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ORIGINAL ARTICLE

Silencing *UHRF1* Inhibits Cell Proliferation and Promotes Cell Apoptosis in Retinoblastoma Via the PI3K/Akt Signalling Pathway

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Abstract

This study aimed to investigate the effect of silencing ubiquitin-like with PHD and RING finger domains 1 (UHRF1) on the proliferation and apoptosis of retinoblastoma (RB) cells and to clarify the molecular mechanism of the *UHRF1* gene in the development of RB. Human RB WERI-Rb-1 cells were selected and assigned into a blank group (WERI-Rb-1 cells with no transfection), NC-shRNA group (WERI-Rb-1 cells infected with NC-shRNA virus) and *UHRF1*-shRNA group (WERI-Rb-1 cells infected with pGC-UHRF1-shRNA-LV-GFP# (39–1) virus). The mRNA and protein expression of UHRF1 was detected by RT-qPCR and Western blot analysis. The effect of silencing *UHRF1* on the proliferation and apoptosis of WERI-Rb-1 cells was assessed by MTT assay, EdU assay, flow cytometry, and Hoechst staining. Furthermore, the expression of cell cycle-related factor (cyclin D1), apoptosis-related factors (caspase-9, Bcl-2 and Bax), and PI3K/Akt signalling pathway-related factors (p-PI3K, PI3K, p-Akt and Akt) were measured via Western blot analysis. The RNA interference plasmid *UHRF1*-shRNA was successfully constructed. After WERI-Rb-1 cells were infected with UHRF1-shRNA, decreased mRNA and protein expression of UHRF1 was found. WERI-Rb-1 cells infected with UHRF1-shRNA showed inhibited proliferative ability and increased apoptosis. In the *UHRF1*-shRNA group, more cells arrested at the G0/G1 phase and less cells at the S and G2/M phases. WERI-Rb-1 cells infected with *UHRF1*-shRNA had increased expression of caspase-9 and Bax and decreased expression of Bcl-2 expression and decreased levels of p-PI3K and p-Akt. In conclusion, our study demonstrated that silencing *UHRF1* could inhibit the proliferation of RB cells and promote apoptosis. The mechanism may be caused by the downregulation of the proportion of Bcl-2/Bax expression and the promotion of the expression of caspase-9 through the PI3K/Akt signalling pathway.

Keywords UHRF1 · Retinoblastoma · PI3K/Akt signalling pathway · Cell proliferation · Cell apoptosis

Introduction

Retinoblastoma (RB) is a rare form of retinal cancer in children. If left untreated, RB can develop into sporadic or hereditary forms, or can even be fatal [1]. RB is characterized by the inaction of functional alleles of the RB1 gene and has a morbidity of one out of 15,000 to 20,000 live births [2, 3]. Recent studies indicated that bilateral disease was found in approximately 40% of patients, while unilateral disease was present in the remaining 60% of patients [4]. Currently, RB contributes to the rising costs of health care among those countries with high birth rates, such as Asia and Africa [5]. RB has many symptoms, including pupil abnormalities, deterioration of vision, leukocoria, red and irritated eyes, growth retardation or stunting [6]. The treatment for RB generally includes plaque radiotherapy, thermotherapy, external radiotherapy, laser photocoagulation, chemotherapy and ophthalmectomy [7]. When RB is detected at later stages, in addition to active treatment and poor efficiency, the main detriment to paediatric patients is the incidence of secondary cancer and retinopathy [8]. Therefore, it is crucial to explore the molecular mechanisms involved in the formation and progression of RB in order to establish a novel therapeutic pathway for RB.

Ubiquitin-like with PHD and ring finger domains 1 (UHRF1), also known as ICBP90, was identified as a protein whose expression is detected only in proliferative cells, but

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not in quiescent cells [9]. Recent findings have demonstrated demonstrating that UHRF1 can regulate gene expression via epigenetic mechanisms, including DNA methylation, histone methylation, histone ubiquitination and histone deacetylation [10–12]. In addition, UHRF1 is highly expressed in many types of cancer cells, and its overexpression has been correlated with tumour promoting effects, such as high proliferation potential and the inhibition of apoptosis [13]. Furthermore, the mRNA and protein expression of UHRF1 are involved in the cell cycle; for example, silencing UHRF1 inhibits S phase entry, and zebrafish with a depletion of function mutation in UHRF1 suppresses the proliferation of hepatocytes and increases apoptosis [12, 14]. Interestingly, a previous study also demonstrated that UHRF1 is highly expressed in RB and functions as a tumour-promoting factor, as demonstrated by reduced colony formation and a reduced size of xenografted tumours upon silencing UHRF1 in RB cells [15]. However, the function of silencing UHRF1 in RB cell biological processes with the involvement of the PI3K/Akt signalling pathway has not been well studied. Thus, we successfully constructed the RNA interference plasmid UHRF1-shRNA to investigate the regulatory role of silenced UHRF1 in WERI-Rb-1 cells through the PI3K/Akt signalling pathway.

Materials and Methods

Transfection and Screening of Recombinant Lentivirus Vector Plasmid

HEK293T human embryonic kidney cells (293 T) were used as a packaging host for the transfection-based production of recombinant lentiviruses. The 293 T cells (Genepharma Co., Ltd., Shanghai, China) were cultured in RPMI 1640 medium containing 10% foetal bovine serum (FBS) in an incubator at 37 °C with 5% CO_2. One day before transfection, 293 T cells were inoculated into 24-wellplates at a density of 1×10^5 cells/mL. The recombinant lentivirus vector pGC-UHRF1-shRNA-LV-GFP# (39–1) and the negative control (NC) lentivirus vector pGC-NC-LV-GFP (Genepharma Co., Ltd., Shanghai, China) were transfected into 293 T cells in accordance with the Lipofectamine 2000 instructions (Invitroge, USA), and a blank group (293 T cells without transfection) was also stablished. After 48 h of transfection, total protein from each group was extracted using a protein extraction kit, and the protein concentration was measured by the BCA method.

The recombinant lentivirus vector and NC vector plasmid were transformed into *Escherichia coli* receptive DH5α cells with the co-plasmids pHelper 1.0 and pHelper 2.0. The bacterial solution was collected, and plasmid DNA was extracted and purified by plasmid extraction kit. The plasmid DNA was transfected into 293 T cells in accordance with the method provided by Lipofectamine 2000 (Invitrogen, USA). After 48 h of transfection, the supernatant was extracted by centrifugation, then the supernatant was filtered, centrifuged and used as the virus concentrate. The 293 T cells were infected by the double dilution method. The number of fluorescent cells and the virus titre were observed under a fluorescence microscope. The virus titre (U/mL) = the number of positive cells expressing GFP × dilution multiple / virus volume.

Cell Transfection and Grouping

The human RB WERI-Rb-1 cell line (purchased from American Type Culture Collection [ATCC], VA, USA) was cultured in RPMI 1640 medium (HyClone Company, Logan, UT, USA) containing 10% FBS and maintained in an incubator with 5% CO_2 at 37 °C. One day before infection, WERI-Rb-1 cells were inoculated into 24-wellplates at a density of 1×10^5 cells/well. After overnight culture, the cells were infected with lentivirus supernatant containing pGC-UHRF1-shRNA-LV-GFP# (39–1) or pGC-NC-LV-GFP with a multiplicity of infection (MOI) of 20. After 48 h of infection, the expression of GFP was observed under a fluorescence microscope. The cells were collected and washed with PBS and the infection efficiency was determined by flow cytometry and CellQuest 2.0 software (BD Biosciences Inc.). If the number of GFP positive cells was >70%, then the following experiment was conducted. The WERI-Rb-1 cells were assigned into three groups: blank group (WERI-Rb-1 cells without transfection), NC-shRNA group (WERI-Rb-1 cells infected with NC virus) and *UHRF1*-shRNA group (WERI-Rb-1 cells infected with pGC-UHRF1-shRNA-LV-GFP# (39–1) virus).

RNA Extraction and Reverse Transcription Quantitative Polymerase Chain Reaction (RT-qPCR)

Total RNA from cells was extracted by TRIzol reagent (Invitrogen, Carlsbad, California, USA) in accordance with the manufacturer's instructions. Total RNA (500 ng) was reverse transcribed in a final volume of 10 mL with the PrimeScript RT Reagent Kit (TaKaRa Biotechnology Co. Ltd., Liaoning China). According to the SYBR Premix Ex Taq instructions (TaKaRa Biotechnology Co. Ltd., Liaoning China), UHRF1 expression was measured. GAPDH was used for normalization. Primer sequences are presented in Table 1.

Table 1 Quantitative polymerase chain reaction primer sequences

Primer name	Primer squence
GAPDH - F	TGACTTCAACAGCGACACCCA
GAPDH - R	CACCCTGTTGCTGTAGCCAAA
UHRF1 - F	CGACGGAGCGTACTCCCTAG
UHRF1 - R	TCATTGATGGGAGCAAAGCA

RT-qPCR was performed three times on an ABI 7500 (ABI, Austen, 166 Texas), and the data were calculated using the comparative cycle threshold (CT) ($2^{-\Delta\Delta Ct}$) method.

3-[4,5-Dimethylthiazol-2-Yl]-2,5-Diphenyl Tetrazolium Bromide (MTT) Assay

The cells were collected, and the concentration was adjusted to 5×10^3 cells/well before culturing at 37 °C with 5% CO_2 for 24 h, 48 h, 72 h and 96 h. Then, 20 μL of MTT (Sigma-Aldrich, Shanghai, China) was added to the cells (5 mg/mL), and the cells were maintained at 37 °C with 5% CO_2 for 4 h before the MTT was removed. The optical density (OD) value was measured at 490 nm when the cells were suspended in 150 μL of dimethyl sulfoxide.

5-Ethynyl-2′-Deoxyuridine (EdU) Incorporation Assay

After 48 h of transfection, an EdU incorporation assay was performed in WERI-Rb-1 cells according to the instructions of the Cell-Light EdU imaging detecting kit (RiboBio, Guangzhou, China). As an alternative thymidine analogue, the incorporation of EdU can label and identify cells undergoing DNA replication. EdU-positive cells were calculated with the following formula = (EdU positive cells/Hoechst stained cells) × 100%.

Flow Cytometry

Cell apoptosis and the cell cycle were measured by flow cytometry. After suspending in 500 μL of binding buffer, the cells were collected and washed twice with PBS. After the cells were incubated in the dark at room temperature for 15 min, Annexin V-fluorescein isothiocyanate (5 μL) and propidium iodide (PI) (5 μL) (Key GEN, China) were added for the cell apoptosis assay, and 500 μL of PBS containing 50 μg/mL PI was added for the cell cycle analysis. Fluorescence was detected by flow cytometry (BD Biosciences Inc.).

Western Blot Analysis

Cells were washed twice with PBS, lysed in sample buffer, and heated at 98 °C for 10 min. The protein concentration was measured by the BCA method. The, the proteins were separated on 9% SDS/polyacrylamide gels, transferred onto PVDF membranes (Millipore, USA), and blocked with 5% non-fat milk for 1 h at room temperature. Subsequently, the membranes were incubated with primary antibodies, namely, anti-UHRF1 (1:500; Novus, USA); anti-caspase-9, Bcl-2, Bax, p-PI3K and p-Akt (1:1000; Santa Cruz, USA); and anti-GAPDH (1:500; Cell Signaling Technology, USA) at 4 °C overnight. The membranes were subsequently incubated with secondary antibodies for 1 h at room temperature. GAPDH was served as an internal control. The ChemiDocTM XRS + Imaging system (Bio-Rad Laboratories, Inc., Hercules, CA, USA) and Image Gauge V3.12 (Fujifilm, Tokyo, Japan) were used to obtain the chemiluminescence measurements and semi-quantitative values.

Tumour Xenograft Experiment

WERI-Rb-1 cells (100 μL, 1×10^6 cells), or WERI-Rb-1 cells from UHRF1-shRNA and NC-shRNA stably transfected lines, were collected and injected subcutaneously into the left side of the neck of each nude mouse (male, 4-week-old, 5 mice per group). Mice were monitored daily, and the tumour volumes were evaluated ($0.5 \times \text{length} \times \text{width}^2$) for four days. After 20 days, the mice were sacrificed, and all tumour grafts were excised and weighed. A portion of the tissues was used for extracting protein and total RNA, and the remaining tissue was fixed in 4% paraformaldehyde for 24 h. All animal experiments were performed in the animal laboratory centre of the College of Veterinary Medicine, Jilin University. The study protocol was approved by the Animal Care and Use Committee of the College of Veterinary Medicine, Jilin University.

Statistical Analysis

All the data in our study were analysed by GraphPad Prism (GraphPad Software Inc., La Jolla, CA) and SPSS 19.0 software (SPSS, Chicago, IL, USA) and are presented as the mean ± standard deviation. Significant differences were assessed using Student's t test or one-way ANOVA. Values of $P < 0.05$ indicate a statistically significant difference.

Results

Determination of Lentivirus Infection Efficiency

After pGC-UHRF1-shRNA-LV-GFP# (39–1) and the NC lentivirus vector pGC-NC-LV-GFP were infected into 293 T cells for 48 h, Western blot analysis (Fig. 1a) was performed to detect the protein expression of UHRF1. The protein expression of UHRF1 in the 293 T cells infected with the pGC-UHRF1-shRNA-LV-GFP# (39–1) vector was significantly lower than that in the cells with pGC-NC-LV-GFP ($P < 0.05$). There was no significant difference between the NC group and the blank group ($P > 0.05$).

By fluorescence microscopy, GFP was expressed in the 293 T cells transfected with *UHRF1*-shRNA or NC-shRNA lentivirus, and the infection efficiency was higher than 90%. No GFP expression was found in the 293 T cells without lentivirus infection (Fig. 1b).

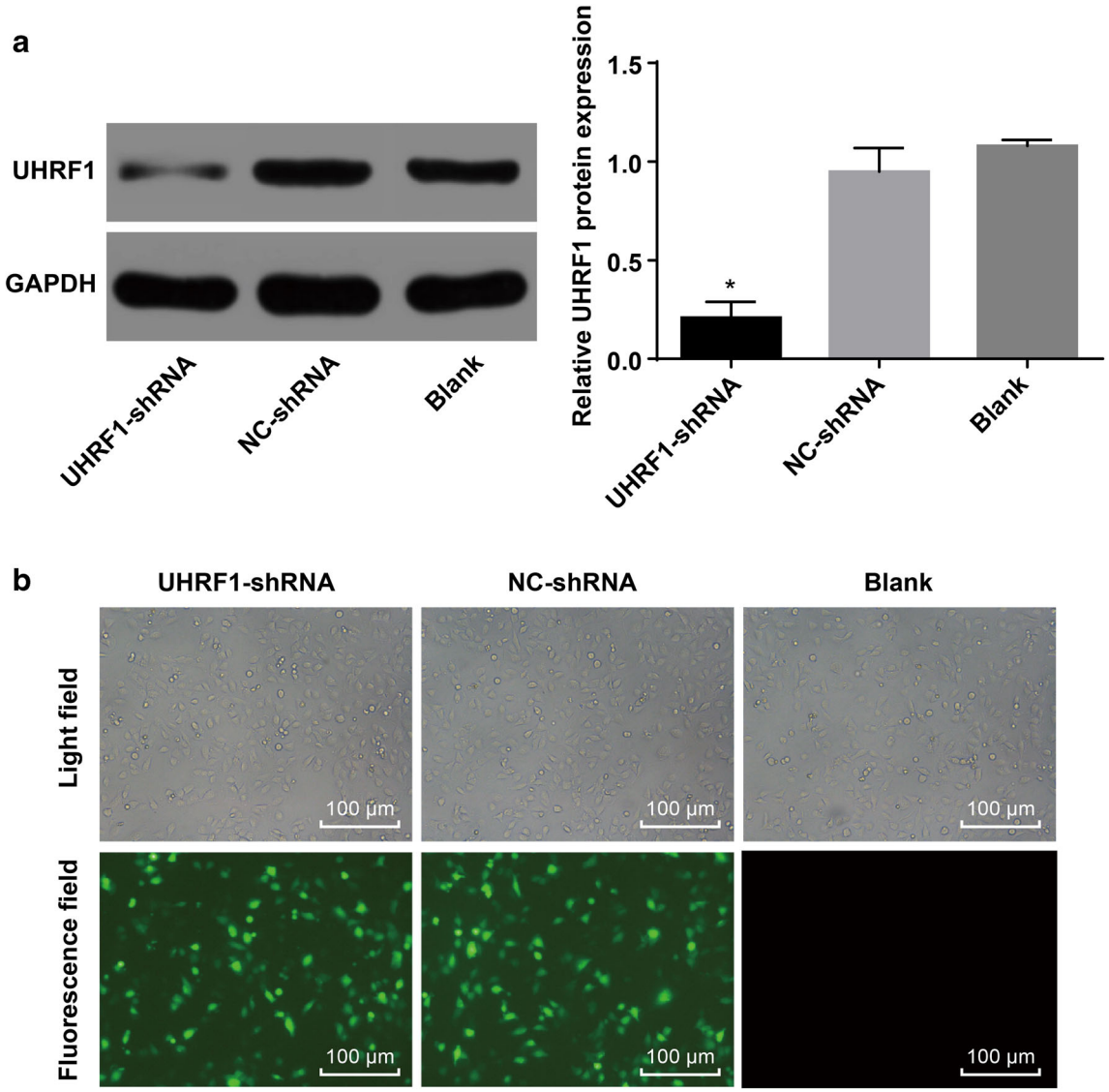

Fig. 1 pGC-UHRF1-sh RNA-LV-GFP#1 is an effective carrier. Note: **a** The protein expression of UHRF1 in 293 T cells was detected by Western blot analysis. **b** Lentivirus infection efficiency of 293 T cells in different groups was observed under a contrast microscope (upper) and a fluorescence microscope (lower) (100 X)

Effect of *UHRF1*-shRNA Infection on the mRNA and Protein Expression of UHRF1

After pGC-UHRF1-shRNA-LV-GFP# (39–1) and the NC lentivirus vector pGC-NC-LV-GFP were infected into 293 T cells for 48 h, WERI-Rb-1 cells carrying GFP were observed in the field of vision of the NC group and the *UHRF1*-shRNA group by fluorescence microscopy (Fig. 2a). The flow cytometry results (Fig. 2b) showed that the rate of GFP-positive cells in the NC group and the *UHRF1*-shRNA group was (85.21 ± 4.21)% and (88.08 ± 5.53)%, respectively, indicating that the lentivirus carrying the recombinant gene was successfully infected into the WERI-Rb-1 cells.

The results of the RT-qPCR and Western blot analysis illustrated that after the WERI-Rb-1 cells were infected with lentivirus, the mRNA and protein expression of UHRF1 in the *UHRF1*-shRNA group decreased significantly (Fig. 2c and d; $P < 0.0001$). There was no significant difference in the mRNA and protein expression of UHRF1 between the NC-shRNA group and the blank group ($P > 0.05$). The results demonstrated that the virus infection itself had no significant effect on UHRF1 content.

Silencing *UHRF1* Inhibits the Proliferation of WERI-Rb-1 Cells

To study the effects of *UHRF1* in cell proliferation, the MTT assay was conducted. No significant difference was found in the proliferation of WERI-Rb-1 cells between the

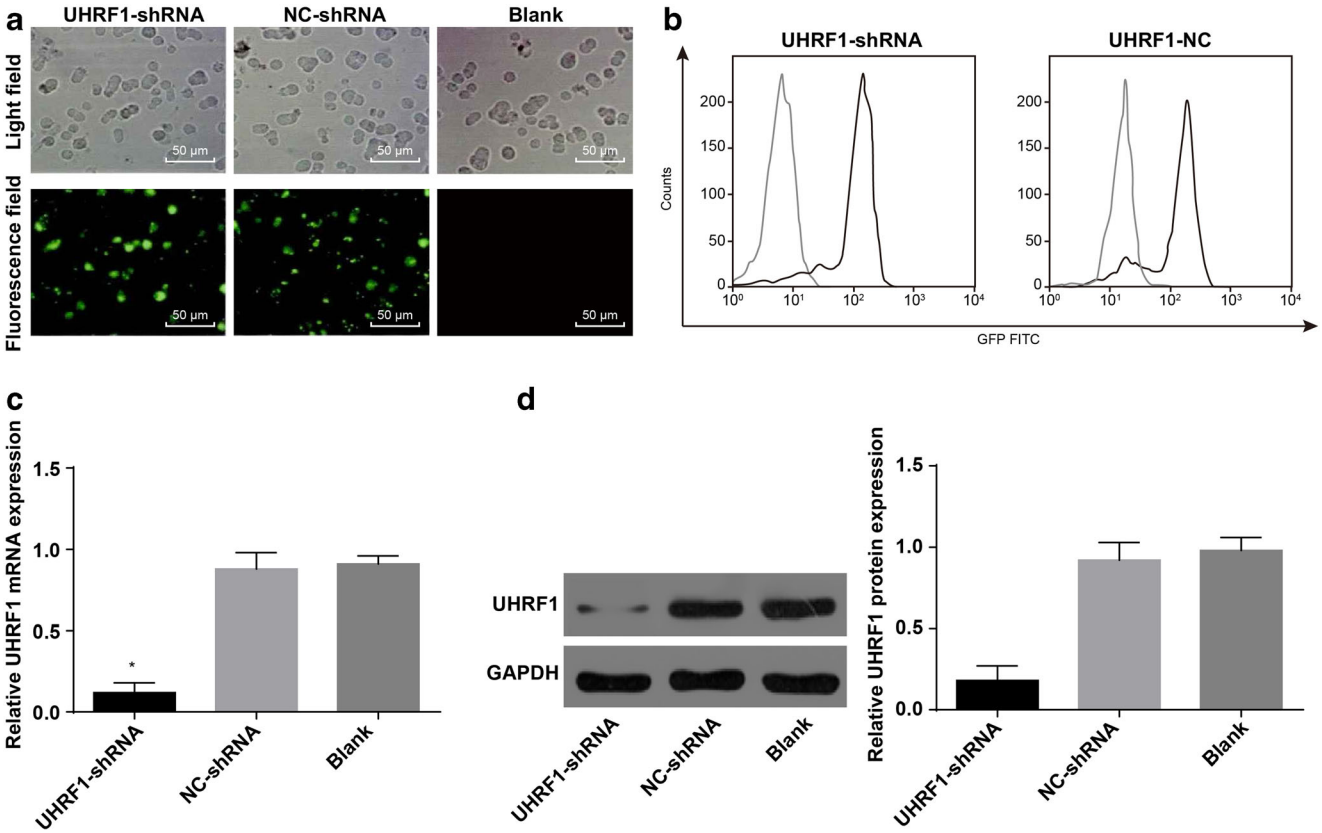

Fig. 2 Effect of *UHRF1*-shRNA infection on the mRNA and protein expression of UHRF1. Note: **a** Lentivirus infection efficiency of WERI-Rb-1 cells in different groups was observed under a contrast microscope (upper) and a fluorescence microscope (lower) (100 X). **b** The infection efficiency of WERI-Rb-1 cells at 48 h after infection with recombinant lentivirus was detected by flow cytometry. **c** The mRNA expression of UHRF1 in WERI-Rb-1 cells after infection with lentivirus was detected by RT-qPCR. **d** The protein expression of UHRF1 in WERI-Rb-1 cells after infection with lentivirus was detected by Western blot analysis

NC-shRNA and blank groups (Fig. 3a; $P > 0.05$), while the proliferative ability of WERI-Rb-1 cells in the *UHRF1*-shRNA group decreased significantly from the second day ($P < 0.05$). An EdU assay further showed that the proliferation rates of the *UHRF1*-shRNA, NC-shRNA and blank groups were (8.85 ± 1.24)%, (35.14 ± 4.68)% and (32.54 ± 4.14)%, respectively. A significant difference was found between the *UHRF1*-shRNA group and the NC-shRNA and blank groups ($P < 0.001$) (Fig. 3b). To investigate the role of UHRF1 on the cell cycle, flow cytometry (Fig. 3c) showed that the number of cells in the G0/G1 phase in the *UHRF1*-shRNA group [(76.54 ± 8.24)%] was higher than that in the NC-shRNA [(65.28 ± 5.87)%] and blank [(66.54 ± 7.41)%] groups ($P < 0.05$). The number of cells in S phase in the *UHRF1*-shRNA group [(12.24 ± 2.52)%] was lower than that in the NC-shRNA [(26.48 ± 2.52)%] and blank [(26.89 ± 2.57)%] groups ($P < 0.01$). No significant difference was found in the cell cycle distribution between the NC-shRNA and blank groups ($P > 0.05$).

In addition, factors related to the cell cycle, such as cyclin D1 were measured by Western blot assay to further confirm our results. As shown in Fig. 3d, cyclinD1 expression was significantly decreased in the *UHRF1*-shRNA group when compared with the NC-shRNA and blank groups. These results showed that *UHRF1* is an important factor that can help promote RB development.

Silencing *UHRF1* Promotes the Apoptosis of WERI-Rb-1 Cells

Flow cytometry was used to determine the effects of UHRF1 on WERI-Rb-1 cell apoptosis after pGC-UHRF1-shRNA-LV-GFP# (39–1) and the NC lentivirus vector pGC-NC-LV-GFP were infected into 293 T cells for 48 h. The results (Fig. 4a) indicated a significant difference in the cell apoptosis rate between the *UHRF1*-shRNA group [(18.59 ± 2.47)%] and the NC-shRNA [(10.14 ± 1.75)%] and blank [(9.86 ± 1.65)%] groups ($P < 0.05$). No significant difference was found between the NC-shRNA and blank groups ($P > 0.05$). Hoechst staining results (Fig. 4b) suggested that the number of bright blue apoptotic cells in the *UHRF1*-shRNA group was higher than that in the NC-shRNA and blank groups,

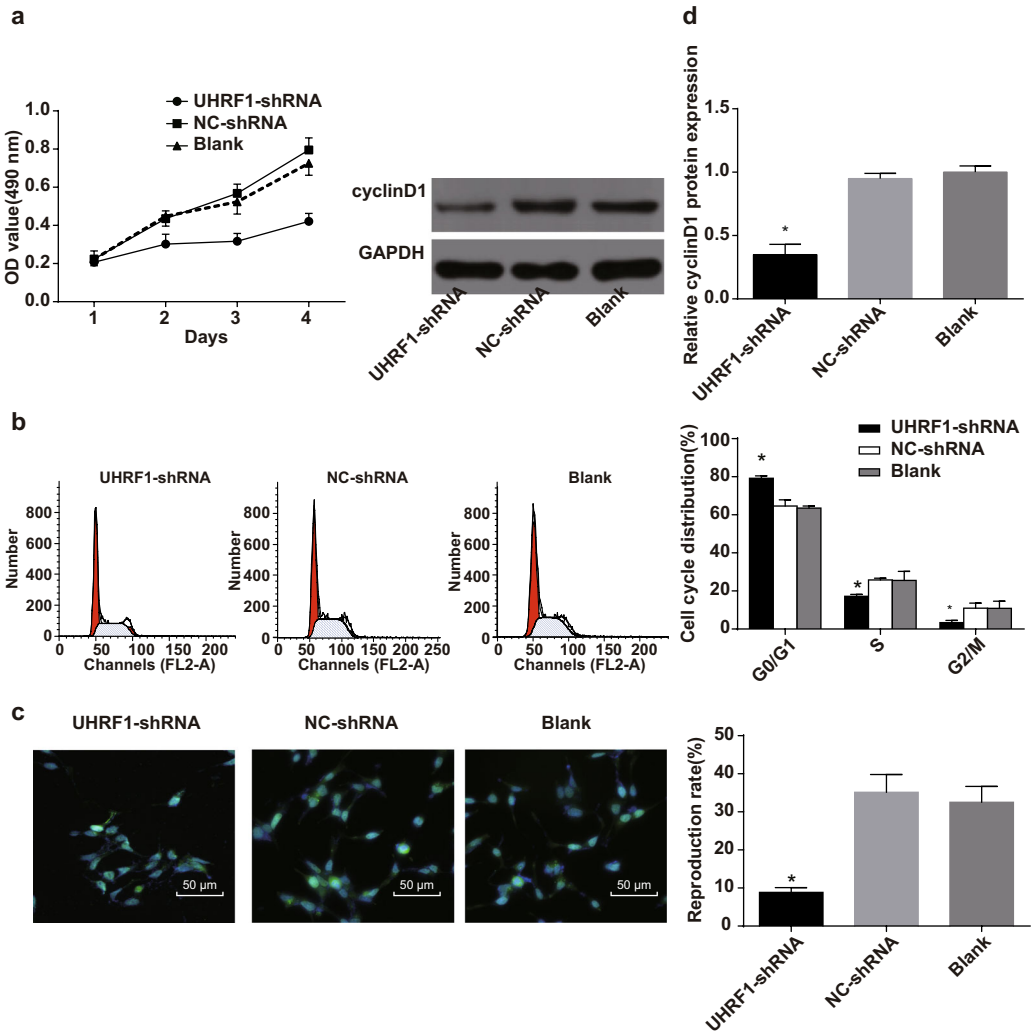

Fig. 3 Silencing *UHRF1* inhibits the proliferation of WERI-Rb-1 cells. Note: **a** The growth curve of WERI-Rb-1 cells detected by MTT assay. **b** Edu assay was used to detect cell proliferation. **c** Flow cytometry was conducted to detect the cell cycle. **d** Western blot analysis was used to detect the expression of a proliferation-related factor (cyclin D1). *$P < 0.05$ compared with the NC-shRNA group and the blank group

and the apoptosis rate increased significantly ($P < 0.05$). No significant difference was found between the NC-shRNA and blank groups ($P > 0.05$).

In addition, Western blot analysis was used to test the expression of apoptosis-related factors (Bax, Bcl-2 and caspase-9). The results showed that the *UHRF1*-shRNA group had increased expression of Bax and caspase-9 and decreased Bcl-2 expression when compared with those levels in the NC-shRNA and blank groups (Fig. 4c; $P < 0.05$). No significant difference was found between the NC-shRNA and blank groups ($P > 0.05$). The above results suggest that silencing *UHRF1* promotes the apoptosis of WERI-Rb-1 cells.

Silencing *UHRF1* Suppresses the Expression of the PI3K/Akt Signalling Pathway in WERI-Rb-1 Cells

A previous study showed that the PI3K/Akt signalling pathway is involved in the regulation of tumour cell proliferation and apoptosis [16]. We verified by Western blot analysis whether *UHRF1* regulates the PI3K/Akt signalling pathway. The results showed that the extent of Akt and PI3K phosphorylation in the WERI-Rb-1 cells in the *UHRF1*-shRNA group was significantly lower than those levels in the NC-shRNA and blank groups ($P < 0.05$), but the total protein levels of Akt and PI3K were not significantly changed in the *UHRF1*-shRNA group ($P > 0.05$; Fig. 5).

Discussion

Previous evidence indicated a high incidence of RB in developing countries [17]. Understanding the underlying pathological mechanisms related to RB are important to designing new targeted therapies to manage RB and preventing metastasis [18]. In the present study, we established the suppression of cell proliferation in

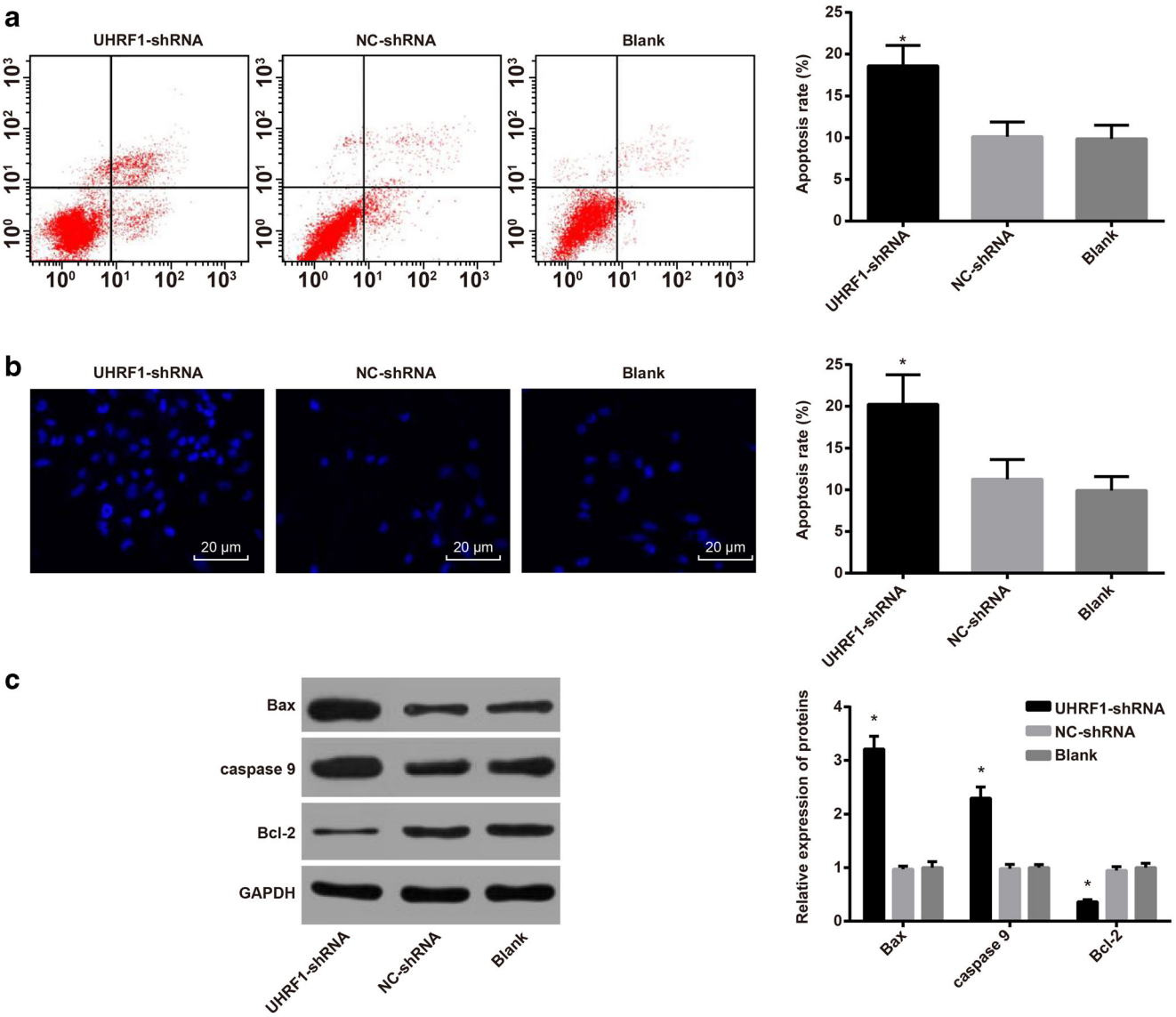

Fig. 4 Silencing *UHRF1* promotes the apoptosis of WERI-Rb-1 cells. Note: **a** The effect of *UHRF1* gene silencing on the apoptosis of WERI-Rb-1 cells was detected by flow cytometry. **b** The effect of *UHRF1* gene silencing on the apoptosis of WERI-Rb-1 cells was detected by Hoechst staining. **c** The effect of *UHRF1* gene silencing on the expression of Bax, Bcl-2 and caspase-9 in WERI-Rb-1 cells was detected by Western blot analysis. * $P < 0.05$ compared with the NC-shRNA group and the blank group

cultured RB WERI-Rb-1 cells using a *UHRF1* gene silencing technique. Specifically, we concluded that silencing *UHRF1* could inhibit RB cell proliferation and promote apoptosis. The mechanism may be caused by the downregulation of the proportion of Bcl-2/Bax and the promotion of the expression of caspase-9 through the PI3K/Akt signalling pathway.

The vector for the downregulation of *UHRF1* was successfully constructed with an infection efficiency of greater than 90%. The successful infection of WERI-RB-1 cells by a lentivirus carrying the recombinant gene was confirmed by flow cytometry. After RT-qPCR and Western blot analysis, the mRNA and protein expression of *UHRF1* in the UHRF1-shRNA group decreased significantly after lentivirus infection, suggesting that the virus infection itself had no significant effect on *UHRF1* content.

In the following experiments, the effects of silencing *UHRF1* on the regulation of the biological functions of RB WERI-Rb-1 cells were investigated. The obtained results demonstrated that silencing *UHRF1* inhibited the proliferation of WERI-Rb-1 cells and arrested more cells at the G0/G1 phase, accompanied with decreased levels of cyclin D1. Previous evidence demonstrated that uncontrolled proliferation is one of the characteristics of tumourigenesis and is usually caused by changes in cell growth signalling pathways or cell cycle checkpoints [19]. Previous evidence suggests that

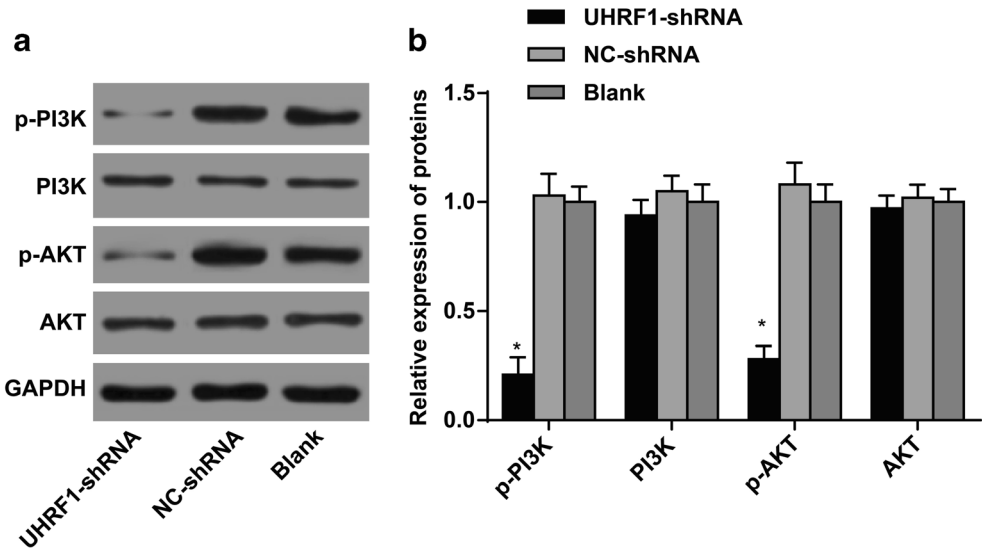

Fig. 5 Silencing *UHRF1* suppresses the expression of the PI3K/Akt signalling pathway in WERI-Rb-1 cells. Note: The effects of *UHRF1* gene silencing on the expression of PI3K, p-PI3K, p-Akt and Akt in WERI-Rb-1 cells was detected by Western blot analysis. * $P < 0.05$ compared with the NC-shRNA group and the blank group

UHRF1 functions as a basic regulator of cell proliferation and maintains DNA methylation; theupregulation of UHRF1 expression can be found in multiple types of human malignancy but is unchanged in differentiated tissues, suggesting that UHRF1 plays a key role in carcinogenesis and can be a useful anticancer drug target [20, 21]. Furthermore, *UHRF1*-deficient cells result in the suppression of the oncogenic properties of cancer cells and an enhanced sensitivity to anticancer drugs and γ-rays [22]. Np95/ICBP90-like RING finger protein (NIRF) has a multi-domain composition, including a NIRF_N domain (ubiquitin-like domain), PHD finger domain, YDG/SRA domain and RING finger domain [23]. As an ubiquitin ligase, NIRF can interact with the CDK2-cyclin E complex and induce G1 arrest [24, 25]. NIRF can interact with multiple cell cycle proteins, including cyclins (A2, B1, D1 and E1), p53 and pRB, indicating its close function with the core cell cycle machinery [26], which agrees with our results.

After pGC-UHRF1-shRNA-LV-GFP# (39–1) and NC lentivirus vector pGC-NC-LV-GFP were infected to 293 T cells for 48 h, flow cytometry was used to detect cell apoptosis. The results revealed that silencing *UHRF1* promotes the apoptosis of WERI-Rb-1 cells (presented by increased expression of Bax and caspase-9 and decreased Bcl-2 expression). Caspase family members play a key role in the process of apoptosis [27]. The activation of caspase-9 after mitochondrial destruction and cytochrome c release is related to the intrinsic or mitochondrial pathway of apoptosis [28]. Additionally, after treatment with UNBS5162, a naphthalimide, WERI-Rb-1 cells showed significantly upregulated Bax expression and downregulated Bcl-2 expression [29]. In agreement with our study, a previous report demonstrated that after transfection with *UHRF1* siRNA, the expression of cleaved caspase-9 and Bax increased, while Bcl-2 expression decreased [27].

Furthermore, the mechanism of the involvement of the PI3K/Akt signalling pathway in RB was investigated. We found that WERI-Rb-1 cells infected with *UHRF1*-shRNA presented decreased levels of p-Akt and p-PI3K, suggesting that silencing *UHRF1* suppressed the PI3K/Akt signalling pathway. The PI3K/Akt pathway is involved in cell growth, proliferation, survival and tumourigenesis [30]. Previous findings have documented a significant correlation between the development of RB and the activation of the PI3K/Akt pathway [31]. Consistent with our results, Cui et al. confirmed the decreased phosphorylation of Akt in a coordinated fashion in *UHRF1*-deficient stage 1 cells [32].

Taken together, we provide potent evidence for a new strategy in RB therapy. The key finding of our study presents significant evidence that the *UHRF1*-shRNA system can specifically and efficiently suppress the proliferation of RB cells and promote apoptosis. The mechanism may be caused by downregulating the proportion of Bcl-2/Bax and promoting the expression of caspase-9 through the PI3K/Akt signalling pathway.

Availability of Data and Materials All data generated or analysed during the present study are included in this published article.

Compliance with Ethical Standards

Competing Interests The authors declare that they have no competing interests.

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