• Basic Research •

DNA hypermethylation of *COL4A1* in ultraviolet-B-induced age-related cataract models *in vitro* and *in vivo*

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Abstract

● **AIM:** To explore the DNA methylation of *COL4A1* in ultraviolet-B (UVB)-induced age-related cataract (ARC) models *in vitro* and *in vivo*.

● **METHODS:** Human lens epithelium B3 (HLEB3) cells and Sprague Dawley rats were exposure to UVB respectively. The MTT assay was utilized to evaluate cell proliferation. Flow cytometry was employed for analysis of cell apoptosis and cell cycle. *COL4A1* expression in HLEB3 cells and anterior lens capsules were assessed using Western blot and reverse transcription-polymerase chain reaction (RT-PCR). The localization of *COL4A1* in HLEB3 cells was determined by immunofluorescence. The methylation status of CpG islands located in *COL4A1* promoter was verified using bisulfite-sequencing PCR (BSP). DNMTs and TETs mRNA levels was examined by RT-PCR.

● **RESULTS:** UVB exposure decreased HLEB3 cells proliferation, while increased the apoptosis rate and cells were arrested in G0/G1 phase. *COL4A1* expression was markedly inhibited in UVB treated cells compared to the controls. Hypermethylation status was detected in the CpG islands within *COL4A1* promoter in HLEB3 cells subjected to UVB exposure. Expressions of DNMTs including DNMT1/2/3 were elevated in UVB treated HLEB3 cells compared to that in the controls, while expressions of TETs including TET1/2/3 showed the opposite trend. Results from the UVB treated rat model further confirmed the decreased expression of *COL4A1*, hypermethylation status of the CpG islands at promoter of *COL4A1* and abnormal expression of DNMT1/2/3 and TET1/2/ in UVB exposure group.

● **CONCLUSION:** DNA hypermethylation of *COL4A1* promoter CpG islands is correlated with decreased *COL4A1* expression in UVB induced HLEB3 cells and anterior lens capsules of rats.

● **KEYWORDS:** human lens epithelium cells; age-related cataract; *COL4A1*; hypermethylation; ultraviolet-B; rat

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INTRODUCTION

Age-related cataract (ARC) has become a major cause of visual impairment and blindness in the global elderly population and presents an increasing visual challenge in China[1-3]. The ARC is divided into nuclear, cortical, and posterior subcapsular base on the position of opacity in the lens[1]. At present, surgery is considered to be an effective method for the treatment of ARC, but its treatment process still has inevitable risks. Posterior capsule opacity, also known as posterior cataract, often occurs after extracapsular cataract extraction surgery, with a postoperative probability of about 20%–30%. The occurrence of ARC is influenced by a range of environmental and genetic factors that contribute to its development[2-3]. Previous studies had shown that in addition to increasing age, a series of risk factors were also closely related to the incidence of ARC, including gene polymorphism, type 2 diabetes mellitus, cigarette smoking, malnutrition and ultraviolet-B (UVB) exposure[4-5].

The association between UVB exposure and ARC had been demonstrated by epidemiological studies[6-8]. The causal relationship was further confirmed by *in vitro* and animal investigations, including human lens epithelial cells[9], rabbit[10], and rat[11]. A comprehensive review of relevant epidemiological reports before 2021 suggested that people exposed to sunlight for a long time had a notably increased risk of ARC. It was

found that participating in outdoor activities was a significant contributing factor in the development of cortical cataract, with an increase in risk noted as the cumulative exposure to UVB radiation accumulated over time[12]. UVB is responsible for a series of pathophysiological changes in lens epithelial cells and could further induce a variety of pathogenic effects, such as ubiquitination[13], methylation[14-15], oxidative stress[16-19], and autophagy[20]. At present, the occurrence of ARC caused by UVB exposure is believed to be influenced by a combination of genetic and environmental factors, but the specific pathogenesis and related molecular mechanisms have not been fully elucidated.

DNA methylation plays an important role in controlling the expression of crucial genes related to the normal development of retinal neurons[21]. Abnormal methylation has been found in various diseases including retinoblastoma, pterygium, age-related macular degeneration, and cataract[22-26]. Previous studies have verified that aberrant status of DNA methylation can cause abnormal expression of Werner syndrome gene, 8-oxoguanine DNA glycosylase 1 (OGG1) and O-6-methylguanine DNA methyltransferase (MGMT) in cataract lens[27-29]. Previous studies had demonstrated that exposure to UVB radiation induced a series of changes in lens epithelial cells by impacting the methylation level of crucial genes. A research on UVB exposure and OGG1 methylation in ARC suggested that the lowered expression of OGG1 was associated with hypermethylation in the CpG islands within the promoter region of the *OGG1* gene in the lens cortex. OGG1 belongs to a group of proteins involved in repairing DNA damage caused by oxidation[30]. In another study, exposure to UVB radiation resulted in increased methylation levels at a specific CpG site located on the promoter region of *ERCC6* gene, subsequently causing a decrease in the expression of ERCC6 in LEC. ERCC6 is an important component protein of nucleotide excision and repair, and its decreased expression leads to increased apoptosis of lens epithelial cells, thus aggravating ultraviolet damage[14]. Taken together, abnormal methylation of core genes is closely related to UVB-induced lens epithelial cell damage.

Our recent investigation revealed that the inhibition of COL4A1 resulted in a halt during the S-phase, leading to suppressed proliferation and increased apoptosis and epithelial-mesenchymal transition (EMT) in human lens epithelium B3 (HLEB3) cells[31]. *COL4A1* is situated on chromosome 13q34 and comprises of 52 exons. It encodes the α1 chain of type IV collagen, which serves as the primary collagenous constituent found in basement membranes[32]. It was found that hypermethylated *COL4A1* in anterior lens capsule membrane samples was associated with ARC. The association between ARC and hypermethylation of the *COL4A1* promoter region was identified through genome-wide methylation analysis[33]. But the underlying mechanisms of UVB exposure on methylation of *COL4A1* in ARC are unclarified and need further research to be confirmed.

Here, we intended to evaluate if the methylation status is vital for the development of UVB-induced ARC. We probed into the likelihood that the gene expression in HLEB3 cells and animal models with UVB-induced rat cataracts could be affected by the hypermethylation of CpG islands located in the promoter region of *COL4A1*. This study may furnish experimental evidence in favor of the application of epigenetic elements, like DNA methylation, in the management and prevention of ARC.

MATERIALS AND METHODS

Ethical Approval All experimental procedures were granted ethical approval by the Ethics Committee of Xi'an Medical University (No.XYLS2021185).

Cells Culture and Treatment HLEB3 cells were acquired from the American Type Culture Collection (ATCC; Rockville, MD, USA) and maintained in Dulbecco's modified Eagle's medium (DMEM; Sigma-Aldrich, USA), with the addition of 10% fetal bovine serum (FBS; Sigma, USA), under conditions of 37℃ and 5% CO_2 environment.

Prior to relative treatment, HLEB3 cells were incubated in 6-well plates using DMEM without FBS for a duration of 24h. Next, the HLEB3 underwent 300 J/m^2 UVB exposure for 200s with a UVB lamp (TFML-20; UVP, Upland, CA, USA), using a UV-M radiometer (Beijing Shida Photoelectric Instrument Co., Beijing, China) to determine the intensity and dose of UVB. Different time intervals were employed to purify the samples, allowing for the retrieval of mRNA, DNA and protein for further analysis.

RT-qPCR Assay TRIzol reagent (Invitrogen) was utilized to extract the total RNA from HLEB3 cells, following the manufacturer's instructions. Subsequently, cDNA synthesis was conducted using the PrimeScript RT reagent kit (Takara, Dalian, China). The reverse transcription-polymerase chain reaction (RT-PCR) assay was performed using the SYBR Green PCR kit (Takara, Dalian, China). GAPDH was selected as the endogenous control gene. The $2^{-\Delta\Delta Ct}$ method was adopted to determine the relative expression levels.

Western Blot Assay The protein samples underwent electrophoresis utilizing a gel composed of 10% sodium dodecyl sulfate-polyacrylamide (SDS-PAGE) and subsequently transferred onto nitrocellulose filter membranes (Millipore, Bedford, MA, USA) at 40 mA for 8h. The blocking buffer (consisting of 5% nonfat milk, 50 mmol/L Tris, 0.05% Tween-20 and 200 mmol/L NaCl) was applied to obstruct the membrane. Subsequently, the membrane underwent incubation with primary antibodies against rat-COL4A1 (diluted at a ratio

of 1:1000; obtained from Abcam) and rat-GAPDH (diluted at a ratio of 1:2000; obtained from Abcam) for a duration of 12h at a temperature of 4℃. The membrane was subjected to three washes with TBST (500 mmol/L NaCl, 20 mmol/L Tris and 0.1% Tween-20) under ambient conditions for a duration of 5min each time prior to incubation with alkaline phosphatase-conjugated secondary antibodies (1:4000; Santa Cruz, USA) at room temperature for a period of 2h. The bands were identified using an ECL Western blot kit from Thermo Fisher Scientific, based in USA, and subsequently evaluated with Lab Works (TM ver4.6) software provided by UVP Bio Imaging Systems located in NY, USA. GAPDH was employed as a control.

Cell Proliferation Assay The cell viability of HLEB3 was assessed using the MTT assay, in which the survival rate was determined under different conditions including UVB exposure. After 24h of treatments with UVB irradiation, cells were incubated with 20 μL of MTT solution at a concentration of 5 mg/mL for a duration of 4h. Subsequently, the culture medium was replaced with 150 μL of dimethyl sulfoxide, and the measurement of absorbance at 490 nm was quantified through a microplate reader.

Cell Apoptosis Assay The analysis of cell apoptosis was conducted through flow cytometry. The measurement of cell apoptosis rate was conducted by employing an Annexin V-APC Apoptosis Detection Kit (Elabscience, CHN) following the instructions provided by the manufacturer. In general, the cultured cells were harvested and suspended in 200 μL of binding buffer. After that, Annexin V-APC and 7-AAD were added for staining at room temperature in the absence of light for a duration of 15min. Subsequently, flow cytometry (FACScan; BD Biosciences) was employed to assess the rates of apoptosis.

Cell Cycle Analysis Cells were subjected to UVB irradiation and harvested after 72h. The cells were subsequently treated with 75% ethanol at a temperature of 4℃ for a duration of 4h. After removing the liquid portion, an RNA enzyme containing iodide was introduced. Afterwards, the cells were rinsed thrice using phosphate buffer saline (PBS) and subsequently underwent cell cycle analysis through FACS Calibur, followed by data interpretation conducted through FACS Diva (BD Biosciences, USA).

Bisulfite-sequencing PCR Cloning and Sequencing Phenol/chloroform extraction method was employed to extract and purify DNA. To convert unmethylated cytosine residues to uracil, a bisulfite conversion kit from Qiagen (Frederick, MD, USA) was utilized following the manufacturer's instructions. The CpG islands in the *COL4A1* promoter region were evaluated through methylation-specific polymerase chain reaction (PCR) analysis to assess methylation status. For amplification, we employed specific primers: 5'-TGGTAGTAAAAGGTGTTTGAGGTTA-3' (forward) and 5'-CCTCCTTCTCCTATACAAATAACTACTA-3' (reverse). The resulting bisulfite-sequencing PCR (BSP) products were cloned into a PMD 20-T vector provided by Takara. Subsequently, three clones per construct were selected for sequencing purposes.

***In Vivo* Assay** UVB exposure was administered to female Sprague Dawley rats with albinism unilaterally at the age of 6wk (17 rats per group). The rats were administered intraperitoneal injection of xylazine (14 mg/kg) and ketamine (94 mg/kg) as anesthesia, with a 10-minute interval before the commencement of the treatment. Both eyes of the animals were subjected to pupillary dilation using tropicamide instillation for 5min, followed by exposing to UVB (15min exposure time, 9 kJ/m^2). Rats were sacrificed with an overdose application of carbon dioxide and subsequent cervical dislocation at 3 and 7d post-exposure, respectively. The eyeballs of rats were enucleated. Then, the lens was separated from the eyeball structures and placed in PBS. The lens equator was observed under a microscope to extract the remnants of the ciliary body. The anterior lens capsules of each sample were isolated for further analysis. The lens epithelial cells are supported by the basement membranes of the anterior lens capsules.

Statistical Analysis The statistical analyses were conducted by SPSS software version 17.0 (SPSS, Inc., Chicago, IL, USA). A one-way analysis of variance (ANOVA) was utilized to assess the variation in means among the groups. The criterion for statistical significance was established at a level of $P<0.05$.

RESULTS

UVB Exposure Decreased Cell Proliferation in HLEB3 Cells The MTT assay results demonstrated a notable decrease in cell viability of HLEB3 cells following UVB treatment in comparison with the control group (Figure 1A). The flow cytometry analysis was conducted to determine the apoptosis rate of HLEB3 cells. The findings revealed a remarkable elevation in the apoptosis level of HLEB3 cells following exposure to UVB radiation (Figure 1B, 1C). Meanwhile, results of the analysis of cell cycle indicated a higher proportion of cells in G0/G1 phase among UVB-treated HLEB3 cells (Figure 1D, 1E). These results imply that the proliferation of HLEB3 cells was reduced following exposure to UVB radiation.

UVB Exposure Decreased COL4A1 Expression in HLEB3 Cells The content of COL4A1 mRNA in HLEB3 cells exposed to UVB and the control group was examined using qRT-PCR analysis. Decreased COL4A1 mRNA (Figure 2A) expression in UVB treated cells was detected compared to the

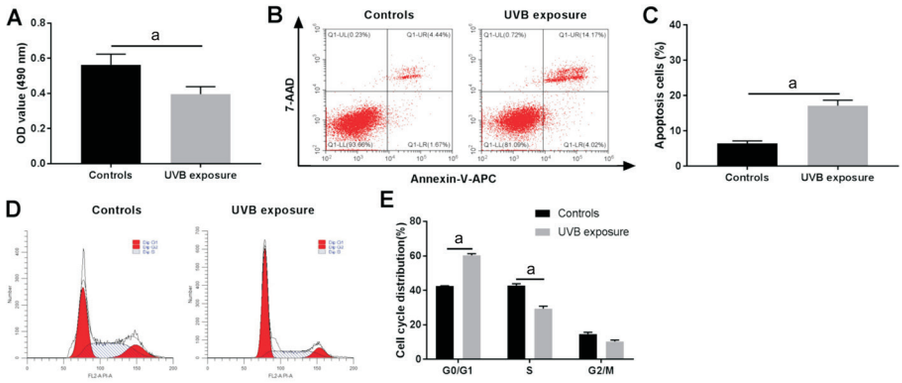

Figure 1 Effect of UVB exposure on cell proliferation of HLEB3 cells A: The viability of HLEB3 cells was assessed using the MTT assay after 24h of exposure to UVB light; B: Flow cytometry was employed to measure the apoptosis rate in HLEB3 cells; C: The proportion of apoptotic HLEB3 cells; D: Flow cytometry analysis revealed the cell cycle profile of HLEB3 cells; E: Cell cycle distribution of HLEB3 cells. [a]*P*<0.05 *vs* control group. UVB: Ultraviolet-B; HLEB3: Human lens epithelium B3.

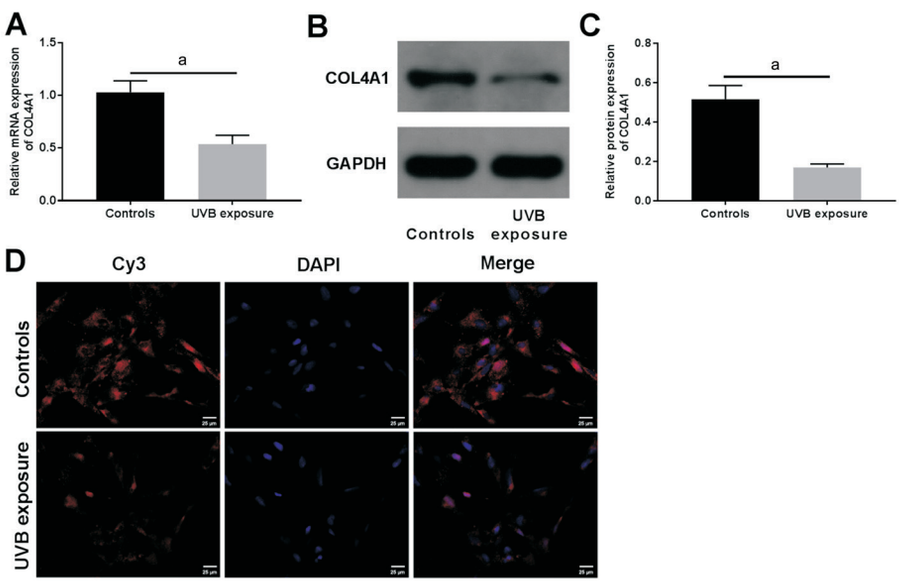

Figure 2 Comparison of mRNA and protein levels of COL4A1 in HLEB3 cells A: Quantitative RT-PCR analysis was conducted to assess the mRNA levels of COL4A1 in both control and UVB-treated HLEB3 cells. The values presented with mean±SD. B: Western blot analysis was performed to evaluate the protein expression of COL4A1 in both control and UVB-treated HLEB3 cells. C: The relative level of COL4A1 protein compared to GAPDH is reported as the mean±SD. D: Immunofluorescence staining using anti-COL4A1 monoclonal antibodies was carried out on HLEB3 cells. Scale bars: 25 μm. [a]*P*<0.05 *vs* control group. UVB: Ultraviolet-B; HLEB3: Human lens epithelium B3; RT-PCR: Reverse transcription-polymerase chain reaction; SD: Standard deviation.

controls. To validate the alteration in protein levels of COL4A1 between cells treated with UVB and control cells, a Western blot analysis was conducted. Figure 2B, 2C illustrated that the expression of COL4A1 protein was decreased in UVB-treated cells relative to the control group. Immunofluorescence was conducted with anti-COL4A1 monoclonal antibodies in HLEB3 cells. The staining intensity of COL4A1 was observed to be reduced in cells treated with UVB relative to the control group (Figure 2D).

To demonstrate the correlation between COL4A1 expression and its methylation status, we conducted a BSP detection to determine the methylation rate in the *COL4A1* promoter in both UVB-exposed HLEB3 cells and control cells. The presence of a CpG island in the promoter region of *COL4A1* was predicted by bioinformatic analysis (Figure 3A). A visual representation of bisulfite genomic sequencing findings is presented in Figure 3B. Each individual clone is depicted in a row, with each CpG site represented by a circle. Filled and unfilled circles indicate methylated and unmethylated CpGs, respectively. As depicted in Figure 3C, the HLEB3 cells treated with UVB exposure had a higher methylation rate at the CpG islands of the *COL4A1* promoter relative to the control group. These findings indicate that the expression of COL4A1 is decreased due to DNA hypermethylation in the promoter region of *COL4A1*.

DNMTs and TETs Expression in HLEB3 Cells Treated with UVB As we aimed to explore the mRNA expression of DNMTs and TETs, which are responsible for catalyzing and maintaining DNA methylation, our investigation focused on

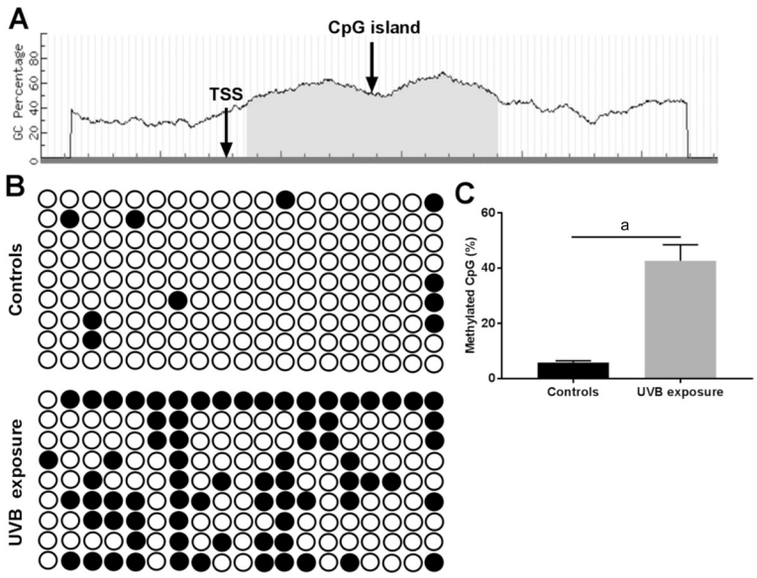

Figure 3 Methylation status of the CpG island located at *COL4A1* promoter in HLEB3 cells A: The location of the CpG island in the *COL4A1* promoter region; B: Bisulfite genomic sequencing was performed on the CG-rich sequence of the *COL4A1* CpG island in DNA samples obtained from both control and UVB treated HLEB3 cells. The representation of each plasmid clone was depicted by a row of circles. Unmethylated and methylated CpG sites were represented by circles that were opened and closed, respectively. C: The group exposed to UVB showed a higher level of methylation in comparison to the control group. [a]*P*<0.05 *vs* control group. UVB: Ultraviolet-B; HLEB3: Human lens epithelium B3.

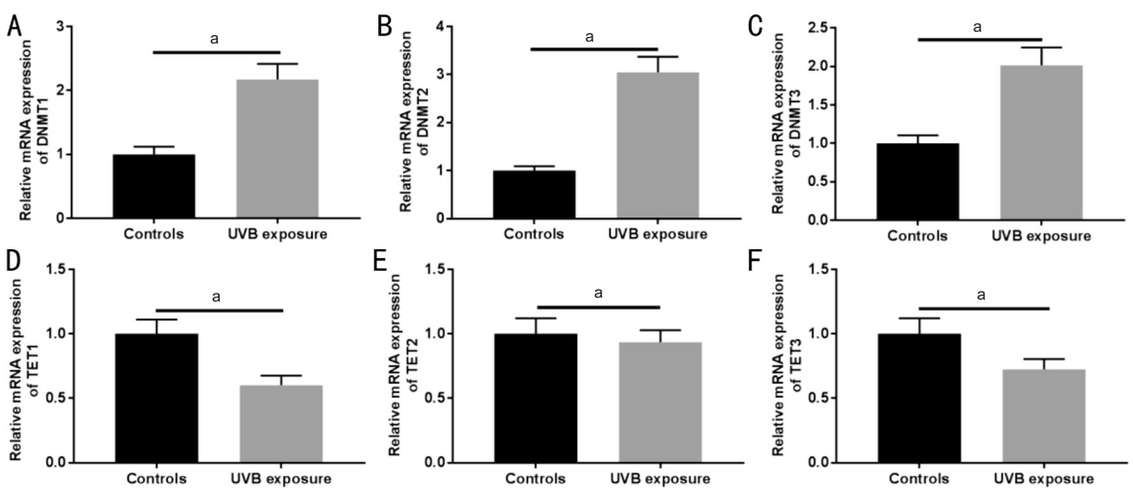

Figure 4 Relative expression of mRNA levels of DNMTs and TETs in HLEB3 cells DNMT1/2/3 (A–C) and TET1/2/3 (D–F) mRNA expression in HLEB3 cells of the control and UVB-exposed group were analyzed using quantitative RT-PCR. Values represent as mean±SD. [a]*P*<0.05 *vs* control group. UVB: Ultraviolet-B; HLEB3: Human lens epithelium B3; RT-PCR: Reverse transcription-polymerase chain reaction; SD: Standard deviation.

these enzymes. DNMTs facilitate the methylation of DNA by introducing methyl groups to the 5-carbon position of cytosine, leading to the formation of 5-methylcytosine (5mC). TET specifically recognizes the methyl group of 5mC on genomic DNA and catalyzes oxidation, which plays an essential part in the process of actively removing methyl groups from genomic DNA. The results indicated that DNMTs, including DNMT1/2/3, were expressed at higher level in HLEB3 cells treated with UVB exposure compared to that in the controls (Figure 4A-4C). On the contrary, the levels of TETs expression, specifically TET1/2/3, exhibited a decrease in HLEB3 cells following exposure to UVB radiation when compared to the control group (Figure 4D-4F).

UVB Reduced COL4A1 Expression and Induced ARC-like Phenotype *via* Methylation at *COL4A1* Promoter in Rats UVB exposure was administered to female Sprague Dawley rats with albinism unilaterally at the age of 6wk. The lens was separated from the eyeball structures. The COL4A1 expression in the anterior lens capsules of both UVB treated rat models and control groups was investigated using RT-PCR and Western blot. Repressed mRNA expression of COL4A1 (Figure 5A) and decreased protein levels (Figure 5B, 5C) were observed in the anterior lens capsules of UVB-exposed rat models relative to the control group. The degree of COL4A1

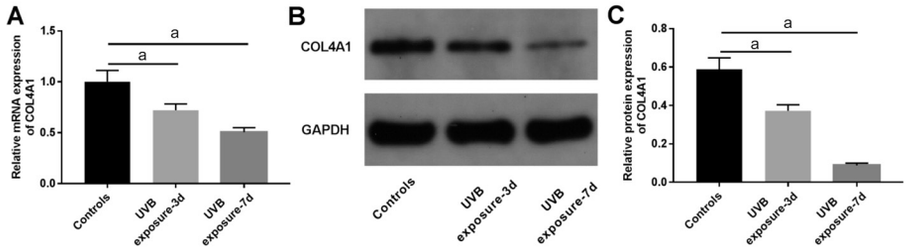

Figure 5 Comparison of mRNA and protein levels of COL4A1 in anterior lens capsules of the UVB treated rat A: The mRNA levels of COL4A1 in the anterior lens capsules of both UVB treated rat model and control groups were analyzed using quantitative RT-PCR; B: Protein expression of COL4A1 in the anterior lens capsules of both UVB treated rat model and control groups was examined through Western blot analysis; C: The relative level of COL4A1 protein to GAPDH was calculated and presented as mean±SD. [a]*P*<0.05 *vs* control group. UVB: Ultraviolet-B; RT-PCR: Reverse transcription-polymerase chain reaction; SD: Standard deviation.

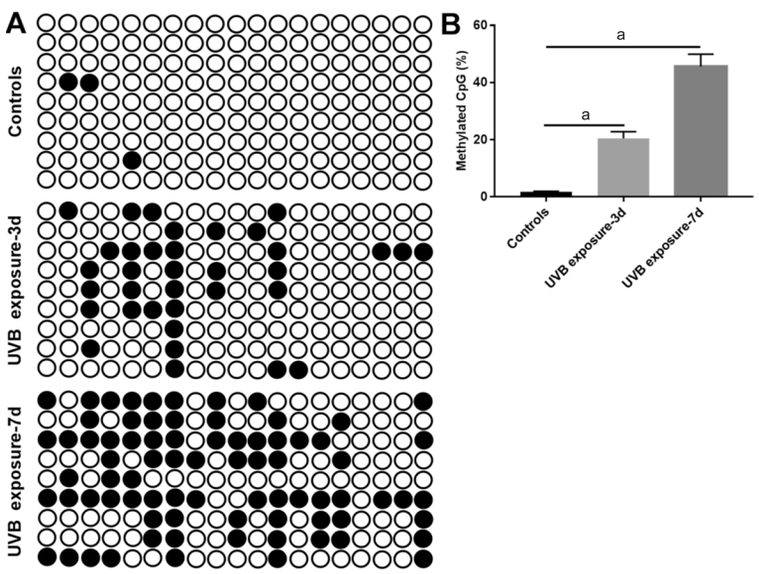

Figure 6 Methylation of the CpG island located at *COL4A1* promoter in anterior lens capsules of the UVB treated rat A: The *COL4A1* CG-rich sequence of the CpG island in the DNA from anterior lens capsules of the UVB treated rat model and the controls was analyzed using bisulfite genomic sequencing. Each row of circular shapes represents an individual plasmid clone. Unmethylated and methylated CpG sites are represented by circles that are opened and closed, respectively. B: The group exposed to UVB showed a dose-dependent increase in hypermethylation compared to the control group, indicating higher levels of methylation. [a]*P*<0.05 *vs* control group.

reduction was positively correlated with the treatment time and dose of UVB.

To validate the correlation between methylation status and COL4A1 expression, we evaluated the degree of methylation of the *COL4A1* promoter region in DNA samples extracted from both UVB-treated rat models and control groups using BSP. As depicted in Figure 6A and 6B, the methylation level observed at the CpG island of COL4A1 promoter was comparatively elevated in rat models subjected to UVB exposure compared to the control group.

We further examined the levels of mRNA expression for DNMTs and TETs. The results indicated that DNMTs, including DNMT1/2/3, were expressed at higher level in UVB treated rat model relative to that in the control group (Figure 7A-7C). Contrarily, the expression of TETs, including TET1/2/3, were reduced in UVB treated rat model compared to that in the controls (Figure 7D-7F).

DISCUSSION

Epigenetic modifications have been demonstrated to contribute to the pathogenesis of age-related macular degeneration and ARC[27-29,34]. Recent advancements in epigenomics and epigenetics indicate that epigenetics might be a crucial regulatory mechanism of environmental factors influencing genome alterations[35]. Diet and environmental exposures had been confirmed to be able to modify the patterning of DNA methylation[36]. It has been demonstrated that the regulation of gene expression in development is linked to DNA methylation[37]. Hence, changes in DNA methylation could be strongly linked to the development of eye disorders associated with aging that are triggered by specific environmental hazards such as excessive exposure to UVB radiation.

In this research, we carried out an investigation to affirm the influence of UVB radiation on the methylation process of *COL4A1* DNA in ARC. We noticed a remarkable decline in

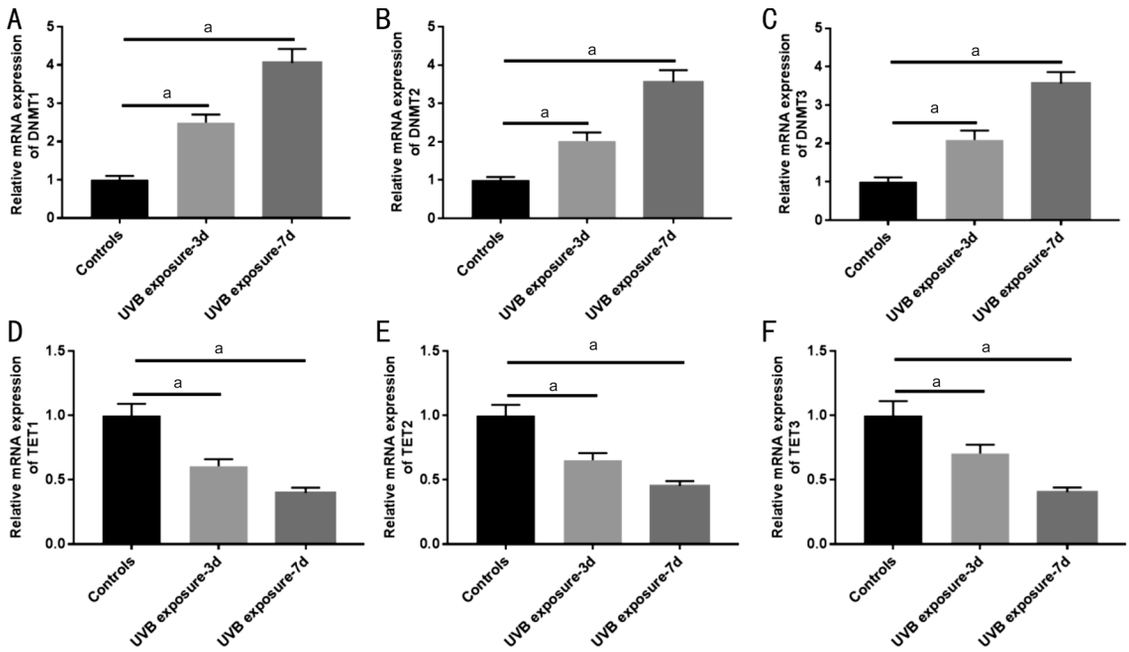

Figure 7 Relative expression of mRNA levels of DNMTs and TETs in anterior lens capsules of the UVB treated rat A: Quantitative RT-PCR analysis of the DNMT1/2/3 (A-C) and TET1/2/3 (D-F) mRNA in anterior lens capsules of control and UVB exposure group were analyzed using quantitative RT-PCR. Values represent as mean±SD. [a]*P*<0.05 *vs* control group. UVB: Ultraviolet-B; RT-PCR: Reverse transcription-polymerase chain reaction; SD: Standard deviation.

both mRNA and protein levels of COL4A1 in lens epithelial cells exposed to UVB, in contrast to the control group. This reduction was discovered to be related to the increased methylation of the CpG islands within the promoter region of *COL4A1*. In conclusion, the results suggested that the decrease in COL4A1 expression caused by DNA methylation is connected to the occurrence of ARC resulting from exposure to UVB radiation.

It has been shown that the lens epithelium in its normal state produces fibrillar collagen, which forms an essential part of the basement membrane located on the anterior surface of the lens capsule. Previous research has shown that human lens epithelium cells in extended cultures produce a consistent layer of material resembling a capsule, indicating that the regeneration of strong cell-to-cell connections or achieving a high cellular density similar to the natural lens environment could play a crucial role in the synthesis of lens capsules[38-39]. The potential involvement of altered DNA methylation in the abnormal expression of COL4A1 may contribute to the progression of ARC induced by UVB exposure. The initial stage of the photodynamic reaction involves the sensitizer absorbing light, resulting in the generation of an excited state known as a triplet state. Subsequently, this excited sensitizer has the capability to directly interact with the substrate, leading to the production of either singlet oxygen or free oxygen radicals. The physical crosslinking of collagen is induced by reactive oxygen species. The mechanical stiffening effect occurs when collagen undergoes crosslinking through riboflavin-ultraviolet A, similar to how cataract formation arises from the ultraviolet-induced crosslinking of crystalline proteins in the lens. Mutations in *COL4A1* had been identified as a contributing factor to the development of cerebral small vessel disease. Additionally, it is common for the eyes and kidneys to be affected by this condition. Results of previous studies indicated that gene mutations of *COL4A1* were associated with eye diseases. The lack of observable traits in mice with a heterozygous genotype for a null allele of *COL4A1* and *COL4A2* implies that the presence of the mutant protein is necessary for disease manifestation[40]. Van Agtamel *et al*[41] observed that mice with a dominant mutation in the *COL4A1* gene exhibited manifestations such as opacity of the cornea, adhesion between the iris and cornea, and hydrophthalmos. Other studies have identified about 13 single mutations in the *COL4A1* gene that can lead to lens abnormality and bubble cataract[42]. However, the precise mechanisms underlying the development of cataracts due to these collagen mutations remain elusive.

In the current study, we did not further explore the function of transcription factors. Further investigation should prioritize the exploration of the impact of DNA methylation on the functionality of associated transcription factors. An investigation in a large, multicenter and prospective cohort is planned to be conducted to illustrate the exact mechanisms of UVB and *COL4A1* promoter hypermethylation in cataract prognosis. We chose to employ the HLEB3 cells for conducting *in vitro* experiments because of their recognized potential as an

appropriate candidate for intervention and ultraviolet radiation studies in previous research. Nevertheless, as a modified immortalized cell line, HLEB3 is still distinct from the primary lens epithelial cells in nature, which might interfere with the outcomes of related *in vitro* experiments. Hence, we will contemplate using the primary lens epithelial cells as the cell model in future further studies.

In conclusion, our results verified the hypermethylation status in *COL4A1* promoter in lens of UVB-induced ARC. Therefore, it is imaginable that epigenetic change in COL4A1 promoter might be involved in the UVB exposure-induced lens damage in ARC. The current results further illustrate the importance of protecting the lens from excessive UVB exposure in healthy people.

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Authors' contributions: Li P contributed to the conception and design of experiments, authored, as well as reviewed drafts of the paper; Wang L, Sun J, and Zhu D performed the experiments, prepared figures along with the tables; Yang Y and He Y analyzed the data; Li YM and Wang ZJ prepared figures. All authors reviewed previous versions of the manuscript, and read and approved the final version of the article.

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Conflicts of Interest: Wang L, None; **Zhu D,** None; **Yang Y,** None; **He Y,** None; **Sun J,** None; **Li YM,** None; **Wang ZJ,** None; **Li P,** None.

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