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MicroRNA-22-3p Regulates the Apoptosis of Lens Epithelial Cells Through Targeting KLF6 in Diabetic Cataracts

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Purpose: The purpose of this study was to identify novel abnormally expressed microRNAs (miRNAs) and their downstream target in diabetic cataract (DC).

Methods: General feature, fasting blood glucose, glycosylated hemoglobin, and type A1c (HbA1c) expression level of patients were collected. DC capsular tissues were obtained from patients and the lens cells (HLE-B3) exposed to different concentrations of glucose were used to simulate the model in vitro. Both mimic and inhibitor of *miR-22-3p* were transferred into HLE-B3 to up- and downregulate *miR-22-3p* expression, respectively. The cellular apoptosis was evaluated by quantitative real-time polymerase chain reaction (qRT-PCR), Western blot, and immunofluorescence. The downstream target gene of *miR-22-3p* was identified by dual luciferase reporter.

Results: In DC capsules and HLE-B3 under hyperglycemia, *miR-22-3p* showed a significant downward trend. The expression of *BAX* was upregulated and the *BCL-2* was downregulated following high glucose. The expression of *BAX* was significantly down- or upregulated in HLE-B3 cells following transfection of mimic or inhibitor of *miR-22-3p*, respectively. Conversely, *BCL-2* was significantly increased or decreased. Dual luciferase reporter assay showed that *miR-22-3p* directly targeted Krüppel Like Factor 6 (*KLF6*) to regulate cell apoptosis. In addition, the expression of *KLF6* were significantly up- or downregulated following transfection of inhibitor or mimic of *miR-22-3p*.

Conclusions: This study suggested that *miR-22-3p* could inhibit lens apoptosis by targeting *KLF6* directly under high glucose condition. The *miR-22-3p/KLF6* signal axis may provide novel insights into the pathogenesis of DC.

Translational Relevance: Differential expression of *miR-22-3p* may account for the pathogenesis of DC and lead to a new therapeutic strategy for DC.

Introduction

Cataract is a main cause of blindness, especially in developing countries.[1] Over the past decades, understanding of the pathogenesis and risk factors of cataract has increased.[1,2] In addition to irreversible risk elements, such as aging, income, sex, race, and myopia,[2,3] the relation between cataract and systemic disease has attracted more attention of many researchers currently, especially diabetes mellitus (DM).[1] Diabetic cataract (DC) is characterized by higher incidence, earlier development, and more complications,[4,5] where high glucose levels can promote its development. The progression of DC has been reported to be involved in complicated pathological mechanisms, such as oxidative stress, autoimmunity, epithelial-mesenchymal transition (EMT), and lens epithelial cells (LECs) apoptosis.[6–10] The generation of polyols from glucose by aldose reductase (AR) was the initial mechanism in DC formation. It is likely that the LECs apoptosis, oxidative stress, and autoimmune theory are the complex mechanism of DC formation.[9] Thus, exploring the

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mechanism of LECs apoptosis under high hyperglycemia is significant to understand the pathology of DC.

MicroRNAs (miRNAs) fall into the category of small, single-stranded, non-protein coding RNAs of about 18 to 23 nucleotides in length, which interact with the 3′-untranslated regions (3′-UTR) of downstream messenger RNAs (mRNAs) and affect the expression of mRNA at the post-translational level, such as mRNA degradation or translational repression.[11,12] A growing body of evidence suggested that miRNAs could have been detected in various ocular tissues and had an important effect on the apoptosis, proliferation, and stress response of multiple ocular cells.[11–13] Studies on cataract also confirmed that miRNAs and their downstream binding genes were involved in regulating LECs' function and significantly affect disease progression.[11,14–16] In recent years, accumulating evidence has showed that abnormal expression of miRNAs, such as *miR-34a*, *miR-15a*, *miR-16-1*, and *miR-125b*, were related to abnormal apoptosis of LECs during the pathogenesis of cataract.[11,17–19] Thus, miRNA's regulation of LECs apoptosis through the binding target gene may be essential and the fundamental mechanisms during the development of the cataract formation in DM.

MiR-22-3p, originally discovered as a tumor suppressor,[20] has recently been linked to DM and various ophthalmic diseases,[21] such as fibrotic cataract[22] and retinal pigment epithelial injury.[23] It was found that *miR-22* was significantly decreased in the H9c2 embryonic cardiac myoblast cell line induced by high glucose, whereas upregulation of *miR-22* could reduce the oxidation and apoptosis of diabetic cardiomyopathy by targeting *Sirt1*.[24] Wang et al.[22] identified that *miR-22-3p* is a critical regulator of lens fibrosis, whose research indicated that *miR-22-3p* was significantly downregulated in posterior capsule opacification cataract and the direct target of *miR-22-3p*, *HDAC6*, was activated under such conditions and contributed to lens fibrosis. More importantly, Liu et al.[14] found that high glucose could induce a decreasing trend in the expression of *miR-22* in lens epithelial cells, indicating that *miR-22* may be a protective factor in DC. However, the specific effect and mechanism of *miR-22-3p* on the progression of DC and the downstream target gene of *miR-22-3p* remains unclear in DC. In this study, we demonstrated that *miR-22-3p* could inhibit the apoptotic progress in LECs by targeting Krüppel Like Factor 6 (*KLF6*) directly under high glucose conditions, which provide novel views into the pathogenic mechanism of DC.

Materials and Methods

Acquisition of Clinical Tissue Samples

Fresh human lens anterior capsular tissues from 20 cases with age-related cataract (ARC) and 20 cases with DC were collected respectively from the Department of Ophthalmology, The Affiliated Yantai Yuhuangding Hospital of Qingdao University (Yantai, Shandong, China). This study was approved by the Ethic Committee of the Affiliated Yantai Yuhuangding Hospital of Qingdao University (No. 2022-206). Patients with ARC was diagnosed by professional ophthalmologists and placed as controls. Patients with DC were diagnosed by professional endocrinologists and ophthalmologists. Patients with a history of systemic metabolic diseases, ocular surgery, or combined with other ocular diseases other than diabetic retinopathy (DR) were excluded from this study. Informed consent was obtained from patients and their families prior to the collection of all clinical tissue samples. After the patients were hospitalized, we collected the general clinical information. The value of fasting blood glucose and glycosylated hemoglobin type A1c (HbA1c) were provided by the Department of Laboratory Medicine.

Cell Culture and Treatment

The human lens epithelial B3 (HLE-B3) cell lines were kindly donated by Eye Hospital of Wenzhou Medical University. HLE-B3 cells were cultured in Roswell Park Memorial Institute1640 (RPMI1640; Biological Industries, Israel) added with 10% fetal bovine serum (Biological Industries, Israel), penicillin (100 U/mL; Biological Industries, Israel), and streptomycin (100 µg/mL) (Sigma, USA) at 37°C cell culture chamber containing 5% CO_2. HLE-B3 cells were cultured under different concentrations of glucose (50, 80, and 100 mmol/L) to simulate the high glucose microenvironment in human eyes, and 5.5 mmol/L glucose served as a control for the other groups. All cells with different glucose concentrations were cultured at 37°C for 48 hours.

Transfection of MicroRNA

When HLE-B3 cells grew to 80% to 90% density, they were treated with trypsin (Biological Industries, Israel) digestion. Then, these cells were plated at an approximate density of 1×10^6 cells per well in 6-well plates. Then, 24 hours after that, *miR-22-3p* mimic, mimic negative control (mimic NC), inhibitor, and inhibitor negative control (inhibitor NC; Guangzhou

Table 1. Primer Sequences of MiRNA and MRNA Utilized for QRT-PCR

The Name of the Primer		Sequence
miR-22-3p	F	5′-AAGCTGCCAGTTGAAGAACTGT-3′
	R	5′-CAGTGCGTGTCGTGGAGT-3′
KLF6	F	5′-GGAGGAGACAGTGGTGTGG-3′
	R	5′-CATGTGGAAGTGTCGGTTCA-3′
BAX	F	5′-TTTCCGAGTGGCAGCTG-3′
	R	5′-CAAGTAGAAAAGGGCGACAAC-3′
BCL-2	F	5′-GGATGCCTTTGTGGAACTGT-3′
	R	5′-CACTTGTGGCTCAGATAGGC-3′
U6	F	5′-CTCGCTTCGGCAGCACA-3′
	R	5′-AACGCTTCACGAATTTGCGT-3′
GAPDH	F	5′-GTGCCAGCCTCGTCTCATAG-3′
	R	5′-CTTTGTCACAAGAGAAGGCAG-3′

RiboBio Co., China) were transfected into cells, respectively, with Lipofectamine RNAiMAX (Thermo Fisher Scientific, USA) following the manufacturers' recommendations. According to the instructions, cells were transfected at a final concentration of 50 nM for *miR-22-3p* mimic, *miR-22-3p* mimic NC and 100 nM for *miR-22-3p* inhibitor, and *miR-22-3p* inhibitor NC. The group only added transfection agent was named as the control. All cells were replaced with fresh complete culture medium 6 hours after transfection. After 1 day, the transfected cells were cultured in high glucose (HG; 50 mmol/L) medium for an additional 48 hours.

RNA Isolation and Quantitative Real-Time Polymerase Chain Reaction

MicroRNA from the anterior capsular tissues of the patient's lens was isolated with miRNA First Strand cDNA Synthesis (Tailing Reaction; Sangon Biotech, China). Total RNA of cells or tissues were purified using 5 × All-In-One RT MasterMix with AccuRT (ABM, Canada) based on the reagent manufacturer's guidebook, and reverse transcribed using ChamQ Universal SYBR Qpcr Master Mix (Vazyme, China). The amplification was performed on Applied Biosystems 7500 Series Real-Time PCR system (Thermo Fisher Scientific, USA). *U6* and *GAPDH* served as the housekeeping genes for miRNA and mRNA detection separately. Three biological replicates were used for evaluating the gene expression, which relative to the reference gene expression via the $2^{-\Delta\Delta CT}$ method. Table 1 lists the primers we used in the present study.

Western Blot

The tissue samples were homogenized in a tissue grinder. The tissue homogenates were placed on ice, lysed with protein lysis buffer (RIPA: PMSF = 50:1; Beyotime Biotechnology, China) for 30 minutes at 4°C. The lysates were centrifuged for 20 minutes at a speed of 16,000 rpm. The cell lysates were collected into new Eppendorf tubes. Protein concentration was detected by BCA Protein Assay Kit (Coolaber, China). Then, 20 ug of protein were resolved with 10% SDS-PAGE (Sparkjade, China) and electro-transferred to nitrocellulose membrane, which was subsequently blocked with 5% defatted milk for 1 hour at room temperature and coated with specific primary antibodies against β-tubulin (1:1000; Affinity, USA), BAX (1:3000; Proteintech, China), BCL-2 (1:2000; Abcam, USA), and KLF6 (1:2000; Proteintech, China) for overnight incubation at 4°C. The membrane was probed by the secondary antibody for 1 hour at 37°C. The electrochemiluminescence (ECL) kit (Affinity, USA) was dropped on the membranes and put into the chemiluminescence instrument ChemiScope 6200 Touch (Clinx, China) for automatic luminescence imaging. Image J software (version 1.53t; URL link: https://imagej.nih.gov/ij/download.html) was used for image analysis and processing.

Cell Counting Kit-8 Assays

The Cell Counting Kit-8 (CCK8; Dojindo, Japan) experiment was used to detect cell viability. Approximately 2000 cells/well were cultured into a 96-well plate. After 48 hours of incubation, a mixture of 90 µL medium and 10 µL CCK8 was added to each well. Then, cells were incubated at 37° for 2 hours and protected from light. Finally, the cell viability was detected with the absorbance at 450 nm using a microplate reader.

Hoechst 33258 Staining

The HLE-B3 cells were planted in HG (50 mmol/L) for 48 hours after transfected with *miR-22-3p* mimic, inhibitor, and corresponding NCs. After aspirating the culture medium, cells were fixed with 500 µl stationary liquid for 20 minutes at room temperature. Cells were then slowly and gently washed in precooled phosphate-buffered saline (PBS) and stained with 500 µL Hoechst 33258 (Beyotime Biotechnology, China) for 20 minutes under light exclusion. The cells were washed with PBS again, and then fluorescence quencher was added. Fluorescence microscope Olympus IX51 (Olympus Corporation, Japan)

were used to examine morphological changes in the cells.

Luciferase Reporter Assay

First, TargetScan (http://www.targetscan.org/), an online website tool, was used to predict possible targets of *miR-22-3p*. The results were subsequently confirmed by dual luciferase reporting assays. *MiR-22-3p* mimic and corresponding negative control were co-transferred with *KLF6*-3′UTR wild plasmids or *KLF6*-3′UTR mutant plasmids into HLE-B3 cell lines to assess whether *miR-22-3p* could target to the downstream *KLF6* gene. Cells were collected 48 hours after transfection and lysed to detect Luciferase activity according to the instructions of the luciferase detection kit (Vazyme, China).

Statistical Analysis

Statistical analysis was performed using GraphPad Prism version 8.0 software. All experimental data were presented as mean ± SD, with each experiment performed repeatedly in triplicate. Experiments with two groups were analyzed using unpaired Student's *t*-test when the data are normally distributed or using Wilcoxon test when the data are not normally distributed. Experiments with more than two groups were analyzed using 1-way ANOVA. The P values < 0.05 were considered statistically significant.

Results

General Feature of Age-Related Cataract and Diabetic Cataract

The opacity of the lens in DC mostly occurs in the anterior capsule (Fig. 1A, left) and the posterior capsule (see Fig. 1A, right). The clinical information of patients with ARC and DC is shown in Table 2. No significant differences were found between ARC and DC in age ($P = 0.748$). However, fasting blood glucose and glycosylated HbA1c in the DC group were significantly higher than those in the ARC group ($P < 0.001$; Figs. 1B, 1C).

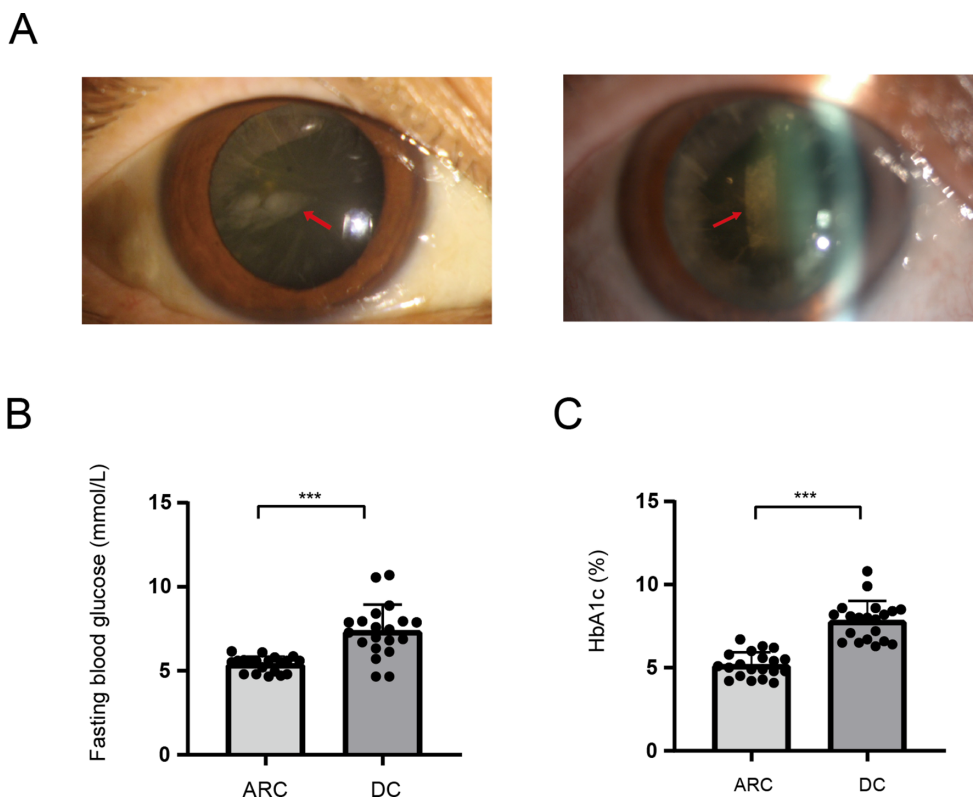

Figure 1. General feature of age-related cataract and diabetic cataract. (**A**) Representative image of the lens capsular opacification in patients with DC. The opacity of the lens in DC occurred in the anterior capsule (*left*) and posterior capsule (*right*). (**B, C**) The fasting blood glucose and HbA1c levels in the ARC group and the DC group. DC, diabetic cataract; ARC, age-related cataract; $n = 20$, ***$P < 0.001$).

Table 2. General Feature of the Two Groups (Mean ± SD)

	ARC	DC	*P* Value
Sex (male/female)	8/12	5/15	0.324
Age, y	66.14 ± 9.72	64.45 ± 10.11	0.748
History of diabetes, y	–	13.10 ± 9.67	–
Fasting blood glucose (mmol/L)	5.39 ± 0.44	7.37 ± 1.57	<0.001
HbA1c (%)	5.18 ± 0.73	7.95 ± 1.29	<0.001

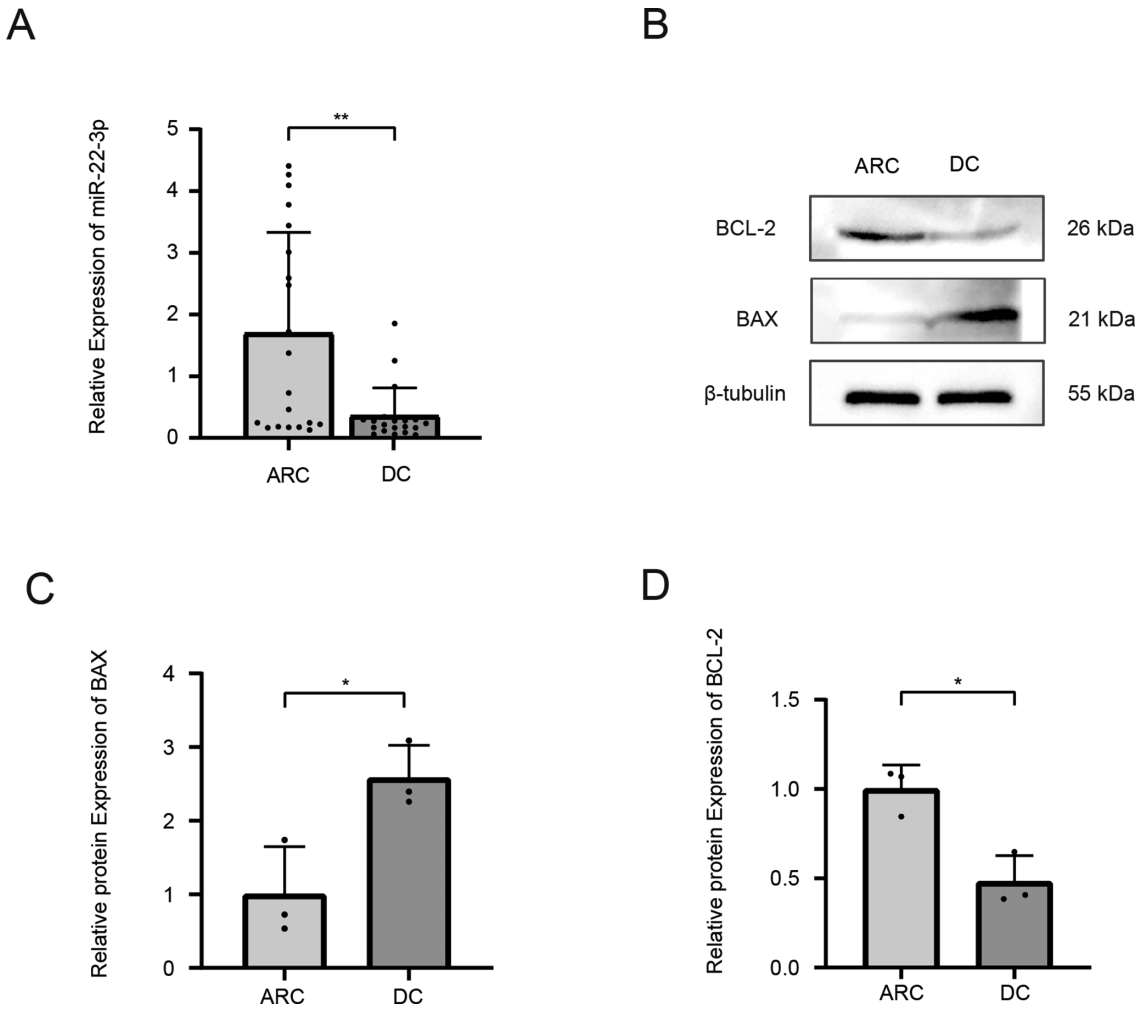

Figure 2. Expression of *miR-22-3p* and markers for apoptosis in human diabetic cataract LECs. (**A**) The qRT-PCR results of miRNA in capsule tissues from patients with DC and patients with ARC ($n = 20$, $^{**}P < 0.01$). (**B, C, D**) The protein expression of apoptosis makers detected by Western blot ($n = 3$, $^{*}P < 0.05$).

MiR-22-3p and Apoptosis-Related Markers' Expression in Human DC Tissues

We collected ARC and DC tissues from the patients who accepted cataract surgery to detect the expression level of *miR-22-3p* and apoptosis-related markers. The results suggested that *miR-22-3p* expression in the DC group was downregulated compared to the ARC group by real-time polymerase chain reaction (qRT-PCR; Fig. 2A). The expression of the pro-apoptotic marker BAX was significantly upregulated in patients with DC, whereas BCL-2, a marker of anti-apoptosis, decreased significantly through Western blot (Figs. 2B, 2C, 2D).

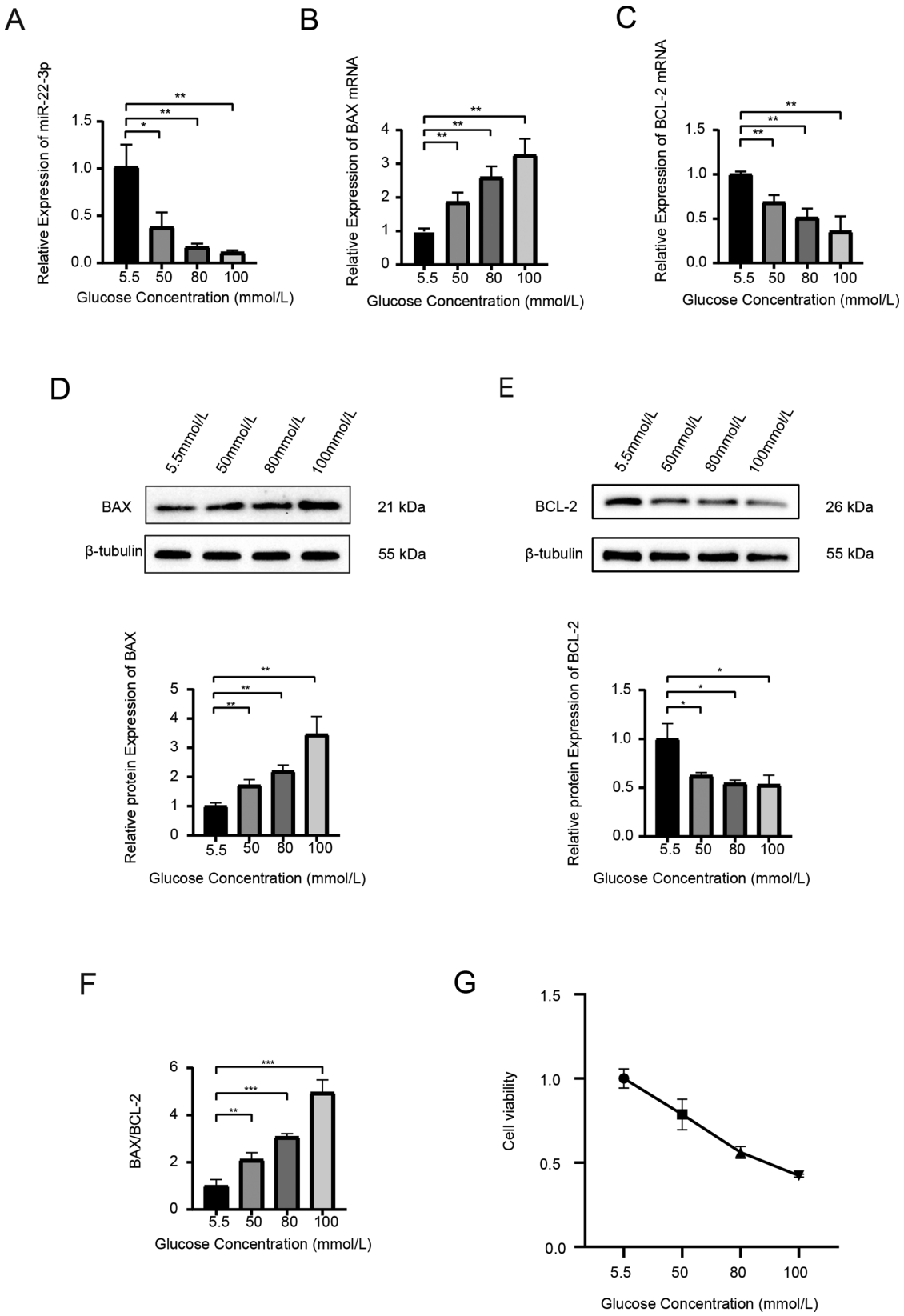

Figure 3. Expression level of *miR-22-3p* and apoptosis in vitro. The relative expression of *miR-22-3p* in HLE-B3 under different glucose concentration (**A**); *BAX* mRNA and protein relative expression levels under different glucose concentration (**B, D**); *BCL-2* mRNA and protein relative expression levels under different glucose concentration (**C, E**); The protein ratio of BAX/BCL-2 (**F**). The cell viability of HLE-B3 under HG detected by the CCK-8 assay(**G**) ($n = 3$, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$).

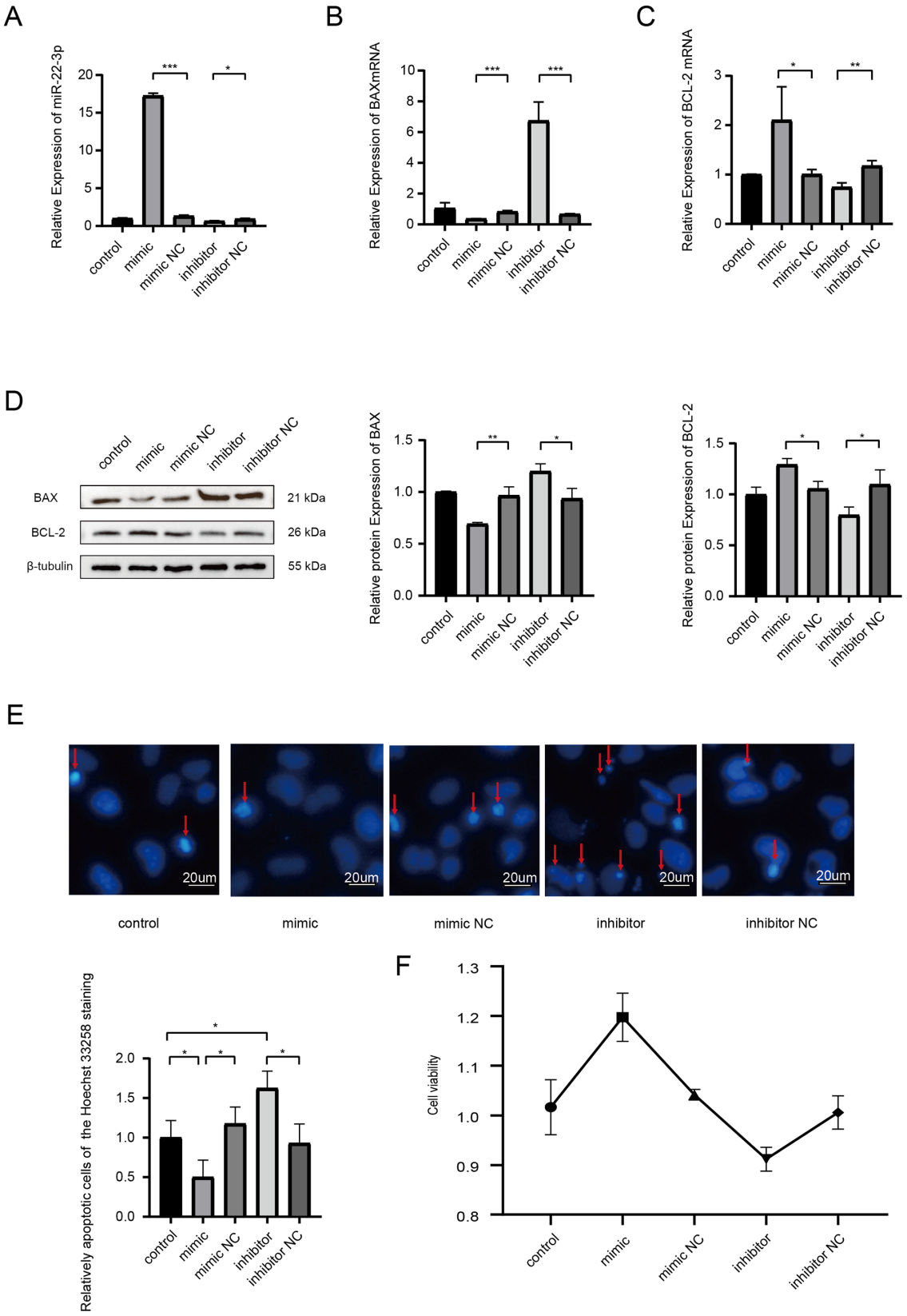

Figure 4. *MiR-22-3p* repressed LECs apoptosis in vitro **(A)**. Validation of transfection efficiency of *miR-22-3p*. (**B, C, D**) The mRNA and protein expression of *BAX* following *miR-22-3p* mimic or inhibitor transfection; (**C, D**) The mRNA and protein expression of the *BCL-2* following *miR-22-3p* mimic or inhibitor transfection. (**E**) Hoechst 33258 staining was performed to show the apoptosis of cells transferred with *miR-22-3p* mimic or inhibitor. (**F**) The activity of cells transferred with *miR-22-3p* mimic or *miR-22-3p* inhibitor ($n = 3$, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$).

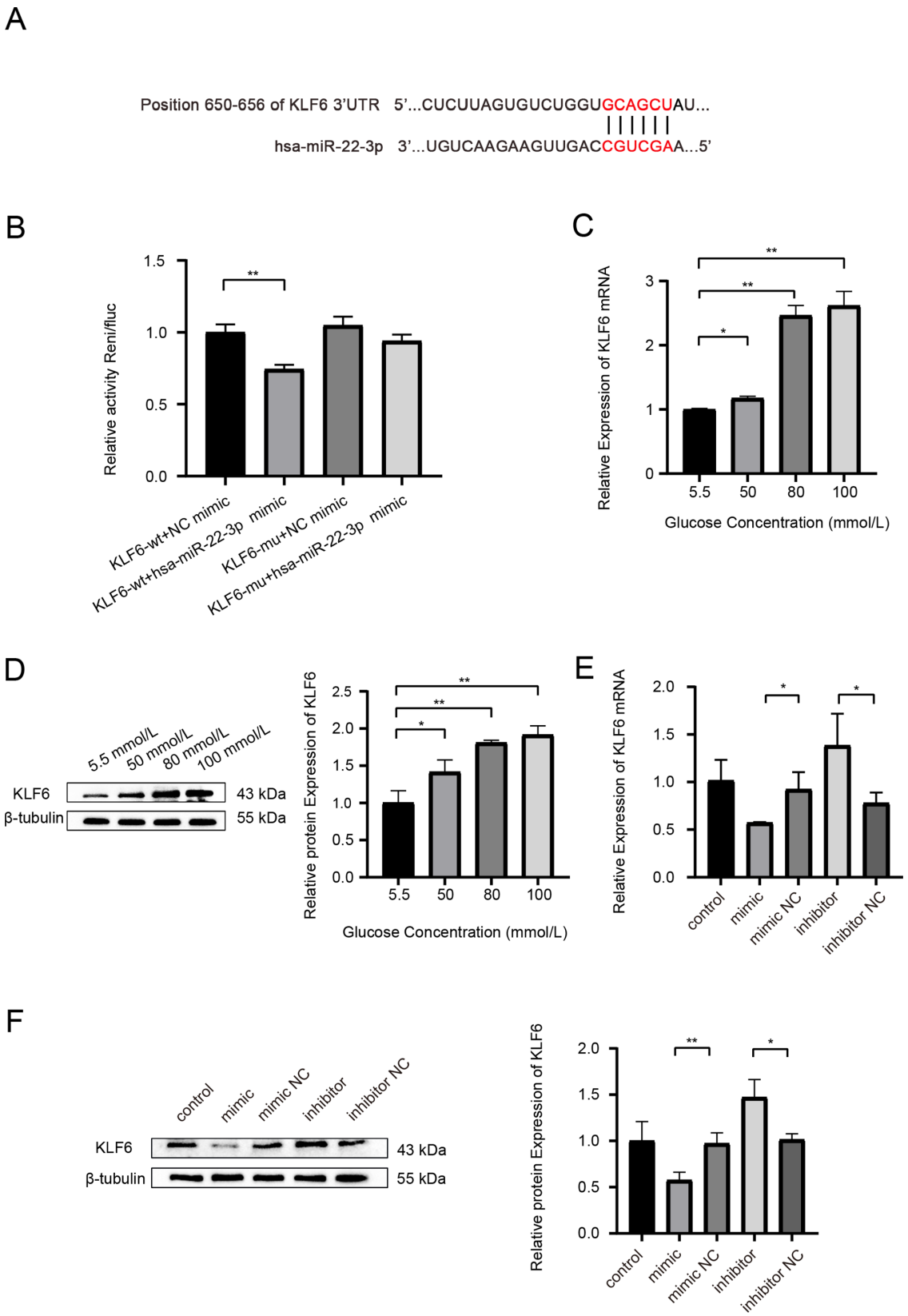

Figure 5. *MiR-22-3p* worked by targeting *KLF6* in vitro (**A**)**.** Online websites prediction results of the *miR-22-3p* target sequence position. (**B**) Luciferase reporter assay showed that the luciferase activity of *KLF6* 3′-UTR-wt significantly decreased with *miR-22-3p* transfection, comparing to that of the NC mimic or *KLF6* 3′- UTR-wt group. (**C, D**) The mRNA and protein expression of *KLF6* following the increase of sugar concentration. (**E, F**) *KLF6* mRNA and protein levels following the *miR-22-3p* mimic or inhibitor transfection ($n = 3$, $^{*}P < 0.05$, $^{**}P < 0.01$).

Expression of *MiR-22-3p* and Apoptosis In Vitro

To further confirm this result, HLE-B3 cells were cultured with HG to determine the expression of *miR-22-3p* and apoptosis levels in vitro. The HLE-B3 cells were cultured with 50 mmol/L, 80 mmol/L, and 100 mmol/L D-glucose to mimic the DC pathological microenvironment, with 5.5 mmol/L D-glucose as the control condition (normal glucose [NG]). From the results of qRT-PCR, it can be found that the expression of *miR-22-3p* was decreased significantly with the increase of glucose concentration (Fig. 3A). The expression of *BAX* increased at both transcription and protein levels under the HG condition (Figs. 3B, 3D), whereas the *BCL-2* expression decreased (Figs. 3C, 3E). The protein ratio of *BAX*/*BCL-2* showed the cells in HG are sensitive to apoptosis (Fig. 3F). The cell viability of HLE-B3 cultured with different concentrations of glucose for 48 hours was decreased with the increase of glucose concentrations (Fig. 3G). Therefore, these results showed that *miR-22-3p* expression level was negatively correlated with the concentration of glucose, whereas the apoptosis level was upregulated under the HG condition.

Downregulation of *MiR-22-3p* Resulted in LECs Apoptosis In Vitro

For the purpose of investigating the potential mechanism of *miR-22-3p* on apoptosis of LECs, *miR-22-3p* mimic, inhibitor, and their respective negative controls were transfected into HLE-B3 cells followed by treatment of HG. First, transfection efficiency was confirmed by qRT-PCR (Fig. 4A). Then, the gene and protein levels of *BAX* and *BCL-2* were detected. As illustrated in Figures 4B, 4C, and 4D, the expression of *BAX* was downregulated both at mRNA and protein level in *miR-22-3p* mimic group under HG (50 mmol/L glucose), whereas the *BCL-2* was upregulated. The *BAX* was upregulated after *miR-22-3p* inhibitor transfection both at mRNA and protein level, whereas the *BCL-2* was downregulated, compared with inhibitor NCs under HG (50 mmol/L glucose). In addition, the Hoechst 33258 staining suggested that the apoptotic cells in the *miR-22-3p* mimic group decreased under the same microscope field, whereas the apoptosis level in the *miR-22-3p* inhibitor group increased (Fig. 4E). The viability of cells analyzed by the CCK-8 experiment, can be seen that the activity of cells in the *miR-22-3p* mimic group increased. On the contrary, the activity of cells in the *miR-22-3p* inhibitor group decreased (Fig. 4F). Accordingly, these results suggested that *miR-22-3p* inhibition contributed to LECs apoptosis in DC.

MiR-22-3p Exerts Anti-Apoptosis Effect by Targeting *KLF6*

It has been found that *miR-22-3p* showed a decreasing trend in DC, thus the regulatory mechanism of *miR-22-3p* in DC was further investigated. *KLF6* could be served as a potential target of *miR-22-3p* through the analysis of the online website (TargetScan, http://www.targetscan.org/; Fig. 5A). Dual luciferase reporter assays were applied to determine whether *miR-22-3p* target the 3′-UTR of *KLF6* mRNA. The results showed that relative level of luciferase activity was significantly inhibited when *KLF6* 3′UTR-wt and *miR-22-3p* mimic were co-transfected into cells, compared with NC mimic or *KLF6* 3′UTR-mu group, which indicated *KLF6* as a representative direct binding target of *miR-22-3p* (Fig. 5B). In addition, we further investigated the regulatory effect of *miR-22-3p* on *KLF6* in vitro. It was found that the *KLF6* mRNA as well as protein expression increased significantly under the high concentrations of glucose (Figs. 5C, 5D). The expression of *KLF6* was largely inhibited after that *miR-22-3p* mimic were transferred into LECs compared with the respective NCs. On the contrary, the downstream gene *KLF6* was increased after *miR-22-3p* inhibitor transfection (Figs. 5E, 5F). These findings demonstrated that *miR-22-3p* could inhibit lens apoptotic progression by targeting *KLF6* directly to affect the DC development.

Discussion

Many epidemiology studies have been reported that DM is a chronic systemic disease, whose incidence keeps increasing over time.[25] Cataract is one of the main reasons for blindness globally, especially in people with diabetes who are two to five times higher in incidence and much earlier in development.[4] The aberrant expression of miRNAs during the cataract development has been reported by many studies, and the role of miRNAs in the formation of DC has attracted more and more attention.[14–16,26] In previous studies, microRNAs, such as *miRNA-199a-5p* and *miRNA-30a*, have been reported to affect the development and progression of DC by targeting downstream genes.[14,16] The development of cataract in patients with diabetes could be partly due to the aging process. In the present study, to control the influence of age bias, we choose the lens capsule of patients with age-related cataract, but not healthy people, as the control samples.

We identified that the expression of *miR-22-3p* was downregulated in the anterior capsules of patients with DC, which was confirmed in vitro as well. We further confirmed that *miRNA-22-3p* regulated the process of apoptosis of LECs by targeting *KLF6*, hoping to provide a basis for the understanding of pathogenesis and development of DC therapies in clinical practice.

MiR-22-3p is an evolutionarily highly conserved microRNAs in vertebrates,[27] which was initially considered as a tumor suppressor gene and played a crucial role in the development and progression of lung cancer, breast cancer, and so on.[28,29] A growing number of studies have demonstrated that *miR-22-3p* was closely related to the hyperproliferative diseases, such as cancer and fibrosis. Recent studies have showed that *miR-22-3p* could inhibit the potential ability of cell proliferation and induce cell apoptosis in several types of tumors.[28,30] In fibrotic cataract, *miR-22-3p* acted as an antifibrotic factor to inhibit the progression of lens fibrosis by targeting downstream gene *HDAC6* and promoting α-tubulin acetylation.[22] It was also closely associated with diabetes and diabetes-related diseases. In diabetes-induced salivary gland dysfunction and HG treated human submandibular glands (SMGs) C6 cells, the *miR-22-3p* showed a decreasing trend and the paracellular permeability decreased in SMG-C6. The overexpression of *miR-22-3p* could reduce the inhibition of paracellular permeability induced by HG.[31] It was found that *miR-22-3p* was downregulated in in the islet tissues of mice with gestational diabetes mellitus (GDM). Upregulation of *miR-22-3p* could improve hepatic insulin resistance in mice with GDM by regulating cytokine signaling 3 (*Socs3*).[32] In diabetic cardiomyopathy, *lncMALAT1* participates in the process of cardiomyocyte apoptosis through the enhancer of zeste 2 polycomb repressive complex 2 subunit (*EZH2*)/*miR-22*/*ATP* binding cassette subfamily a member 1 (*ABCA1*) signaling pathway, and the aberrant upregulation of *miR-22* can alleviate cardiomyocytes injury, which was in keeping in line with the fact that *miR-22-3p* reduces the apoptotic damage of LECs in our experiment.[33] Although *miR-22* has been shown to be involved in multiple metabolic pathways of glucose,[34] how *miR-22-3p* affect DC progression remains a mystery, and the specific mechanism of *miR-22-3p* in the progression of DC still was an attractive topic.

Apoptosis was a common cellular pathological mechanism for initiation of DC, which altered the balance required for LECs homoeostasis and led to cataract formation.[9] The presence of a high-glucose microenvironment may serve to further strengthen or reinforce these effects by inducing endoplasmic reticulum (ER) stress and causing oxidative stress damage to lens fibers.[9] Moreover, recent studies also found that *microRNA-211* upregulation promotes the proliferation and inhibits apoptosis of LECs in DC mice by targeting downstream gene *SIRT1*.[15] Likewise, the study proved that *miR-22-3p* played an inhibitory role in the apoptosis process of LECs by targeting *KLF6* in patients with DC.

The specificity protein/Krüppel-like factor (SP/KLF) transcription factor family played several important roles in a variety of biological cellular functions. *KLF6*, as an important member of this family, had a special zinc finger structure and played a special role in cell proliferation and apoptosis.[35] It was a nuclear transcriptional regulatory factor initially recognized in placental cells and subsequently found to be ubiquitously expressed in various tissues.[36] *KLF6* has been demonstrated to exert an antineoplastic effect through various mechanism,[37] such as induction of apoptosis,[38] cell-cycle arrest,[39,40] and inhibition of angiogenesis.[41] In addition to cancers, the roles of *KLF6* in ophthalmic diseases have attracted increasing attention in recent years. Nakamura et al. initially reported that *KLF6* was detected in the mouse cornea and lens and played a core effect in lens development.[42] Subsequently, it was found that *KLF6* aberrant upregulation led to LECs apoptosis under ultraviolet radiation-B by activating transcription factor 4 (*ATF4*) - activating transcription factor 3 (*ATF3*) - DNA Damage inducible transcript 3 (*CHOP*) axis,[43] and *miR-181* could promote the ability to proliferate and migrate of retinal endothelial cells by targeting *KLF6* in DR.[44] Moreover, recent research has revealed that *miR-22-3p* also could moderate fatty infiltration involved in muscle atrophy by regulating target KLF6 gene, and the *miR-22-3p*/*KLF6*/matrix metallopeptidase 14 (*MMP-14*) axis could be likely to act as a potential therapeutic target for muscle degenerative diseases.[45] Therefore, our research is the first time to find that *miRNA-22-3p* could regulate apoptosis in DCs through targeting *KLF6*, which complements the possible pathogenic mechanism of *miR-22-3p*/*KLF6* axis in DC disease development.

In conclusion, our work indicated that *miR-22-3p* was down expression in DC, and regulated cell apoptosis by targeting the *KLF6* gene. *MiR-22-3p*/*KLF6* axis could be served as a novel underlying pathway for the diagnosis and therapy of DC. Nevertheless, further experimental work in vivo and clinical researches are needed to validate our findings, which is a limitation of this study.

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