Yang J, Li J, Guo H, et al. MSC-derived exosomes protect against oxidative stress-induced skin injury via adaptive regulation of the NRF2 defense system. *Biomaterials*. 2020;257:120264.
26. He J, Liu B, Du X, Wei Y, Kong D, Feng B, et al. Amelioration of diabetic nephropathy in mice by a single intravenous injection of human mesenchymal stromal cells at early and later disease stages is associated with restoration of autophagy. *Stem Cell Res Ther*. 2024;15(1):66.
27. Liu B, Wei Y, He J, Feng B, Chen Y, Guo R, et al. Human umbilical cord-derived mesenchymal stromal cells improve myocardial fibrosis and restore miRNA-133a expression in diabetic cardiomyopathy. *Stem Cell Res Ther*. 2024;15(1):120.
28. Augustine J, Pavlou S, O'Hare M, Harkin K, Stitt A, Curtis T, et al. Characterization of a spontaneously immortalized murine Müller glial cell line QMMuC-1. *Invest Ophthalmol Vis Sci*. 2018;59(3):1666–74.
29. Shih JY, Lin YW, Fisch S, Cheng JT, Kang NW, Hong CS, et al. Dapagliflozin suppresses ER stress and improves subclinical myocardial function in diabetes: from bedside to bench. *Diabetes*. 2021;70(1):262–7.
30. Kay AG, Long G, Tyler G, Stefan A, Broadfoot SJ, Piccinini AM, et al. Mesenchymal stem cell-conditioned medium reduces disease severity and immune responses in inflammatory arthritis. *Sci Rep*. 2017;7(1):18019.
31. Yau JW, Rogers SL, Kawasaki R, Lamoureux EL, Kowalski JW, Bek T, et al. Global prevalence and major risk factors of diabetic retinopathy. *Diabetes Care*. 2012;35(3):556–64.
32. Li Y, Mitchell W, Elze T, Zebardast N. Association between diabetes, diabetic retinopathy, and glaucoma. *Curr Diab Rep*. 2021;21(10):38.
33. Bohler F, Bohler L, Taranikanti V. Targeting pericyte retention in diabetic retinopathy: a review. *Ann Med*. 2024;56(1):2398200.
34. Kumar S, Zhuo L. Longitudinal in vivo imaging of retinal gliosis in a diabetic mouse model. *Exp Eye Res*. 2010;91(4):530–6.
35. Feit-Leichman RA, Kinouchi R, Takeda M, Fan Z, Mohr S, Kern TS, et al. Vascular damage in a mouse model of diabetic retinopathy: relation to neuronal and glial changes. *Invest Ophthalmol Vis Sci*. 2005;46(11):4281–7.

36. Lv J, Bao S, Liu T, Wei L, Wang D, Ye W, et al. Sulforaphane delays diabetes-induced retinal photoreceptor cell degeneration. *Cell Tissue Res*. 2020;382(3):477–86.
37. Martin PM, Roon P, Van Ells TK, Ganapathy V, Smith SB. Death of retinal neurons in streptozotocin-induced diabetic mice. *Invest Ophthalmol Vis Sci*. 2004;45(9):3330–6.
38. Ichikawa Y, Imamura Y, Ishida M. Inner nuclear layer thickness, a biomarker of metamorphopsia in epiretinal membrane, correlates with tangential retinal displacement. *Am J Ophthalmol*. 2018;193:20–7.
39. Huang H, Kolibabka M, Eshwaran R, Chatterjee A, Schlotterer A, Willer H, et al. Intravitreal injection of mesenchymal stem cells evokes retinal vascular damage in rats. *FASEB J*. 2019;33(12):14668–79.
40. Tassoni A, Gutteridge A, Barber AC, Osborne A, Martin KR. Molecular mechanisms mediating retinal reactive gliosis following bone marrow mesenchymal stem cell transplantation. *Stem Cells*. 2015;33(10):3006–16.
41. Barber AJ, Antonetti DA, Kern TS, Reiter CE, Soans RS, Krady JK, et al. The Ins2Akita mouse as a model of early retinal complications in diabetes. *Invest Ophthalmol Vis Sci*. 2005;46(6):2210–8.
42. McLenachan S, Chen X, McMenamin PG, Rakoczy EP. Absence of clinical correlates of diabetic retinopathy in the Ins2Akita retina. *Clin Exp Ophthalmol*. 2013;41(6):582–92.
43. Elshaer SL, Evans W, Pentecost M, Lenin R, Periasamy R, Jha KA, et al. Adipose stem cells and their paracrine factors are therapeutic for early retinal complications of diabetes in the Ins2(Akita) mouse. *Stem Cell Res Ther*. 2018;9(1):322.
44. Wang T, Li HC, Ma J, Yu XL. Effect of sonic hedgehog gene-modified bone marrow mesenchymal stem cells on graft-induced retinal gliosis and retinal ganglion cells survival in diabetic mice. *Int J Ophthalmol*. 2024;17(1):34–41.
45. Simó R, Hernández C. Novel approaches for treating diabetic retinopathy based on recent pathogenic evidence. *Prog Retin Eye Res*. 2015;48:160–80.
46. Zakrzewski W, Dobrzyński M, Szymonowicz M, Rybak Z. Stem cells: past, present, and future. *Stem Cell Res Ther*. 2019;10(1):68.
47. Gehart H, Clevers H. Tales from the crypt: new insights into intestinal stem cells. *Nat Rev Gastroenterol Hepatol*. 2019;16(1):19–34.
48. Abdel-Kawi SH, Hashem KS. Administration of melatonin in diabetic retinopathy is effective and improves the efficacy of mesenchymal stem cell treatment. *Stem Cells Int*. 2022;2022:6342594.
49. Ling L, Feng X, Wei T, Wang Y, Wang Y, Wang Z, et al. Human amnion-derived mesenchymal stem cell (hAD-MSC) transplantation improves ovarian function in rats with premature ovarian insufficiency (POI) at least partly through a paracrine mechanism. *Stem Cell Res Ther*. 2019;10(1):46.
50. Fu X, Liu G, Halim A, Ju Y, Luo Q, Song AG. Mesenchymal stem cell migration and tissue repair. *Cells*. 2019;8(8):784.
51. Bronckaers A, Hilken P, Martens W, Gervois P, Ratajczak J, Struys T, et al. Mesenchymal stem/stromal cells as a pharmacological and therapeutic approach to accelerate angiogenesis. *Pharmacol Ther*. 2014;143(2):181–96.
52. Zhang S, Zhang W, Li Y, Ren L, Deng H, Yin X, et al. Cotransplantation of human umbilical cord mesenchymal stem cells and endothelial cells for angiogenesis and pulp regeneration in vivo. *Life Sci*. 2020;255:117763.
53. Peng BY, Dubey NK, Mishra VK, Tsai FC, Dubey R, Deng WP, et al. Addressing stem cell therapeutic approaches in pathobiology of diabetes and its complications. *J Diabetes Res*. 2018;2018:7806435.
54. Rong L, Wei W, Fang Y, Liu Y, Gao T, Wang L, et al. Clinical-grade human embryonic stem cell-derived mesenchymal stromal cells ameliorate diabetic retinopathy in db/db mice. *Cytotherapy*. 2024;26(6):606–15.
55. Liang Q, Li Q, Ren B, Yin ZQ. Intravenous infusion of small umbilical cord mesenchymal stem cells could enhance safety and delay retinal degeneration in RCS rats. *BMC Ophthalmol*. 2022;22(1):67.
56. Yang Z, Li K, Yan X, Dong F, Zhao C. Amelioration of diabetic retinopathy by engrafted human adipose-derived mesenchymal stem cells in streptozotocin diabetic rats. *Graefes Arch Clin Exp Ophthalmol*. 2010;248(10):1415–22.
57. Mead B, Logan A, Berry M, Leadbeater W, Scheven BA. Paracrine-mediated neuroprotection and neuritogenesis of axotomized retinal ganglion cells by human dental pulp stem cells: comparison with human bone marrow and adipose-derived mesenchymal stem cells. *PLoS One*. 2014;9(10):e109305.
58. Guan Y, Cui L, Qu Z, Lu L, Wang F, Wu Y, et al. Subretinal transplantation of rat MSCs and erythropoietin gene modified rat MSCs for protecting and rescuing degenerative retina in rats. *Curr Mol Med*. 2013;13(9):1419–31.
59. Chen SN, Xu ZG, Ma YX, Chen S, He GH, Han M, et al. Protective effect of LIF-huMSCs on the retina of diabetic model rats. *Int J Ophthalmol*. 2021;14(10):1508–17.
60. Gu X, Yu X, Zhao C, Duan P, Zhao T, Liu Y, et al. Efficacy and safety of autologous bone marrow mesenchymal stem cell transplantation in patients with diabetic retinopathy. *Cell Physiol Biochem*. 2018;49(1):40–52.
61. Kim H, Goh YS, Park SE, Hwang J, Kang N, Jung JS, et al. Preventive effects of exosome-rich conditioned medium from amniotic membrane-derived mesenchymal stem cells for diabetic retinopathy in rats. *Transl Vis Sci Technol*. 2023;12(8):18.
62. Ezquer M, Urzua CA, Montecino S, Leal K, Conget P, Ezquer F. Intravitreal administration of multipotent mesenchymal stromal cells triggers a cytoprotective microenvironment in the retina of diabetic mice. *Stem Cell Res Ther*. 2016;7:42.
63. Kong JH, Zheng D, Chen S, Duan HT, Wang YX, Dong M, et al. A comparative study on the transplantation of different concentrations of human umbilical mesenchymal cells into diabetic rats. *Int J Ophthalmol*. 2015;18(2):257–62.
64. Scalinci SZ, Scorolli L, Corradetti G, Domanico D, Vingolo EM, Meduri A, et al. Potential role of intravitreal human placental stem cell implants in inhibiting progression of diabetic retinopathy in type 2 diabetes: neuroprotective growth factors in the vitreous. *Clin Ophthalmol*. 2011;5:691–6.
65. Rajashekhar G, Ramadan A, Abburi C, Callaghan B, Traktuev DO, Evans-Molina C, et al. Regenerative therapeutic potential of adipose stromal cells in early stage diabetic retinopathy. *PLoS One*. 2014;9(1):e84671.
66. Yu C, Yang K, Meng X, Cao B, Wang F. Downregulation of long noncoding RNA MIAT in the retina of diabetic rats with Tail-vein injection of human Umbilical-cord mesenchymal stem cells. *Int J Med Sci*. 2020;17(5):591–8.
67. Hoogwerf BJ, Sfera J, Donley BG. Donley, Diabetes mellitus—overview. *Foot Ankle Clin*. 2006;11(4):703–15.
68. Yuan S, Liu Y, Zhu L. Vascular complications of diabetes mellitus. *Clin Exp Pharmacol Physiol*. 1999;26(12):977–8.
69. Ajoalabady A, Liu S, Klionsky DJ, Lip GYH, Tuomilehto J, Kavalakatt S, et al. ER stress in obesity pathogenesis and management. *Trends Pharmacol Sci*. 2022;43(2):97–109.
70. Hetz C, Saxena S. ER stress and the unfolded protein response in neurodegeneration. *Nat Rev Neurol*. 2017;13(8):477–91.
71. Zundell JA, Fukumoto T, Lin J, Fatkhudinov N, Nacarelli T, Kossenkova AV, et al. Targeting the IRE1α/XBP1 Endoplasmic Reticulum Stress Response Pathway in ARID1A-Mutant Ovarian Cancers. *Cancer Res*. 2021;81(20):5325–35.
72. Zu K, Bihani T, Lin A, Park YM, Mori K, Ip C. Enhanced selenium effect on growth arrest by BiP/GRP78 knockdown in p53-null human prostate cancer cells. *Oncogene*. 2006;25(4):546–54.
73. Bernales S, Papa FR. Intracellular signaling by the unfolded protein response. *Annu Rev Cell Dev Biol*. 2006;22:487–508.
74. Song S, Hu T, Shi X, Jin Y, Liu S, Li X et al. ER stress-perturbed intracellular protein O-GlcNAcylation aggravates podocyte injury in diabetes nephropathy. *Int J Mol Sci*. 2023;24(24).
75. Yuan M, Gong M, He J, Xie B, Zhang Z, Meng L, et al. IP3R1/GRP75/VDAC1 complex mediates endoplasmic reticulum stress-mitochondrial oxidative stress in diabetic atrial remodeling. *Redox Biol*. 2022;52:102289.
76. Wang W, Zhang J. Teneligliptin alleviates diabetes-related cognitive impairment by inhibiting the endoplasmic reticulum (ER) stress and NLRP3 inflammation in mice. *Aging*. 2023;16(9):8336–47.
77. Zhong Y, Li J, Chen Y, Wang JJ, Ratan R, Zhang SX. Activation of endoplasmic reticulum stress by hyperglycemia is essential for Müller cell-derived inflammatory cytokine production in diabetes. *Diabetes*. 2012;61(2):492–504.
78. Perico N, Remuzzi G, Griffin MD, Cockwell P, Maxwell AP, Casiraghi F, et al. Safety and preliminary efficacy of mesenchymal stromal cell (ORBCEL-M) therapy in diabetic kidney disease: a randomized clinical trial (NEPHSTROM). *J Am Soc Nephrol*. 2023;34(10):1733–51.
79. Yang T, Zhang N, Yang N. Single-cell sequencing in diabetic retinopathy: progress and prospects. *J Transl Med*. 2025;23(1):49.

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