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Original Article

miR-143 promotes cell proliferation, invasion and migration via directly binding to BRD2 in lens epithelial cells

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Abstract: Objective: Cataract causes the greatest number of blindnesses worldwide. This study aims to investigate the role of miR-143 in lens epithelial cells. Methods: Clustering analysis was conducted to systematically compare miRNA expression levels across cataract and myopia. The levels of miR-143 and Bromodomain containing 2 (BRD2) were determined using real-time quantitative PCR (RT-qPCR) assay in lens epithelial cells. Transwell and wound healing assays were conducted to detect cell invasive and migratory abilities. The regulation relationship between MiR-143 and BRD2 was assessed using dual-luciferase reporter gene assays. BRD2 was knocked down using siRNA-BRD2, and siRNA-BRD2, and miR-143 inhibitors were transfected into cells with lipofectamine 2000. Results: Through retrieving five databases, 2690 miRNAs were selected. Volcano plot results demonstrated that 200 miRNAs were differentially expressed between cataract and myopia, in which 152 miRNAs were upregulated and 48 miRNAs downregulated in myopia compared with cataract. MiR-143 was upregulated in cataract compared with myopia ($P<0.05$). MiR-143 inhibitor suppressed the proliferation, invasion and migration of lens epithelial cells (all $P<0.05$). Luciferase reporter assays confirmed that BRD2 was a miR-143 target gene in SRA01/04 cells. Knockdown of BRD2 promoted SRA01/04 cell proliferation, invasion and migration (all $P<0.05$). In addition, silencing of BRD2 partially reversed the functions of miR-143 inhibitor on proliferation, invasion and migration (all $P<0.05$). Conclusion: MiR-143 suppresses lens epithelial cell proliferation, invasion and migration by regulating BRD2, which may support a novel therapeutic strategy for cataract patients.

Keywords: Cataract, miR-143, BRD2, clustering analysis, invasion

Introduction

Cataracts, opacifications of the lens, are the leading cause of blindness worldwide [1]. Crystalline lenses, composed only of monolayers of lens epithelium and fibers arranged in an orderly manner, play a key role in focusing images on the retina [2]. Currently, although surgery is the sole successful remedy, the frequently occurred complication of posterior capsular opacification (PCO) may lead to the reoccurrence of visual axis opacification [3]. Globally, there are numerous eye disorders caused by lens fibrosis, including anterior subcapsular cataracts (ASC) and posterior capsule opacifications (PCO) [4]. Although the most common cause of cataract is aging, other reasons, such as trauma, medications and genetic predisposition, can also cause cataracts [5]. Thus, it is of great importance to explore the biomarkers for diagnosis and treatment of cataract. In this study, lens epithelium cell line served as a model for cataract to understand the basic properties of cataract.

MicroRNAs (miRNAs) are a group of small non-coding RNAs with 20-25 nucleotides in length [6]. MiRNAs can post-transcriptionally regulate gene expression via targeted binding to mRNAs' 3'-untranslated region (3'-UTR) [7]. Accumulating evidence demonstrated miRNAs disorders and aberrant expression in patients with various diseases, such as several cancers, Alzheimer's disease and cataract. Increasing studies have reported that a variety of miRNAs were abnormally expressed in cataract, including miR-22, miR-630, miR-261, and miR-26b [8-10]. MiR-143 is low expressed and functions

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as a tumor suppressor in a variety of cancers, including gastric cancer, pancreatic cancer, gallbladder cancer, and colorectal cancer [11-14]. MiR-143 can increase the radiosensitivity of breast cancer cells through fibroblast growth factor (FGF1) [15]. Overexpression of miR-143-3p was substantiated to inhibit the viability and promote cell apoptosis of cervical cancer cells, and also inhibited tumorigenicity *in vivo* [16]. Similarly, miR-143 inhibited cell metastasis, invasion, and epithelial-mesenchymal transition (EMT)-related genes and promoted cell apoptosis in gastric cancer [17]. However, the expression and functions of miR-143 are still unclear.

Bromodomain containing 2 (BRD2), at chromosome 6p21.3, encodes a transcriptional regulator that belongs to BET protein family [18]. During mitosis, BRD2 is associated with transcription complexes and exhibits specific affinity with the acetylated lysine-12 residue in histone H4 [19]. BRD2 was low expressed in 3T3-L1 adipocytes and inhibited adipogenesis through increasing extracellular signal-regulated kinase 1/2 (ERK1/2) phosphorylation [20]. The etiology of cataract formation is multifaceted and commonly attributed to various factors. LEC apoptosis serves as a prevalent cytological foundation for the pathogenesis of non-congenital cataracts. Research on age-related cataracts has consistently demonstrated a substantial increase in LEC apoptosis, thereby highlighting its significance in the advancement and progression of cataracts [21]. Changes in the processes of proliferation, migration, and adhesion within lens epithelial cells can potentially influence the development of cataracts [22]. Thus, our study aims to examine the effect of miR-143 on the progression of cataracts by quantifying the invasion and proliferation of lens epithelial cells.

Material and methods

Cell culture

Human lens epithelial SRA01/04 cell line was purchased from American Type Culture Collection (ATCC, Rockville, USA). Dulbecco's modified Eagle's medium/F-12 (DMEM, Gibico, Carlsbad, USA) with 20% fetal bovine serum (FBS, Thermo Fisher Scientific, NY, USA) was utilized for culturing the cells. To prevent contamination, the medium was supplemented with 100 U/mL penicillin and 100 μg/mL streptomycin. A 5% CO_2 atmosphere was used for culture at 37°C.

Cell transfection

GenePharma Technology (Shanghai, China) synthesized and commercially obtained specific small interfering RNA (siRNA) BRD2, scramble oligonucleotides, miR-143 mimic, and miR-143 inhibitor. The sequences were as follows: siRNA BRD2 5'-GGGCAGUACAUGAACAACUTT-3'; scramble oligonucleotides 5'-GCACAAUCAAGUCUAAACUTT-3'; mimic control 5'-UUCUCCGAACGUGUCACGUTT-3'; miR-143 mimic 5'-ATCCAGTGCAGGGTCCGAGG-3'; inhibitor control 5'-CAGUACUUUUGUGUAGUACAA-3' and miR-143 inhibitor 5'-AGGGAUUCCUGGGAAAACUGGAC-3. 10^6 cells per well were seeded into six-well plates and cultivated at 37°C in 5% CO_2 in an incubator for 24 hours until 80-90% cell confluence was reached. In order to transfect cells, a solution containing oligonucleotides at a final concentration of 50 nM or vectors weighing 2 mg was combined with 10 mL Lipofectamine 2000 and mixed for a duration of 10 minutes. The transfected cells were gathered for follow-up experiments after transfection.

miRNA sequencing and bioinformatics analysis

An extraction Kit (Haigene, Harbin, China) was used to extract the total RNAs. RNA quantification was performed through A Nanodrop 2000 bioanalyzer (Thermo Fisher Scientific). Reverse transcription of cDNA libraries was performed using the Illumina TruSeq RNA Sample Preparation Kit (Illumina, San Diego, CA, USA). Sequencing was performed on an Illumina HiSeq 2500 system using paired-end readings. CASAVA tool v1.8 (Illumina) and webservers FastQC were employed to obtain the raw read files and check the sequence reads quality. The purpose was to get miRNA sequences, so other ncRNAs, including siRNA, lncRNA, and circRNA, were filtered out. Subsequently, miRBase 22.0 (http://www.mirbase.org) and miRWalk (http://mirwalk.uni-hd.de) were applied to analyze the remaining sequences to detect the miRNAs. MiRanda database was employed to predict the target genes of miRNAs.

Cell viability assay

Cell counting Kit 8 (CCK-8, Beyotime, Jiangsu, China) was used to calculate cell viability. Cells were placed at a density of 2×10^3 cells/well in 200 μL growth medium and cultivated for 24, 48, 72 or 96 h in a 96-well plate. The cells were cultured at 37°C with 5% CO_2. Following a 2-hour incubation at 37°C, we measured the absorbance at 450 nm after adding 10 μL of CCK-8 solution to each well.

RNA extraction and real-time quantitative PCR (RT-qPCR)

Total RNA extraction kit (Tiangen Biotech, Beijing, China) was employed to extract total RNA from HLECs, following the guidelines provided by the manufacturer. The extracted RNA was quantified using a NanoDrop 2000 spectrophotometer (NanoDrop Technologies; Thermo Fisher Scientific, Inc.), which was stored at 80°C before use. First-strand cDNA was synthesized from 2.0 grams of total RNA using an M-MLV reverse transcriptase kit from Promega Corporation, and the cDNA was stored at 20°C before use. SYBR Premix Ex Taq™ (Takara Biotechnology Co., Ltd.) was utilized to carry out qPCR on StepOnePlus™ Real-Time PCR system (Applied Biosystems; Thermo Fisher Scientific, Inc.). The primers were: miR-143 Forward, 5'-GGGGTGAGATGAAGCACTG-3', Reverse, 5'-CAGTGCGTGTCGTGCAGT-3'; U6 Forward, 5'-CTCGCTTCGGCAGCACA-3', Reverse, 5'-AACGCTTCACGAATTTGCGT-3'; BRAD2 Forward, 5'-CCACGAAAAGACTTGCCTGA-3', Reverse, 5'-CAGCGTGCTTCTTTGAGAGC-3'; GAPDH Forward, 5'-TCCCTCAAGATTGTCAGCAA-3', Reverse, 5'-AGATCCACAACGGATACATT-3'. Thermocycling was performed by heating the cells to 95°C for three minutes, then cooling them to 62°C for 40 seconds, for 40 cycles. In the final dissociation stage, 95°C was applied for 15 s, 65°C was applied for 1 minute, and 95°C was applied for 15 s. The level of BRAD2 in SRA01/04 cell line was detected using GAPDH as an internal control. An analysis of relative gene expression was carried out using the $2^{-\Delta\Delta Ct}$ method.

Wound healing

SRA01/04 cells were seeded into 6-well plates to measure the ability of cell migration. After replacing fresh medium, a 10 uL pipette tip was used to scratch wounds across the monolayer cells. The images were captured, and the time of wound infliction was considered to be 0 h. After 48 h, a microscope connected to a digital camera was used to capture images of the cells.

Transwell assay

Transwell inserts (Corning, Beijing, China) were used for cell invasion assay. Transwell inserts with matrigel (Corning, Jiangsu, China) were placed in a 24-well plate and thus produced upper and lower chambers. In brief, the upper chambers were seeded with 200 μL HLE SRA01/04 cells, while the lower wells contained 500 μL medium with 10% FBS as the chemoattractant. Using cotton swabs, we moved the nonmigratory or noninvasive cells on the upper filter surfaces after culturing for 24 h. The lower surfaces of the filter were fixed for 30 minutes with paraformaldehyde and stained for 20 minutes with crystal violet. The invaded or migrated cells were counted using a microscope (Olympus Corporation, Tokyo, Japan).

Protein extraction and western blot analysis

The cellular proteins were extracted for western blot analysis. The protein concentration was determined using the BCA protein assay kit (Thermo Scientific, USA). Subsequently, a total of 100 μg of proteins were subjected to separation using a 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) technique. Next, the resulting gel was transferred onto a polyvinylidene fluoride (PVDF) membrane (Millipore, Billerica, MA, USA). After blocking the membrane with phosphate buffer saline (PBS), the primary antibody was incubated overnight at 4°C. Following three washes with tris buffered saline tween (TBST), secondary antibodies were introduced, and the membranes were subjected to shake for a duration of 2 h at room temperature. Next, the visualization of protein bands on the membranes was achieved using the ECL reagent (WBKLS0500; Merck KGaA, Darmstadt, Germany), followed by imaging with the ImageQuant LAS4000 mini analysis system (GE Healthcare Life Sciences, Little Chalfont, UK).

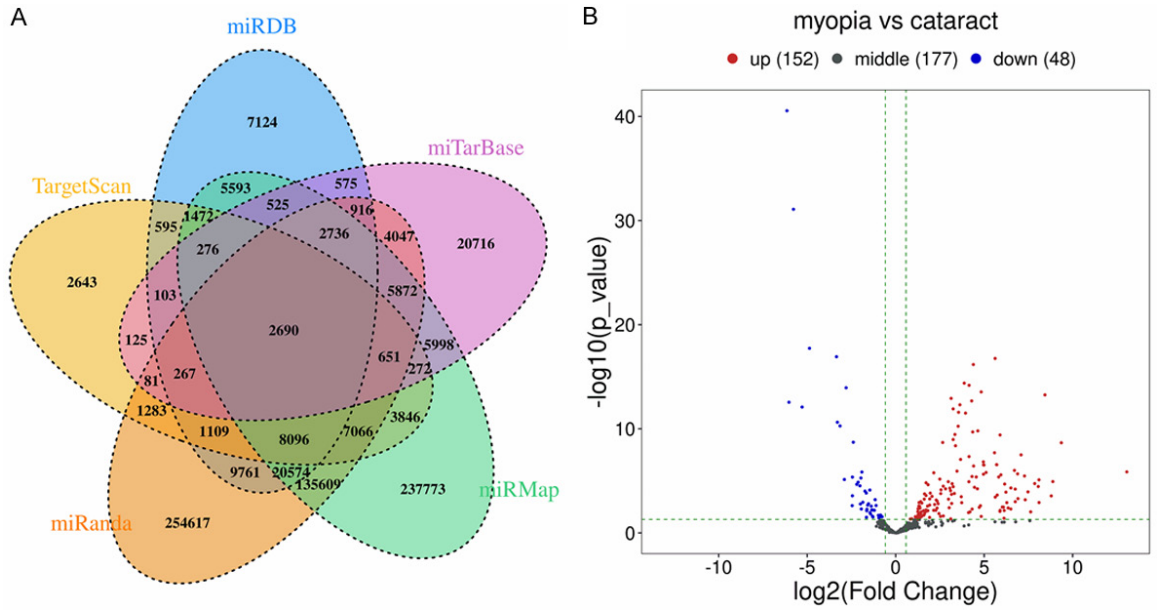

Figure 1. Identification of dysregulated miRNAs in cataract. A: Veen results showed that 2690 miRNAs were filtered out from five databases; B: Volcano results revealed that 200 miRNAs were differentially expressed between cataract and myopia, in which 152 miRNAs were upregulated and 48 miRNAs were downregulated in myopia compared with cataract.

Dual-luciferase reporter assay

TargetScan predicted the targeted correlation between miR-143 and BRD2. To verify this, the predicted miR-143 binding sequences on BRD2 mRNA 3'UTR were mutated from UGAUGUCA to AGUAGAG. HMGA1's wild-type and mutant 3'-UTRs were inserted into the pGL3 reporter vector by Youbio (Changsha, China), which were named pGL3-BRD2-wt and pGL3-BRD2-mut. Subsequently, miR-143 mimics or NC mimics and the pGL3-BRD2-wt or pGL3-BRD2-mut were co-transfected into SRA01/04 cells using Lipofectamine 2000 Reagent (Invitrogen, USA). The firefly luciferase activity was measured with a dual luciferase reporter assay system (Promega, Beijing, China) after 24 h of culture, with renilla luciferase activity as an internal control.

Statistical analysis

GraphPad Prism was used to perform statistical analysis. From three independent experiments, data were presented as mean ± SD. Two groups were compared using students' t-tests. Multiple groups were compared using one-way ANOVA coupled with a post hoc Bonferroni test. $P<0.05$ indicated statistically significant differences.

Results

Identification of dysregulated miRNAs in cataract

Five miRNA databases, including miRDB, miTarBase, miRMap, miRanda and TargetScan were used to select miRNAs. Veen results showed that 2690 miRNAs were filtered out from five databases (**Figure 1A**). The dysregulated miRNAs in cataract should be aberrantly expressed between cataract and myopia. Thus, for differential expression analysis, miRNAs were quantified at a count-based level. In total, volcano results revealed that 200 miRNAs were differentially expressed between cataract and myopia, in which 152 miRNAs were upregulated and 48 miRNAs were downregulated in myopia compared with cataract (adjusted *P*-value <0.1 and fold-change >2 or <1/2) (**Figure 1B**).

Bioinformatics analysis of cataract and myopia

Therewith, miRNA levels across cataract and myopia were systematically compared using clustering analysis. The differentially expressed miRNAs were anzlyed across the clusters of cataract patients and the myopia patients, showing that the miRNAs exhibited a distinct ability to differentiate between cataract and

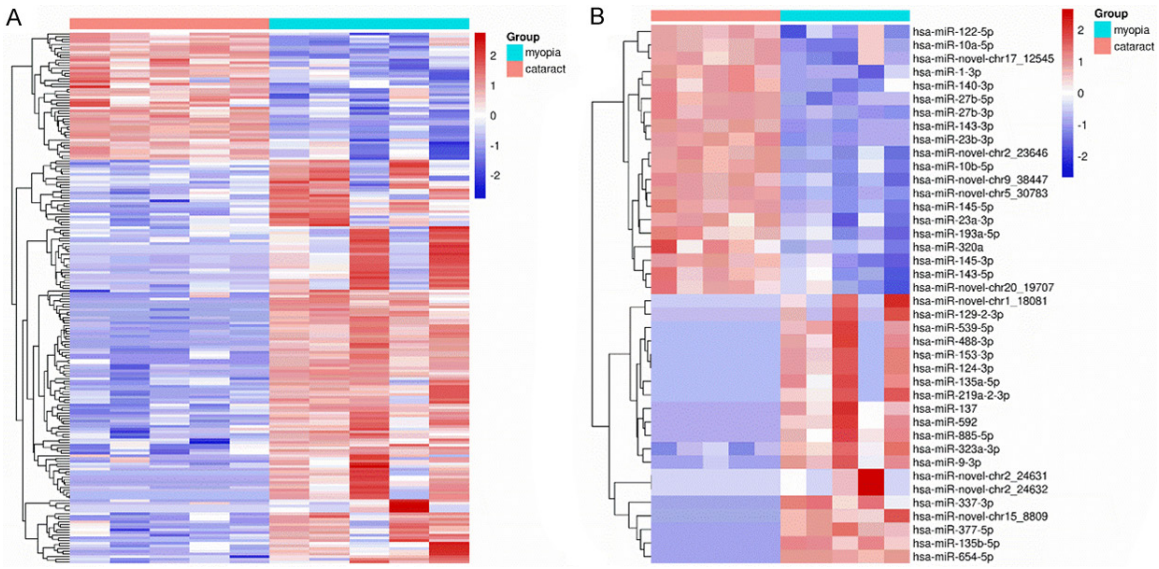

Figure 2. Bioinformatics analysis of cataract and myopia. A: The differentially expressed miRNAs were analzyed in the clusters of cataract patients and the myopia patients; B: Heatmap was conducted to select the top 40 differentially expressed genes (DEGs) between cataract and myopia.

myopia (**Figure 2A**). Heatmap revealed the top 40 differentially expressed genes (DEGs) between cataract and myopia (**Figure 2B**).

The top 5 upregulated and downregulated miRNAs in cataract compared to myopia

To identify whether these ncRNAs were present in cataract, the levels of the selected miRNAs were measured in cataract and myopia population from the publicly available RNAseq dataset. Compared with myopia patients, the top 5 upregulated miRNAs in cataract were miR-1, miR-10b, miR-23b, miR-143 and miR-145 (**Figure 3**). On the contrary, the top 5 downregulated miRNAs in cataract were miR-9, miR-124, miR-129, miR-219a and miR-654 compared with myopia (**Figure 4**).

Silencing of miR-143 suppressed cell proliferation and metastasis

Through bioinformatics analysis, miR-143 was found to be the most upregulated miRNA in cataract compared with myopia. MiR-143 inhibitor was employed to silence miR-143 in human lens epithelial SRA01/04 cells ($P<0.05$; **Figure 5A**). Cell proliferation was found to be suppressed after miR-143 inhibitor transfection ($P<0.05$; **Figure 5B**). Cell invasive and migratory activities were calculated using transwell and wound healing assays. As we expected, cell invasion ($P<0.05$) and migration ($P<0.05$) were inhibited after knocking down miR-143 (**Figure 5C** and **5D**). All the results illustrated that silencing of miR-143 suppressed cell proliferation, invasion and migration in SRA01/04 cells.

Network graphs indicated the target genes of miRNAs

To identify the functional miRNAs responsible for cataract progression, miRanda was applied to predict the miRNA binding sites of the dysregulated miRNAs. The target genes of upregulated (**Figure 6A**) and downregulated (**Figure 6B**) miRNAs were predicted. It illuminated 6 target mRNAs of miR-143, including BRD2, KRAS, MAPK7, MAP3K7 and GXYLT1.

BRD2 was proved to be a target of miR-143

To explore that miR-143 targeted BRD2, 3'-UTR of BRD2 mRNA was mutated from UGAUGUCA to AGUAGAG in SRA01/04 cells (**Figure 7A**). To confirm that miR-143 directly binds BRD2, a luciferase reporter gene assay was conducted. The results indicated that wild type BRD2 mRNA co-transfected with miR-143 mimic showed reduced luciferase compared with wild type BRD2 mRNA co-transfected with NC mimic ($P<0.05$). In contrast, the luciferase of miR-143 mimic co-transfected with mutant BRD2

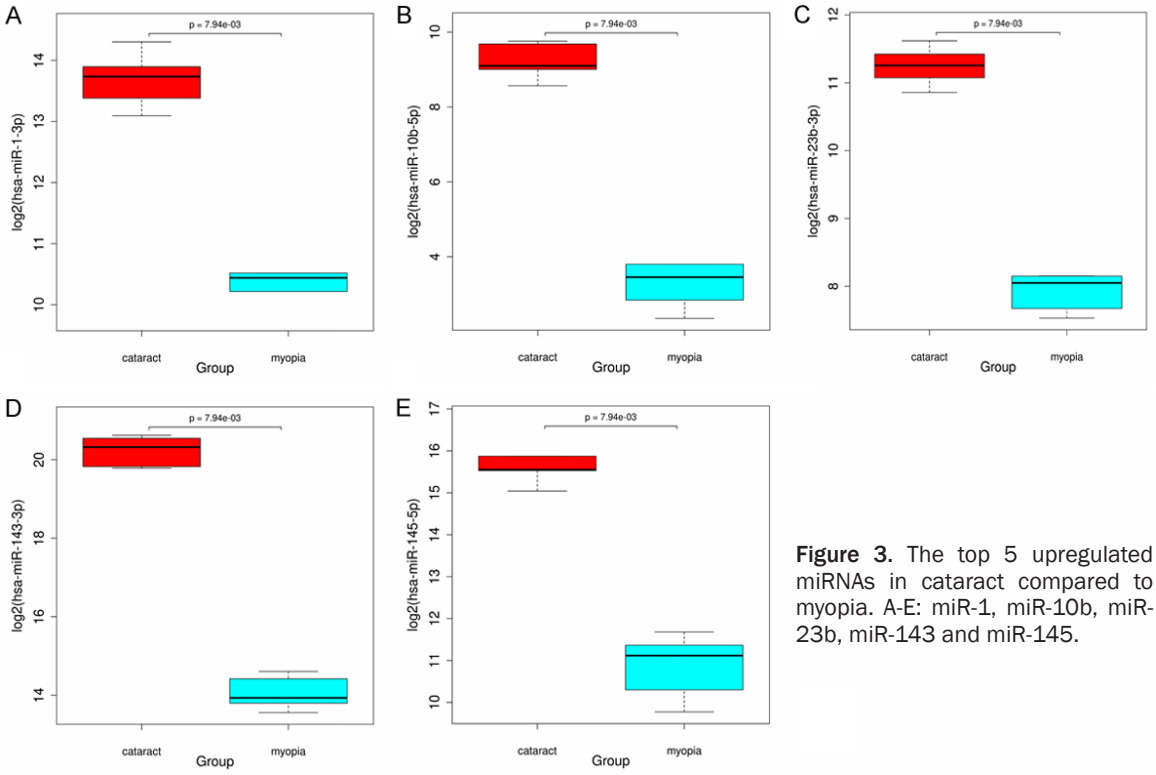

Figure 3. The top 5 upregulated miRNAs in cataract compared to myopia. A-E: miR-1, miR-10b, miR-23b, miR-143 and miR-145.

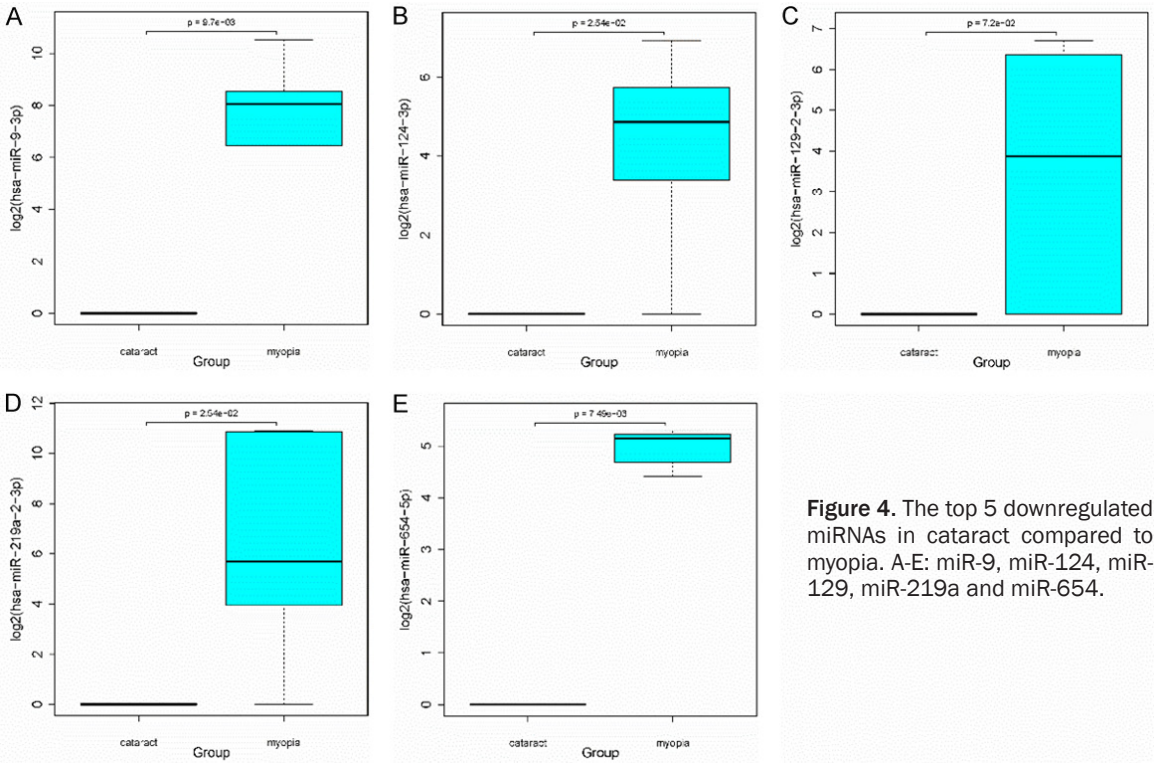

Figure 4. The top 5 downregulated miRNAs in cataract compared to myopia. A-E: miR-9, miR-124, miR-129, miR-219a and miR-654.

mRNA showed no alteration compared with NC mimic co-transfected with mutant BRD2 mRNA (P>0.05; **Figure 7B**). Moreover, the mRNA and protein levels of BRD2 were significantly elevat-

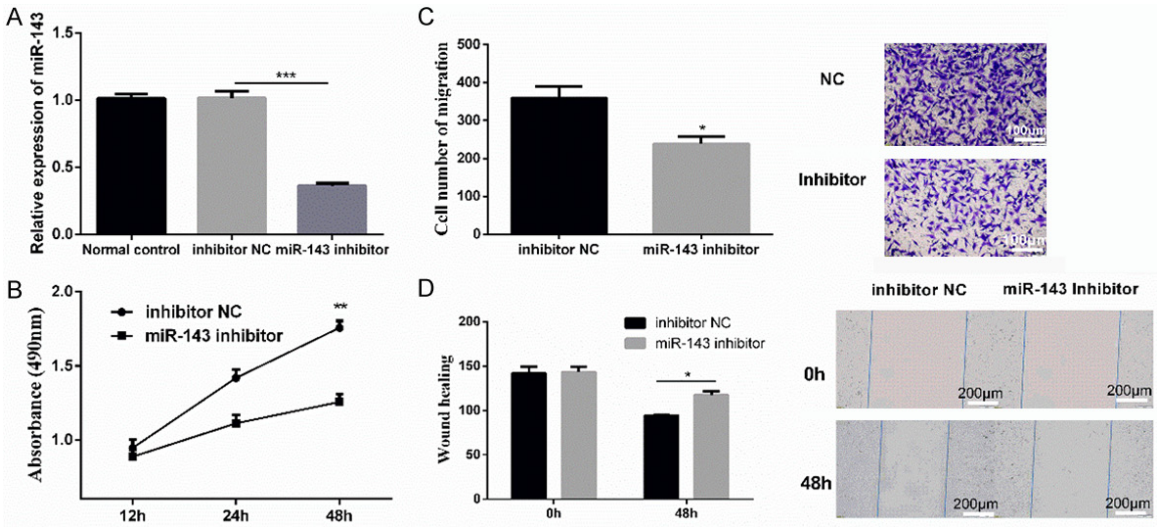

Figure 5. Knockdown of miR-143 inhibited cell proliferation and metastasis. A: MiR-143 inhibitor was employed to silence miR-143 in human lens epithelial SRA01/04 cells; B: Cell proliferation was found to be suppressed after transfecting the miR-143 inhibitor; C and D: Cell invasive and migratory activities were calculated using transwell (200×) and wound healing (100×) assays. NC: normal control. *P<0.05, **P<0.01, ***P<0.001.

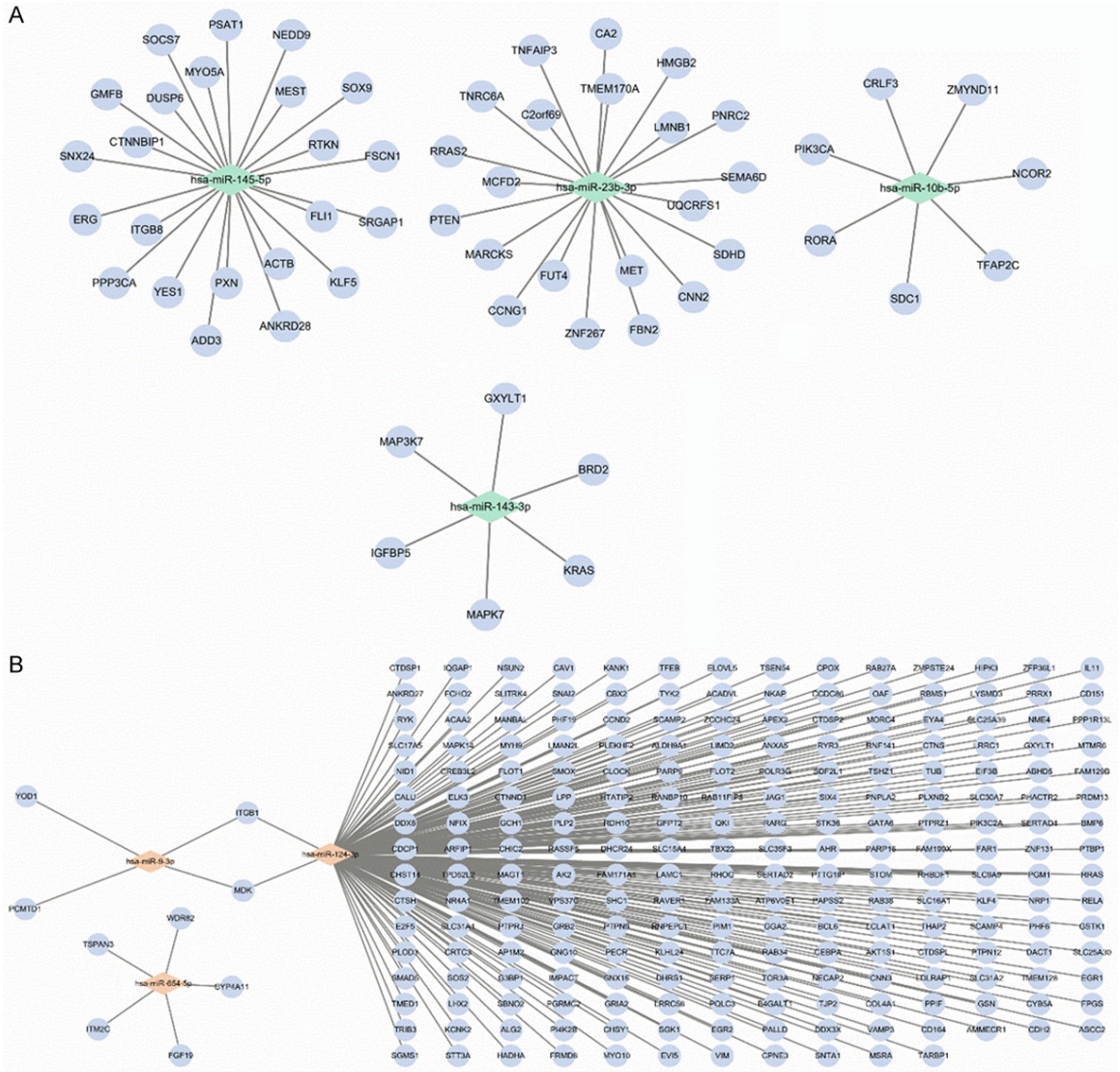

Figure 6. Network graphs indicated the target genes of miRNAs. The target genes of miRNAs were predicted. A: The target genes of some upregulated miRNAs in cataract compared to myopia; B: The target genes of some downregulated miRNAs in cataract compared to myopia.

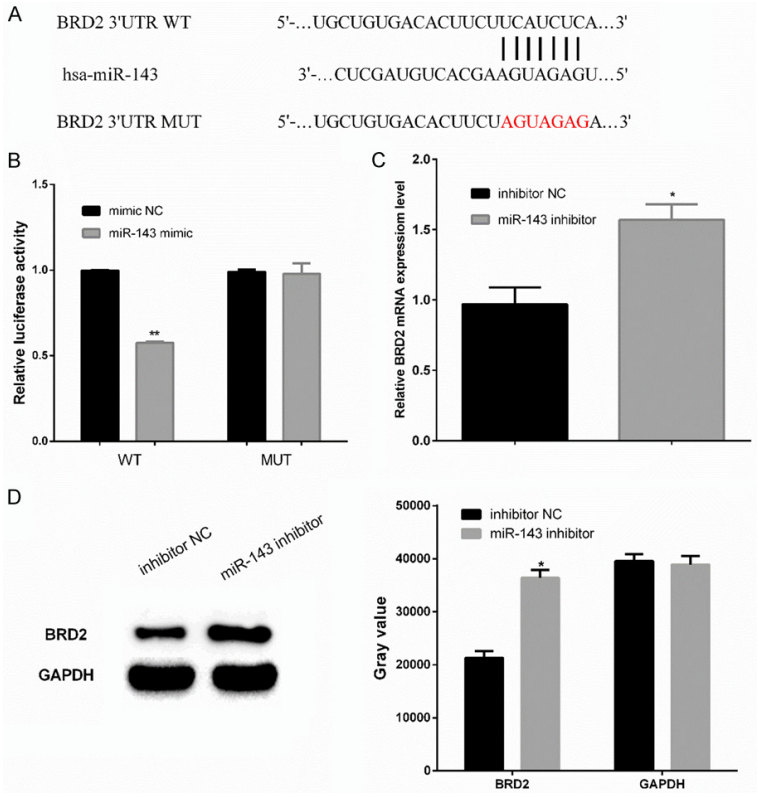

Figure 7. BRD2 was proved to be a target of miR-143. A: Tagetscan predicted the binding sequences between mIR-143 and BRD2, and 3'-UTR of BRD2 mRNA was mutated from UGAUGUCA to AGUAGAG in SRA01/04 cells; B: Luciferase reporter gene assay was performed to verify miR-143 direct binding to BRD2; C: The mRNA level of BRD2 after transfecting miR-143 inhibitor; D: The protein level of BRD2 after transfecting miR-143 inhibitor. NC: normal control; WT: wild type. *P<0.05, **P<0.01.

ed after transfecting miR-143 inhibitor (both P<0.05; **Figure 7C** and **7D**).

Silencing of BRD2 partially reversed the functions of miR-143 inhibitor on proliferation and metastasis

By using RT-qPCR, we detected mRNA and protein levels of BRD2 following co-transfection with an inhibitor of miR-143 and si-BRD2 (P<0.05; **Figure 8A**). Cell proliferation was enhanced by silencing of BRD2, and combined transfections of miR-143 inhibitor and si-BRD2 reversed the proliferation-inhibiting effect of miR-143 inhibitor (P<0.05; **Figure 8B**). Transwell assay illustrated that cell invasion was reduced by silencing of BRD2 (P<0.05). Also, knockdown of BRD2 partially reversed the roles of miR-143 inhibitor on cell invasion (P< 0.05; **Figure 8C**). The knockdown of miR-143 enhanced the migration (P<0.05), and knockdown of BRD2 reversed the effects of miR-143 inhibitor on cell migration (P<0.05) in wound healing assays (**Figure 8D**). All the findings showed that knockdown BRD2 improved cell proliferation, invasion and migration. In addition, silencing of BRD2 partially reversed the functions of miR-143 inhibitor on proliferation, invasion and migration.

Discussion

A cataract refers to the opacification of the crystalline lens of the eye, resulting in a reduction in visual acuity. This ocular condition represents the most widespread and manageable etiology of visual impairment and blindness globally [23, 24]. Lens epithelial cells (LEC) from the outermost layer of the crystal are the only single layer of the lens. While aging is widely recognized as the primary factor, cataract formation can also be attributed to various other factors such as disease, trauma, medications, and genetic predisposition [25]. Cataract pathogenesis is complicated, and fibrosis to human lens epithelial cells (HLECs) is the most important factor contributing to cataract development [26, 27]. MicroRNAs exert a significant influence on crucial biological processes, including cell migration and metabolism, through their ability to negatively modulate the translation and stability of target mRNAs [28, 29]. To explore the underlying mechanisms of cataract formation, a total of 2690 miRNAs were filtered out from five miRNA databases. Volcano plot differentiated the dysregulated miRNAs bet-

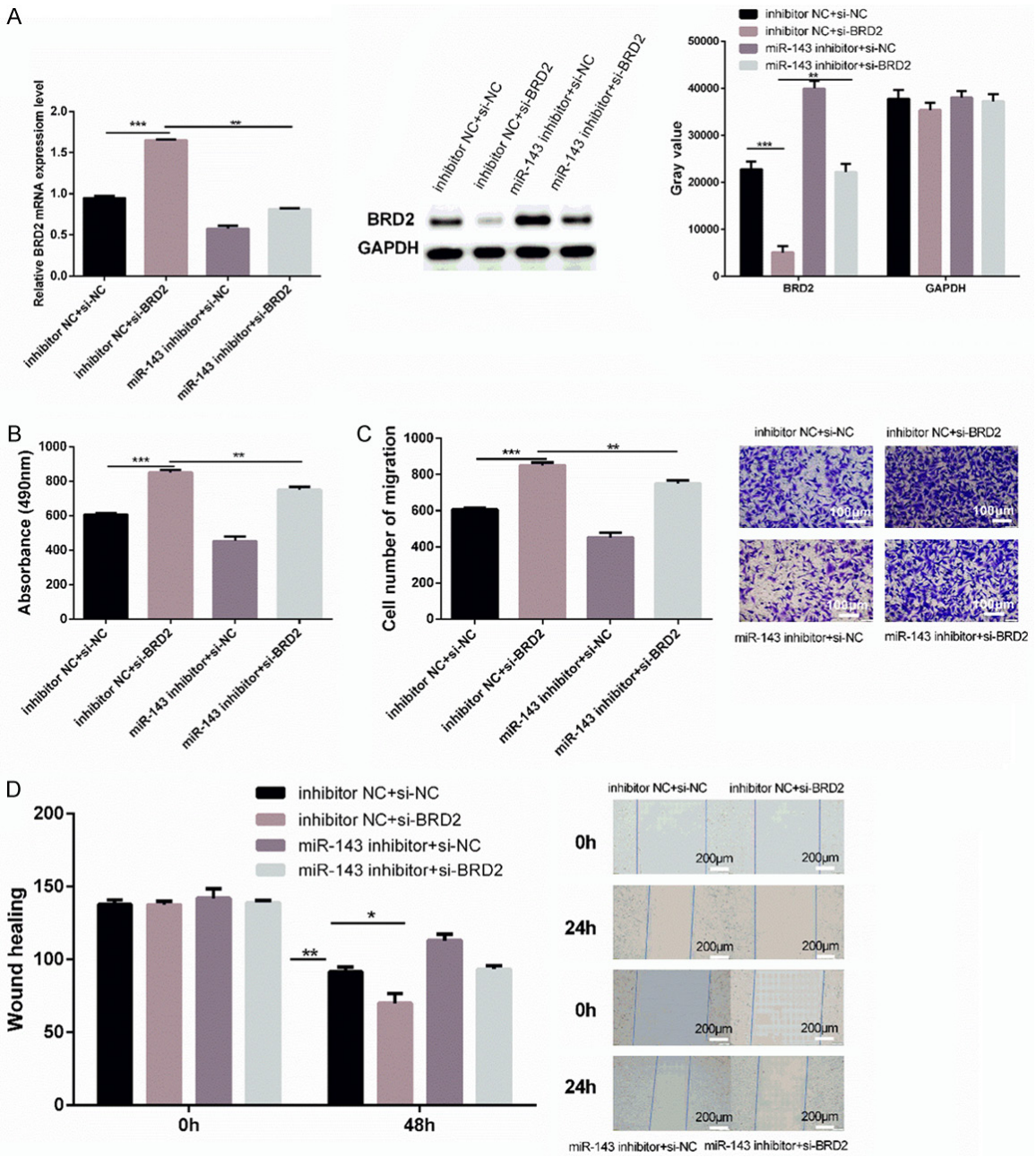

Figure 8. Silencing of BRD2 partially reversed the functions of miR-143 inhibitor on proliferation and metastasis. A: The mRNA and protein levels of BRD2 were detected after co-transfection of miR-143 inhibitor and si-BRD2; B: Co-transfection of miR-143 inhibitor and si-BRD2 reversed the effect of miR-143 inhibitor on cell proliferation; C and D: Knockdown of BRD2 reversed the effects of miR-143 inhibitor on cell invasion (200×) and migration (100×). NC: normal control. *P<0.05, **P<0.01, ***P<0.001.

ween cataract and myopia. Clustering analysis was subsequently performed to systematically compare miRNA levels across cataract and myopia. miR-143 exhibited the most significant disparity between cataract and myopia. Consequently, miR-143 was selected as the candidate for further experimentation.

Shortly after cataract extraction surgery, the remaining lens epithelial cells may undergo proliferation. Simultaneously, a significant portion of the anterior LECs, located on the internal surface of the anterior lens capsule, migrate towards the previously unoccupied posterior capsule. Thus, hindering the proliferation and

migration of LECs could serve as a viable approach to prevent posterior capsule opacification (PCO) [30]. MiR-143 exhibited inhibitory effects on cell proliferation and metastasis but promoted apoptosis in endometrial cancer [31]. However, an upregulation of miR-143 was observed in patients with aqueous humor [32]. In order to investigate the expression levels of miR-143 in cataract, a comprehensive analysis was conducted using five databases to identify miRNAs. The analysis revealed the co-existence of 2690 miRNAs across the five databases. Through comparing the miRNAs between myopia and cataract, we identified 152 upregulated miRNAs and 48 downregulated miRNAs in myopia. The heat map analysis revealed 20 most significantly upregulated and downregulated miRNAs. Consistent with previous research, our findings demonstrated an upregulation of miR-143 in cataract tissues compared to myopia. Numerous studies have been conducted on the effects of miR-143, revealing its impact on cell migration and proliferation. The migration and proliferation of melanoma cancer cells were effectively inhibited, and apoptosis was induced, by the presence of miR-143 [33]. What's more, miR-143 demonstrated the ability to suppress cell proliferation, migration and invasion in breast cancer [34]. In the present study, miR-143 was found to be upregulated in LECs. Our study presents empirical evidence that miR-143 inhibitor has inhibitory effects on the proliferation and migration of LEC SRA01/04 cells.

The Bromo/ExtraTerminal-domain proteins (BRD2, 3, and 4) have been identified as influential determinants of transcription programs. BRD2 actively participates in the process of cell mitosis, forming associations with transcription complexes and acetylated chromatin throughout mitosis. Notably, BRD2 exhibits a specific affinity for the acetylated lysine-12 residue of histone H4 through its two bromodomains [35, 36]. The occurrence of mutations in the Brd2 gene results in embryonic lethality during the process of neurulation. To investigate the underlying mechanism by which Brd2 regulates neuronal differentiation, the phenotype of Brd2 - E2F1 double mutants was examined. The study revealed that the absence of Brd2 resulted in irregularities in the differentiation of neurons and progression of the cell cycle, which were subsequently rectified upon the elimination of the E2F1 gene. These findings indicate that Brd2 plays a crucial role in the cessation of the cell cycle and the differentiation of neuroepithelial cells in the central nervous system of mice, primarily through the E2F1 pathway [37]. The upregulation of BRD2 was observed in chemotherapy-resistant adult T-LBL tissues, and this was found to be significantly correlated with poorer progression-free survival and overall survival outcomes in a cohort of 85 adult T-LBL patients. In both *in vitro* and *in vivo* conditions, apoptosis induced by doxorubicin is suppressed by BRD2 through RasGRP1/Ras/ERK signaling pathway [38]. In vitro, Brd4 nullizygous embryos are incapable of maintaining an inner cell mass and die shortly after implantation [39]. It seems that there is currently no research available on the role of BRD2 in cataracts. Further studies may be needed to determine if there is any connection between the two. In this research, we found that miR-143 downregulated BRD2 expression by regulating its mRNA. BRD2 was predicted to be a target gene of miR-143, and a luciferase reporter assay was performed to verify the forecast. Silencing of miR-143 upregulated BRD2 expression in SRA01/04 cells. In addition, to prove the potential inhibitory function of miR-143 on SRA01/04 cell proliferation through downregulation of BRD2, we employed a targeted siRNA approach to modulate BRD2 in SRA01/04 cells. Our results from BRD2 knockdown experiments revealed a significant reduction in the expression of BRD2 transfected with BRD2 siRNA compared to control in SRA01/04 cells. Additionally, we found that co-transfection of miR-143 inhibitor and BRD2 siRNA led to an increase in BRD2 expression when compared to co-transfection with inhibitor NC and BRD2 siRNA. This further supports the idea that miR-143 can downregulate BRD2 expression. We performed CCK-8, wound-healing, and Transwell assays to investigate the functional significance of miR-143 and BRD2 in LEC proliferation and metastasis. It was observed that knockdown of BRD2 partially reversed the effects of miR-143 inhibitor on these processes in SRA01/04 cells. These findings suggest that miR-143 may play a regulatory role in LEC proliferation and metastasis by directly binding to the mRNA of BRD2.

In conclusion, the research findings suggest that miR-143 has the potential to suppress the

proliferation, invasion, and migration of lens epithelial cells by regulating BRD2 expression. This could potentially lead to the development of a new therapeutic strategy for cataract patients. However, it is essential to note that all the experiments were performed in cells, and further *in vivo* studies are necessary to validate the findings in a more clinically relevant setting. Nonetheless, this research provides a strong foundation for future studies that may lead to the development of more effective therapies for cataracts.

Disclosure of conflict of interest

None.

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References

[1] Stevens GA, White RA, Flaxman SR, Price H, Jonas JB, Keeffe J, Leasher J, Naidoo K, Pesudovs K, Resnikoff S, Taylor H and Bourne RRA; Vision Loss Expert Group. Global prevalence of vision impairment and blindness: magnitude and temporal trends, 1990-2010. Ophthalmology 2013; 120: 2377-2384.

[2] Eldred JA, Dawes LJ and Wormstone IM. The lens as a model for fibrotic disease. Philos Trans R Soc Lond B Biol Sci 2011; 366: 1301-1319.

[3] Awasthi N, Guo S and Wagner BJ. Posterior capsular opacification: a problem reduced but not yet eradicated. Arch Ophthalmol 2009; 127: 555-562.

[4] Kruger AJ, Amon M, Schauersberger J, Abela-Formanek C, Schild G and Kolodjaschna J. Anterior capsule opacification and lens epithelial outgrowth on the intraocular lens surface after curettage. J Cataract Refract Surg 2001; 27: 1987-1991.

[5] Shichi H. Cataract formation and prevention. Expert Opin Investig Drugs 2004; 13: 691-701.

[6] Bartel DP. MicroRNAs: target recognition and regulatory functions. Cell 2009; 136: 215-233.

[7] Blahna MT and Hata A. Smad-mediated regulation of MicroRNA biosynthesis. FEBS Lett 2012; 586: 1906-1912.

[8] Wang X, Wang L, Sun Y, Chen B, Xiong L, Chen J, Huang M, Wu J, Tan X, Zheng Y, Huang S and Liu Y. MiR-22-3p inhibits fibrotic cataract through inactivation of HDAC6 and increase of α-tubulin acetylation. Cell Prolif 2020; 53: e12911.

[9] Kadkhoda S and Ghafouri-Fard S. The importance of miRNA-630 in human diseases with an especial focus on cancers. Cancer Cell Int 2022; 22: 105.

[10] Chen X, Xiao W, Chen W, Liu X, Wu M, Bo Q, Luo Y, Ye S, Cao Y and Liu Y. MicroRNA-26a and -26b inhibit lens fibrosis and cataract by negatively regulating Jagged-1/Notch signaling pathway. Cell Death Differ 2017; 24: 1431-1442.

[11] Guoping M, Ran L and Yanru Q. miR-143 inhibits cell proliferation of gastric cancer cells through targeting GATA6. Oncol Res 2018; 26: 1023-1029.

[12] Ma Z, Luo Y and Qiu M. miR-143 induces the apoptosis of prostate cancer LNCap cells by suppressing Bcl-2 expression. Med Sci Monit 2017; 23: 359-365.

[13] He M, Zhan M, Chen W, Xu S, Long M, Shen H, Shi Y, Liu Q, Mohan M and Wang J. MiR-143-5p deficiency triggers EMT and metastasis by targeting HIF-1α in gallbladder cancer. Cell Physiol Biochem 2017; 42: 2078-2092.

[14] Su J, Liang H, Yao W, Wang N, Zhang S, Yan X, Feng H, Pang W, Wang Y, Wang X, Fu Z, Liu Y, Zhao C, Zhang J, Zhang CY, Zen K, Chen X and Wang Y. MiR-143 and MiR-145 regulate IGF1R to suppress cell proliferation in colorectal cancer. PLoS One 2014; 9: e114420.

[15] Wang D, Wang C, Sun L, Lu X, Shi J, Chen J and Zhang X. MiR-143-3p increases the radiosensitivity of breast cancer cells through FGF1. Cell Mol Biol (Noisy-le-grand) 2022; 67: 256-262.

[16] Tang J, Pan H, Wang W, Qi C, Gu C, Shang A and Zhu J. MiR-495-3p and miR-143-3p co-target CDK1 to inhibit the development of cervical cancer. Clin Transl Oncol 2021; 23: 2323-2334.

[17] Asghariazar V, Kadkhodayi M, Mansoori B, Mohammadi A and Baradaran B. Restoration of miR-143 reduces migration and proliferation of bladder cancer cells by regulating signaling pathways involved in EMT. Mol Cell Probes 2022; 61: 101794.

[18] Peng J, Dong W, Chen L, Zou T, Qi Y and Liu Y. Brd2 is a TBP-associated protein and recruits TBP into E2F-1 transcriptional complex in response to serum stimulation. Mol Cell Biochem 2007; 294: 45-54.

[19] Samelson AJ, Tran QD, Robinot R, Carrau L, Rezelj VV, Kain AM, Chen M, Ramadoss GN, Guo X, Lim SA, Lui I, Nuñez JK, Rockwood SJ, Wang J, Liu N, Carlson-Stevermer J, Oki J, Maures T, Holden K, Weissman JS, Wells JA, Conklin BR, TenOever BR, Chakrabarti LA, Vignuzzi M, Tian RL and Kampmann M. BRD2

inhibition blocks SARS-CoV-2 infection by reducing transcription of the host cell receptor ACE2. Nat Cell Biol 2022; 24: 24-34.
[20] Zang K, Wang J, Dong M, Sun R, Wang Y, Huang Y, Liu X, Li Y, Wang F and Yu M. Brd2 inhibits adipogenesis via the ERK1/2 signaling pathway in 3T3-L1 adipocytes. PLoS One 2013; 8: e78536.
[21] Yan Q, Liu JP and Li DW. Apoptosis in lens development and pathology. Differentiation 2006; 74: 195-211.
[22] Vigneux G, Pirkkanen J, Laframboise T, Prescott H, Tharmalingam S and Thome C. Radiation-induced alterations in proliferation, migration, and adhesion in lens epithelial cells and implications for cataract development. Bioengineering (Basel) 2022; 9: 29.
[23] Thompson J and Lakhani N. Cataracts. Prim Care 2015; 42: 409-23.
[24] Chylack LT Jr, Wolfe JK, Singer DM, Leske MC, Bullimore MA, Bailey IL, Friend J, McCarthy D and Wu SY. The lens opacities classification system III. The longitudinal study of cataract study group. Arch Ophthalmol 1993; 111: 831-6.
[25] Shichi H. Cataract formation and prevention. Expert Opin Investig Drugs 2004; 13: 691-701.
[26] Lovicu FJ, Shin EH and McAvoy JW. Fibrosis in the lens. Sprouty regulation of TGFβ-signaling prevents lens EMT leading to cataract. Exp Eye Res 2016; 142: 92-101.
[27] VanSlyke JK, Boswell BA and Musil LS. ErbBs in lens cell fibrosis and secondary cataract. Invest Ophthalmol Vis Sci 2023; 64: 6.
[28] Huang Y. The novel regulatory role of lncRNA-miRNA-mRNA axis in cardiovascular diseases. J Cell Mol Med 2018; 22: 5768-5775.
[29] Hou Q, Tang J, Wang Z, Wang C, Chen X, Hou L, Dong XD and Tu LL. Inhibitory effect of microRNA-34a on retinal pigment epithelial cell proliferation and migration. Invest Ophthalmol Vis Sci 2013; 54: 6481-6488.
[30] Dong N, Tang X and Xu B. miRNA-181a inhibits the proliferation, migration, and epithelial-mesenchymal transition of lens epithelial cells. Invest Ophthalmol Vis Sci 2015; 56: 993-1001.
[31] Chang L, Zhang D, Shi H, Bian Y and Guo R. MiR-143 inhibits endometrial cancer cell proliferation and metastasis by targeting MAPK1. Oncotarget 2017; 8: 84384-84395.
[32] Hubens WHG, Krauskopf J, Beckers HJM, Kleinjans JCS, Webers CAB and Gorgels TGMF. Small RNA sequencing of aqueous humor and plasma in patients with primary open-angle glaucoma. Invest Ophthalmol Vis Sci 2021; 62: 24.
[33] Nabipoorashrafi SA, Shomali N, Sadat-Hatamnezhad L, Mahami-Oskouei M, Mahmoudi J, Sandoghchian Shotorbani B, Akbari M, Xu H and Sandoghchian Shotorbani S. miR-143 acts as an inhibitor of migration and proliferation as well as an inducer of apoptosis in melanoma cancer cells in vitro. IUBMB Life 2020; 72: 2034-2044.
[34] Xia C, Yang Y, Kong F, Kong Q and Shan C. MiR-143-3p inhibits the proliferation, cell migration and invasion of human breast cancer cells by modulating the expression of MAPK7. Biochimie 2018; 147: 98-104.
[35] Belkina AC, Blanton WP, Nikolajczyk BS and Denis GV. The double bromodomain protein Brd2 promotes B cell expansion and mitogenesis. J Leukoc Biol 2014; 95: 451-60.
[36] Umehara T, Nakamura Y, Jang MK, Nakano K, Tanaka A, Ozato K, Padmanabhan B and Yokoyama S. Structural basis for acetylated histone H4 recognition by the human BRD2 bromodomain. J Biol Chem 2010; 285: 7610-8.
[37] Tsume M, Kimura-Yoshida C, Mochida K, Shibukawa Y, Amazaki S, Wada Y, Hiramatsu R, Shimokawa K and Matsuo I. Brd2 is required for cell cycle exit and neuronal differentiation through the E2F1 pathway in mouse neuroepithelial cells. Biochem Biophys Res Commun 2012; 425: 762-768.
[38] Tian XP, Cai J, Ma SY, Fang Y, Huang HQ, Lin TY, Rao HL, Li M, Xia ZJ, Kang TB, Xie D and Cai QQ. BRD2 induces drug resistance through activation of the RasGRP1/Ras/ERK signaling pathway in adult T-cell lymphoblastic lymphoma. Cancer Commun (Lond) 2020; 40: 245-259.
[39] Houzelstein D, Bullock SL, Lynch DE, Grigorieva EF, Wilson VA and Beddington RS. Growth and early postimplantation defects in mice deficient for the bromodomain-containing protein Brd4. Mol Cell Biol 2002; 22: 3794-3802.