

# Long noncoding RNA UCA1 facilitates cell proliferation and inhibits apoptosis via activating PI3K/Akt pathway in retinoblastoma

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## Research article

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# Abstract

**Background** This study aimed to explore the effect of lncRNA-UCA1 on retinoblastoma (RB) and its potential molecular mechanisms. **Methods** In our study, the expression of lncRNA-UCA1 was measured by qRT-PCR in both RB tissues and RB HXO-RB44 and Y79 cells. The relationship between lncRNA-UCA1 expression and clinical parameters in RB patients were evaluated. Cell proliferation, cell clones, apoptosis and cell cycle of HXO-RB44 and Y79 cells were measured by cell counting kit-8 (CCK-8) assay, colony formation assay and flow cytometry, respectively. In addition, the expressions of PCNA, Caspase-3, survivin, p16, p21, CDK2, PI3K, p-PI3K, Akt, p-Akt and S6k in HXO-RB44 and Y79 cells were measured by western blot. **Results** lncRNA-UCA1 was highly expressed in both RB tissues and RB HXO-RB44 and Y79 cells. Moreover, the expression of lncRNA-UCA1 in RB patients was remarkably correlated with tumor size, optic nerve invasion and pathologic grade. lncRNA-UCA1 markedly facilitated cell proliferation and cell cycle progression, as well as inhibited cell apoptosis in HXO-RB44 and Y79 cells. lncRNA-UCA1 dramatically increased the expression of S6k and the phosphorylation of PI3K and Akt in RB cells. LY294002 (PI3K inhibitor) reversed the effects of lncRNA-UCA1 on RB cell proliferation, apoptosis and cell cycle progression. **Conclusions** Our study indicated that lncRNA-UCA1 could promote cell proliferation and cell cycle progression, as well as inhibit cell apoptosis in RB via activating PI3K/Akt pathway.

## Background

Retinoblastoma (RB) is the most common intraocular cancer and mainly affects children under 5 years old.<sup>1,2</sup> The incidence of RB is about 1/15000 all over the world and has no gender predilection.<sup>3</sup> Although the therapeutic techniques have greatly improved, the survival rate of RB patients still remains poor, mainly due to its insensitivity to chemotherapy and radiotherapy.<sup>4</sup> Therefore, it is urgent to investigate new diagnostic markers and therapeutic targets for RB treatment.

Long noncoding RNAs (lncRNAs) are defined as RNA transcripts longer than 200 nts that have no protein-encoding ability.<sup>5</sup> Accumulating evidence has demonstrated that lncRNAs play important roles in the development and progression of cancers including RB.<sup>6</sup> More and more researches have confirmed that lncRNAs participate in numerous biological activities, such as cell proliferation, migration, invasion and cell cycle arrest.<sup>7,8</sup> For example, lncRNA BDNF-AS has been reported to suppress RB cell proliferation, migration and cell cycle transition.<sup>9</sup> Su et al.<sup>10</sup> have indicated that lncRNA BANCR could suppress cell proliferation, migration and invasion in RB. Dong et al.<sup>11</sup> have confirmed that lncRNA-HOTAIR could promote cell proliferation and invasion via regulating Notch pathway in RB. It is reported that lncRNA-H19 inhibited RB cell proliferation, migration and invasion, and promoted cell apoptosis.<sup>12</sup> However, the effect of lncRNA-UCA1 was still fully unclear in RB.

The phosphatidylinositol 3'-kinase/protein kinase B (PI3K/Akt) pathway is reported to play a crucial part in a variety of malignancies.<sup>13-15</sup> In addition, some lncRNAs may exert their functions by regulating PI3K/Akt pathway in cancer.<sup>5</sup> Previous researches have confirmed that lncRNA-UCA1 could promote

osteosarcoma metastasis by activating PI3K/Akt pathway.<sup>16</sup> Li et al.<sup>17</sup> have indicated that lncRNA-UCA1 could facilitate cell proliferation and suppress apoptosis through regulating PI3K/Akt pathway in gastric cancer. Therefore, we investigate whether lncRNA-UCA1 could affect RB via regulating PI3K/Akt pathway.

In this research, we explored the functions of lncRNA-UCA1 on cell proliferation and apoptosis in RB, and its related molecular mechanisms. Our results indicated that lncRNA-UCA1 could facilitate cell proliferation and inhibit apoptosis through activating PI3K/Akt pathway in RB. Our results indicate that lncRNA-UCA1 may serve as a new therapeutic target for RB.

## Methods

### *Clinical samples*

Total 42 fresh RB specimens and the paired adjacent normal retina specimens from the patients of RB were provided by the Ophthalmology Department of our hospital. None of the patients received anti-tumor treatment, chemotherapy and radiotherapy before surgery. In addition, the characteristics of RB patients were presented in Table 1. This research was permitted by the Ethics Committee of our hospital. All patients or their guardians have signed informed consent.

### *Cell cultures*

Two RB cell lines (HXO-RB44 and Y79) and normal retinal vascular endothelial cell line (ACBRI-181) were supplied by American Type Culture Collection (ATCC, USA). The cell lines were cultured in RPMI-1640 medium (Gibco, USA) containing 10% FBS and 1% penicillin/streptomycin. All cells were cultured in a humidified incubator with 5% CO<sub>2</sub> at 37°C.

### *Cell transfection assay*

The pcDNA3.1-UCA1, UCA1 siRNA and their corresponding negative control (pcDNA3.1-Mock and UCA1 siRNA control) were obtained from Thermo Fisher, USA. LY294002 (PI3K inhibitor) was purchased from Sigma, USA. The pcDNA3.1-UCA1, pcDNA3.1-Mock, UCA1 siRNA and UCA1 siRNA control were separately transfected into HXO-RB44 and Y79 cells by Lipofectamine<sup>®</sup> 3000 Reagent (Invitrogen, USA). The transfected HXO-RB44 and Y79 cells were randomly divided into six groups: BLANK group (no-treated group), pcDNA-UCA1 group (transfected with pcDNA3.1-UCA1), pcDNA-Mock group (transfected with pcDNA3.1-Mock), si-UCA1 group (transfected with UCA1 siRNA), si-NC group (transfected with UCA1 siRNA control) and pcDNA-UCA1 + LY294002 group (transfected with pcDNA-UCA1 and treated with 50 μM LY294002). Finally, all cells were cultured in 37°C for 48 hours.

### *Cell counting kit-8 assay*

The cell proliferation abilities of HXO-RB44 and Y79 cells were tested by cell counting kit-8 (CCK-8) kit (Sigma, USA). In brief, the transfected HXO-RB44 and Y79 cells (1 × 10<sup>4</sup> cells/well) were planted into 96-

well plates. At different time points of 24, 48, 72 and 96 h, 10  $\mu$ L CCK-8 was added into each well. Subsequently, the cells were then cultured at room temperature for 1 h. Finally, the absorbance was measured at 450 nm by a microplate reader (Bio-Rad, USA).

### ***Colony formation assay***

The transfected HXO-RB44 and Y79 cells were respectively seeded into 6-well plates at a density of 300 cells per well and cultivated for 14 d. After fixed in 4% cold formaldehyde for 30 min, the colonies were stained with 0.1% crystal violet (Beyotime, China) for 20 min. Finally, the colonies was photographed and counted under an optical microscope (Olympus, Tokyo, Japan).

### ***Cell cycle analyses***

The transfected HXO-RB44 and Y79 cells were harvested, washed with PBS, fixed in pre-cold 70% (v/v) ethanol for 2 h at  $-20^{\circ}\text{C}$ . Subsequently, the cells were stained with Muse<sup>TM</sup> Cell Cycle Reagent (Merck Millipore, USA) in dark for 30 min. Finally, the cells were observed and analyzed by MUSE<sup>TM</sup> flow cytometry (Merck Millipore, USA).

### ***Apoptosis assay***

The apoptosis of HXO-RB44 and Y79 cells was determined by Annexin V-FITC apoptosis detection kit (Invitrogen, USA). In brief, the transfected HXO-RB44 and Y79 cells were collected and resuspended in Binding buffer. Then, the transfected HXO-RB44 and Y79 cells were stained by using Annexin V-FITC and propidium iodide (PI) for 15 min in a dark room. Subsequently, the apoptotic cells were observed using MUSE<sup>TM</sup> flow cytometer.

### ***Real-Time fluorogenic PCR assays***

Total RNA from RB tissue, adjacent normal retina tissue, HXO-RB44 cells, Y79 cells and ACBRI-181 cells was extracted using TRIZOL (Invitrogen, USA). Then, cDNA was synthesized from total RNA using Revert Aid First Strand cDNA Synthesis Kit (Thermo, USA). Subsequently, the qRT-PCR was performed using SYBR green qPCR Master Mix (Thermo Scientific, USA) according to the manufacturer's protocol. Primers used in this study were as follows: lncRNA-UCA1 F: 5'-CCACACCCAAAACAAAAAATCT-3', R: 5'-TCCCAAGCCCTCTAACAAACA-3'; GAPDH F: 5'-TGTTTCGTCATGGGTGTGAAC-3', R: 5'-ATGGCATGGACTGTGGTCAT-3'.

### ***Western blot analysis***

The total proteins were extracted by RIPA buffer (Beyotime, Jiangsu, China). 50  $\mu$ g of protein samples were separated by 10% SDS-PAGE and then transferred onto nitrocellulose membrane. Subsequently, the membranes were blocked with 5% milk in TBST for 2 h, followed by overnight incubations at  $4^{\circ}\text{C}$  with the primary antibody (PI3K, 1:1000; Akt, 1:1000; PCNA, 1:1000; GAPDH, 1:1000, Sino Biological, USA. p-PI3K, 1:500; p-Akt, 1:500; S6K, 1:1000, Abcam, USA. survivin, 1:1000; CDK2, 1:1000; Caspase-3, 1:1000; p16,

1:1000; p21, 1:1000, Cell Signal, USA). Subsequently, the membranes were incubated with the peroxidase-labeled secondary antibody (anti-rabbit IgG, 1:5000, I5381MSDS, Sigma, USA) for 1 h. Finally, the protein bands were detected by an enhanced chemiluminescence (ECL) kit (Thermo Fisher, USA).

## Statistical analysis

All statistical analyses were performed using SPSS 22.0 Statistical Software (Chicago, IL). The results were presented as the mean  $\pm$  SD. Statistical significance was tested using the one-way ANOVA or Student's t-test.  $P < 0.05$  was considered to be statistically significant.

## Results

### *The expression of lncRNA-UCA1 is upregulated and associated with clinical parameters in RB*

The results of qRT-PCR showed that lncRNA-UCA1 expression in RB tissue was markedly higher than that in paracancerous tissue ( $P < 0.01$ ) (**Fig 1A**). Similarly, lncRNA-UCA1 expression was remarkably increased in HXO-RB44 and Y79 cells compared with ACBRI-181 cells ( $P < 0.01$ ) (**Fig 1B**). All the results suggested that lncRNA-UCA1 was highly expressed in RB. We also found that the expression of lncRNA-UCA1 was remarkably correlated with tumor size ( $P < 0.01$ ), optic nerve invasion ( $P < 0.01$ ) and pathologic grade ( $P < 0.01$ ) (**Table 1**). Meanwhile, the expression of lncRNA-UCA1 had no significantly correlation with age and gender in the patients with RB ( $P > 0.05$ ) (**Table 1**). In addition, the expression of lncRNA-UCA1 in transfected HXO-RB44 and Y79 cells was detected by qRT-PCR. The results showed that lncRNA-UCA1 expression of HXO-RB44 and Y79 cells was significantly increased in pcDNA-UCA1 group compared with BLANK and pcDNA-Mock group ( $P < 0.01$ ) (**Fig 1C**). Meanwhile, when compared with BLANK and si-NC group, lncRNA-UCA1 expression of HXO-RB44 and Y79 cells was markedly decreased in si-UCA1 group ( $P < 0.01$ ) (**Fig 1C**), suggesting that the transfection was successful.

### *lncRNA-UCA1 promotes cell proliferation in HXO-RB44 and Y79 cells*

The results of CCK-8 assay showed that the proliferation ability of HXO-RB44 cells was markedly decreased in si-UCA1 group compared with BLANK and si-NC group at 48 h ( $P < 0.05$ ), 72 h and 96 h ( $P < 0.01$ ) (**Fig 2A**). When compared with BLANK and pcDNA-Mock group, the proliferation ability of Y79 cells was remarkably increased in pcDNA-UCA1 group at 48 h ( $P < 0.05$ ), 72 h and 96 h ( $P < 0.01$ ) (**Fig 2A**). Furthermore, colony formation assay also confirmed that silencing lncRNA-UCA1 expression markedly decreased the cell clones number of HXO-RB44 cells ( $P < 0.01$ ) (**Fig 2B**), while lncRNA-UCA1 overexpression significantly increased the cell clones number of Y79 cells ( $P < 0.01$ ) (**Fig 2B**). To further confirm the function of lncRNA-UCA1 on RB cell proliferation, we also measured the expression of PCNA in both HXO-RB44 and Y79 cells. The expression of PCNA of HXO-RB44 cells was dramatically decreased in si-UCA1 group compared with BLANK and si-NC group ( $P < 0.01$ ) (**Fig 2C**). When compared with BLANK and pcDNA-Mock group, the expression of PCNA of Y79 cells was markedly increased in pcDNA-UCA1 group ( $P < 0.01$ ) (**Fig 2C**). All the results indicated that lncRNA-UCA1 could promote cell proliferation in RB cells.

### ***lncRNA-UCA1 inhibits cell apoptosis in HXO-RB44 and Y79 cells***

As shown in **Fig 3A**, silencing lncRNA-UCA1 expression dramatically increased HXO-RB44 cells apoptosis ( $P < 0.01$ ) (**Fig 3A**). However, lncRNA-UCA1 overexpression significantly decreased the apoptosis of Y79 cells ( $P < 0.01$ ) (**Fig 3A**). To further confirm the effect of lncRNA-UCA1 on RB cell apoptosis, we detected the expression of Caspase-3 and survivin in both HXO-RB44 and Y79 cells. Silencing lncRNA-UCA1 expression remarkably increased Caspase-3 expression and decreased survivin expression in HXO-RB44 cells ( $P < 0.01$ ) (**Fig 3B**). On the contrary, lncRNA-UCA1 overexpression markedly decreased Caspase-3 expression and increased survivin expression in Y79 cells ( $P < 0.01$ ) (**Fig 3B**). The results above suggested that lncRNA-UCA1 could inhibit cell apoptosis in RB cells.

### ***lncRNA-UCA1 promotes cell cycle procession in HXO-RB44 and Y79 cells***

As shown in **Fig 4A**, silencing lncRNA-UCA1 in HXO-RB44 cells dramatically increased the ratio of G0/G1 phase cells ( $P < 0.01$ ), and notably reduced the ratio of S ( $P < 0.01$ ) and G2/M phase cells ( $P < 0.01$ ). Meanwhile, lncRNA-UCA1 overexpression in Y79 cells dramatically decreased the ratio of G0/G1 phase cells ( $P < 0.01$ ), and markedly increased the ratio of S ( $P < 0.01$ ) and G2/M phase cells ( $P < 0.01$ ). This suggests that lncRNA-UCA1 could arrest RB cells at the G0/G1 phase. To further confirm the effect of lncRNA-UCA1 on RB cell cycle, we measured the expression of p16, p21 and CDK2 in both HXO-RB44 and Y79 cells. Our results indicated that silencing lncRNA-UCA1 significantly increased p16 and p21 expression, as well as decreased CDK2 expression in HXO-RB44 cells ( $P < 0.01$ ) (**Fig 4B**). Nevertheless, lncRNA-UCA1 overexpression dramatically decreased p16 and p21 expression, and increased CDK2 expression in Y79 cells ( $P < 0.01$ ) (**Fig 4B**). All the results indicated that lncRNA-UCA1 could promote cell cycle procession in RB cells.

### ***lncRNA-UCA1 activates PI3K/Akt signaling pathway in HXO-RB44 and Y79 cells***

The expression of p-PI3K, p-Akt and S6K in HXO-RB44 cells in si-UCA1 group was dramatically lower than that in BLANK and si-NC group ( $P < 0.01$ ) (**Fig 5**). Meanwhile, the expressions of BLANK and si-NC group in Y79 cells were remarkably increased in pcDNA-UCA1 group compared with BLANK and pcDNA-Mock group ( $P < 0.01$ ) (**Fig 5**), suggesting that lncRNA-UCA1 could activate PI3K/Akt signaling pathway in HXO-RB44 and Y79 cells.

### ***lncRNA-UCA1 promotes cell proliferation and cell cycle procession and inhibits apoptosis via activating PI3K/Akt signaling pathway in RB***

To further confirm whether lncRNA-UCA1 exerted its function on RB through activating PI3K/Akt signaling pathway, we performed a variety of experiments in Y79 cells. The results of CCK-8 showed that Y79 cells proliferation was markedly promoted in pcDNA-UCA1 group compared with BLANK and pcDNA-Mock group at 48 ( $P < 0.05$ ), 72 ( $P < 0.01$ ) and 96 h ( $P < 0.01$ ) (**Fig 6A**). LY294002 markedly reversed the function of lncRNA-UCA1 on cell proliferation ( $P < 0.01$ ) (**Fig 6A**). Colony formation assay results also confirmed that the cell clones number of Y79 cells was dramatically increased in pcDNA-UCA1 group

compared with BLANK and pcDNA-Mock group ( $P < 0.01$ ) (**Fig 6B**). Meanwhile, when compared with pcDNA-UCA1 group, the cell clones number of Y79 cells was notably decreased in pcDNA-UCA1 + LY294002 group ( $P < 0.01$ ) (**Fig 6B**). As shown in **Fig 6C**, Y79 cells apoptosis was dramatically lower in pcDNA-UCA1 group than that in BLANK and pcDNA-Mock group ( $P < 0.01$ ). When compared with pcDNA-UCA1 group, the apoptosis of Y79 cells was remarkably increased in pcDNA-UCA1 + LY294002 group ( $P < 0.01$ ). In addition, we also confirmed that lncRNA-UCA1 overexpression in Y79 cells dramatically decreased the ratio of G0/G1 phase cells ( $P < 0.01$ ), and markedly increased the ratio of S ( $P < 0.01$ ) and G2/M phase cells ( $P < 0.01$ ) (**Fig 6E**). LY294002 significantly reversed the effect of lncRNA-UCA1 on cell cycle ( $P < 0.01$ ) (**Fig 6E**). The results of western blot showed that Caspase-3, p16 and p21 expressions of Y79 cells in pcDNA-UCA1 group were markedly lower than those in BLANK and pcDNA-Mock group ( $P < 0.01$ ) (**Fig 6D and F**), while survivin and CDK2 expressions were significantly higher ( $P < 0.01$ ) (**Fig 6D and F**). When compared with pcDNA-UCA1 group, Caspase-3, p16 and p21 expressions of Y79 cells were remarkably higher in pcDNA-UCA1 + LY294002 group ( $P < 0.01$ ) (**Fig 6D and F**), while survivin and CDK2 expressions were significantly lower ( $P < 0.01$ ) (**Fig 6D and F**). All the results indicated that lncRNA-UCA1 could promote cell proliferation and cell cycle procession, as well as inhibit apoptosis via activating PI3K/Akt pathway in RB.

## Discussion

RB, the most common intraocular malignancy, is diagnosed in approximately 8000 children all over the world each year.<sup>18</sup> In developing countries, the survival rate of RB patients is still below 70%.<sup>19</sup> It is urgent to investigate new and effective molecular targets for the treatment of RB. In this study, we confirmed that lncRNA-UCA1 could facilitate cell proliferation and inhibit apoptosis through activating PI3K/Akt pathway in RB.

More and more evidences have indicated that lncRNAs play a carcinogenic or anticancer role in various malignant tumors, such as RB.<sup>20,21</sup> In addition, lncRNAs are reported to be abnormally expressed in tumors. Previous researches have confirmed that the expression of lncRNA-UCA1 is upregulated in gastric cancer,<sup>16</sup> ovarian cancer<sup>22</sup> and lung cancer.<sup>23</sup> Furthermore, Sun et al.<sup>24</sup> have reported that lncRNA-UCA1 is highly expressed in laryngeal squamous cell carcinoma. In the present study, we assessed the expression of lncRNA-UCA1 in both RB tissues and RB HXO-RB44 and Y79 cells, and found that lncRNA-UCA1 was highly expressed in both RB tissues and RB HXO-RB44 and Y79 cells. Furthermore, we also found lncRNA-UCA1 expression was associated with some clinical parameters of RB patients, such as tumor size, optic nerve invasion and pathologic grade. Accumulating evidences have confirmed that lncRNAs participate in the regulation of cell proliferation, apoptosis, migration and cell cycle in various diseases.<sup>7,25</sup> For example, lncRNA CCAT1 could promote cell proliferation, migration and invasion, as well as suppress cell apoptosis of RB cells by modulating miR-218-5p.<sup>26</sup> Li et al.<sup>27</sup> have indicated that lncRNA 00152 could promote cell proliferation and migration, and suppress apoptosis in RB. lncRNA H19 could suppress RB cell proliferation and promote cell cycle arrest and apoptosis through counteracting miR-17-92 cluster.<sup>28</sup> In this study, lncRNA-UCA1 promoted cell proliferation and cell cycle

procession, and inhibited cell apoptosis in RB, indicating that lncRNA-UCA1 may serve as a potential prognostic biomarker for RB.

More and more researches have suggested that PI3K/Akt signaling pathway affects various biological behaviour in the process of malignancies including cell proliferation, migration, invasion, apoptosis and cell cycle.<sup>29</sup> S6K is a downstream target molecule of PI3K/Akt pathway. Once PI3K/Akt pathway is activated, S6K may be activated and then regulate important process of cell biology.<sup>17</sup> Moreover, previous findings have indicated that lncRNAs could participate in cell biological behaviour by modulating PI3K/AKT pathway in many kinds of cancers. For instance, lncRNA-UCA1 has been reported to regulate cell cycle progression through PI3K signaling pathway in bladder carcinoma cells.<sup>30</sup> A study of Li et al.<sup>17</sup> has indicated that lncRNA-UCA1 significantly promotes cell proliferation and suppresses apoptosis by activating PI3K-Akt-mTOR signaling pathway in gastric cancer. Ma et al.<sup>16</sup> have suggested that lncRNA-UCA1 could promote osteosarcoma metastasis through the activation of PI3K/AKT signaling pathway. In the present study, silencing lncRNA-UCA1 significantly decreased the phosphorylation of PI3K and Akt, as well as the expression of S6k in HXO-RB44 cells, while lncRNA-UCA1 overexpression dramatically promoted the phosphorylation of PI3K and Akt, and S6k expression in Y79 cells, suggesting that lncRNA-UCA1 could activate PI3K/Akt signaling pathway in RB cells. In addition, we also confirmed that LY294002 reversed the function of lncRNA-UCA1 on RB cell proliferation, apoptosis and cell cycle procession. All results indicated that lncRNA-UCA1 could promote cell proliferation and cell cycle procession, as well as inhibit apoptosis via activating PI3K/Akt pathway in RB.

## Conclusions

In conclusion, our work confirmed that lncRNA-UCA1 expression was dramatically upregulated in both RB tissue and RB HXO-RB44 and Y79 cells. In addition, we found that lncRNA-UCA1 could promote cell proliferation and cell cycle procession, as well as inhibit apoptosis via activating PI3K/Akt pathway in RB. Our research provides an innovatively regulatory mechanism about lncRNA-UCA1 in RB and point a new way to RB treatment.

## Abbreviations

retinoblastoma (RB)

Long noncoding RNAs (lncRNAs)

American Type Culture Collection (ATCC)

## Declarations

Ethics approval and consent to participate: This study was conducted after obtaining local ethical committee approval of The Second People's Hospital of Jinan and written informed consent from the

patients.

Consent for publication: Not applicable.

Availability of data and material: All data generated or analyzed during this study are included in this published article [and its supplementary information files].

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

Funding: Not applicable.

Authors' contributions:

ZFY: Substantial contributions to conception and design, data acquisition, drafting the article, final approval of the version to be published;

ZNL: Drafting the article or critically revising it for important intellectual content.

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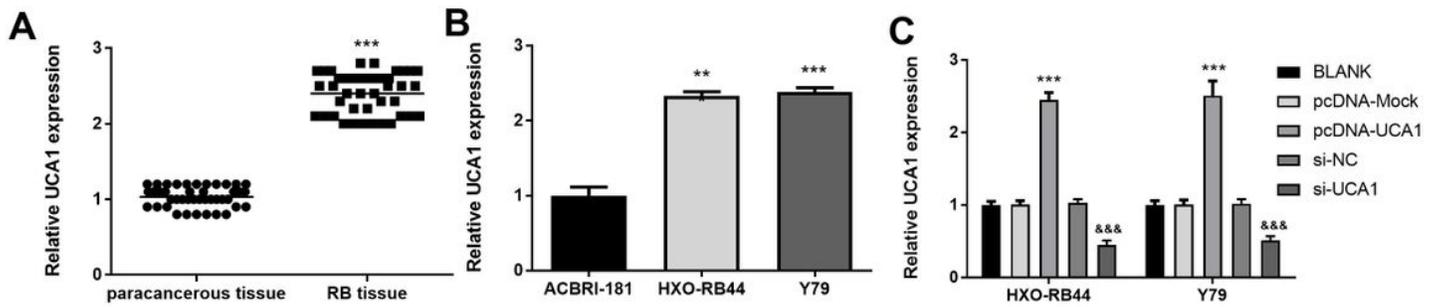
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## Table

Table 1 Association between **lncRNA-UCA1** expression and clinical parameters in RB patients

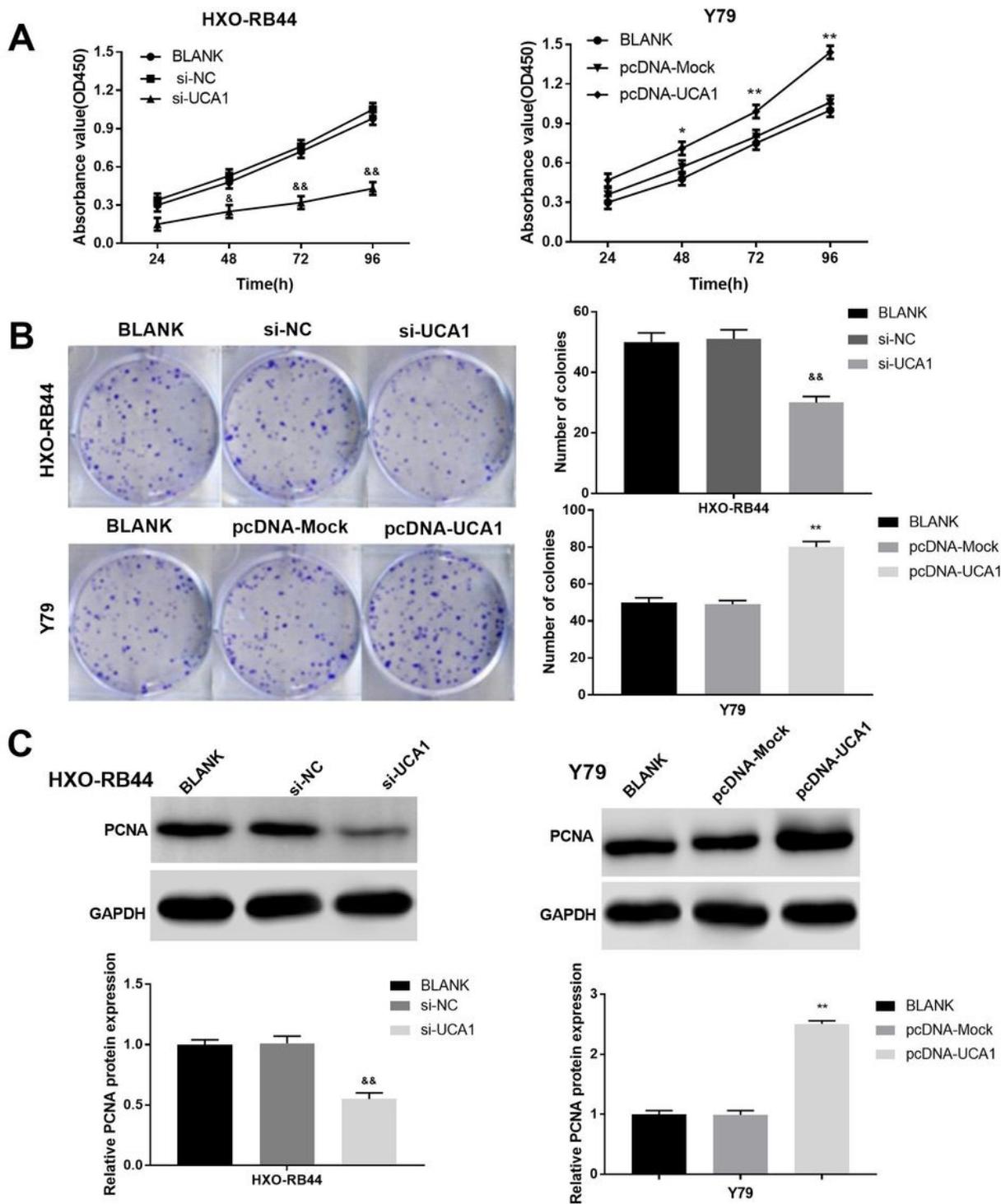
clinical parameters	n	UCA1 expression	P value
Age (years)			
≤ 2	22	2.4 ± 0.268	0.7679
> 2	20	2.4 ± 0.300	
Gender			
Male	20	2.4 ± 0.300	0.8395
Female	22	2.4 ± 0.299	
Tumor size			
≤ 10 mm	22	2.6 ± 0.100	0.0074**
> 10 mm	20	2.2 ± 0.105	
optic nerve invasion			
Yes	25	2.6 ± 0.101	0.0015**
No	17	2.1 ± 0.108	
pathologic grade			
Poorly differentiated	20	2.2 ± 0.101	0.0078**
Well differentiated	22	2.6 ± 0.102	

## Figures



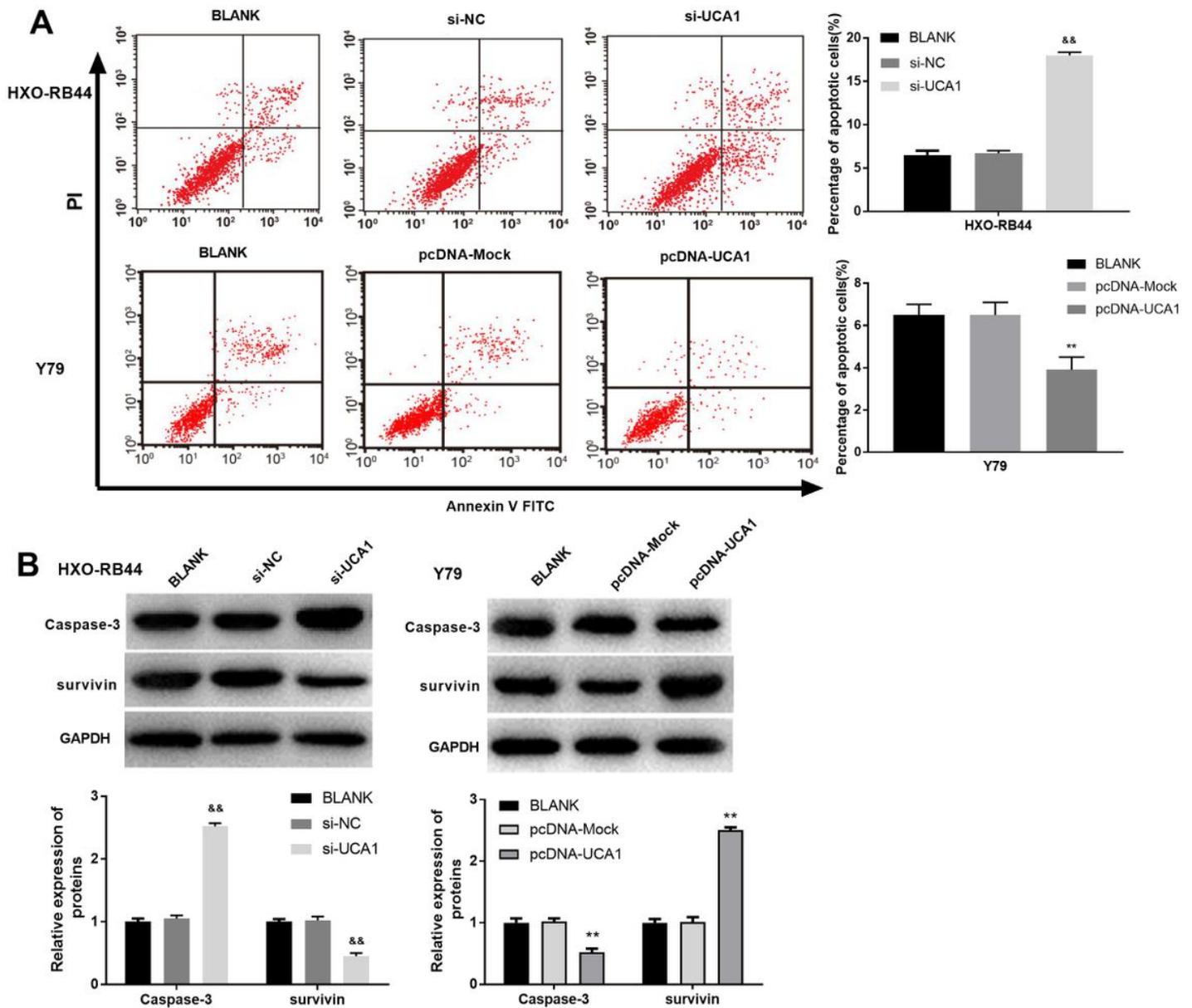
**Figure 1**

The expression of lncRNA-UCA1 in both RB tissues and RB HXO-RB44 and Y79 cells. (A) The expression of lncRNA-UCA1 in RB tissues and adjacent tissue. (B) The expression of lncRNA-UCA1 in ACBRI-181, HXO-RB44 and Y79 cells. (C) The expression of lncRNA-UCA1 in transfected HXO-RB44 and Y79 cells. \*\*P < 0.01, vs. paracancerous tissue group (A); \*\*P < 0.01, vs. ACBRI-181 group (B); \*\*P < 0.01, vs. BLANK and pcDNA-Mock group; &&P < 0.01, vs. BLANK and si-NC group (C).



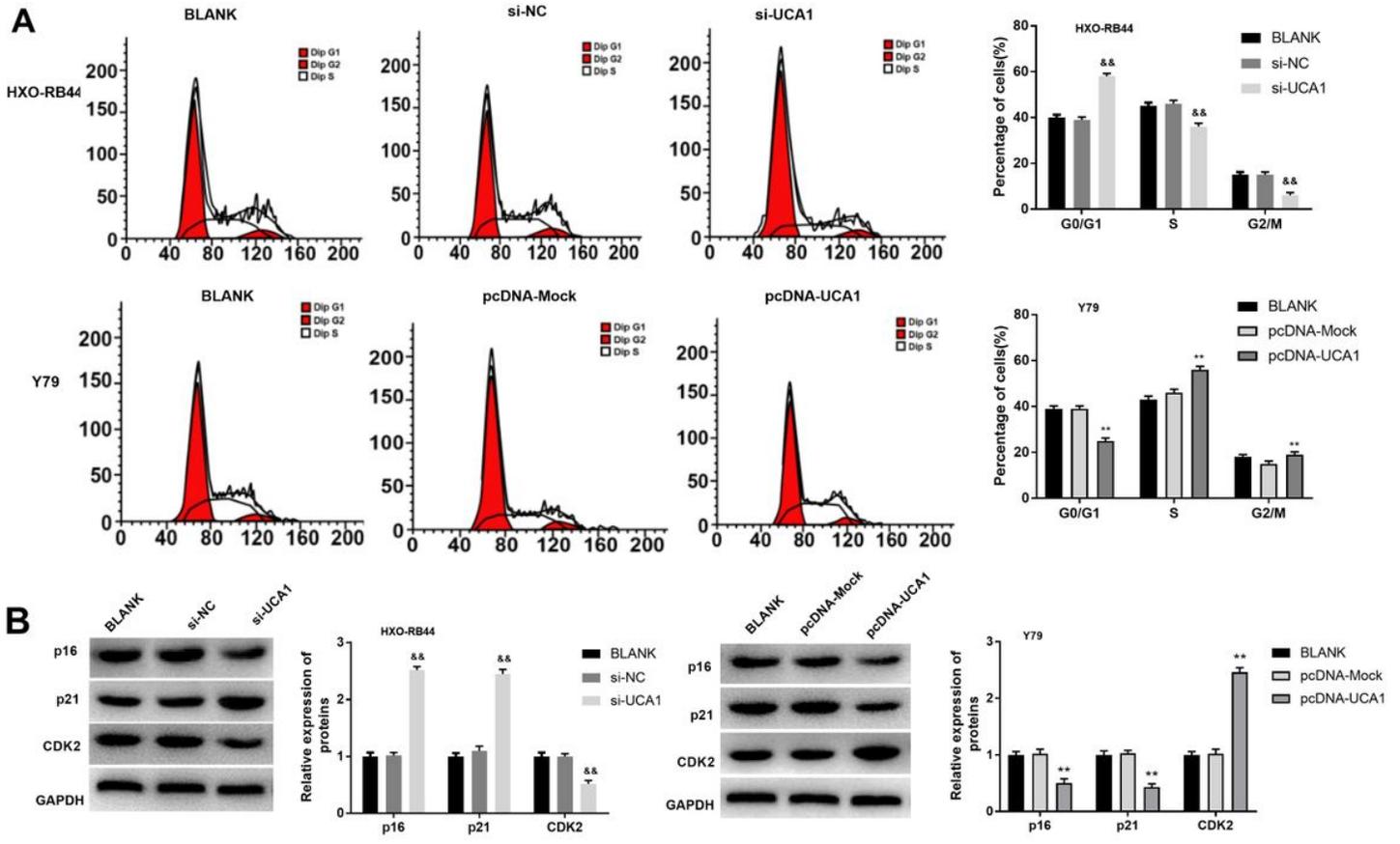
**Figure 2**

lncRNA-UCA1 promoted cell proliferation in HXO-RB44 and Y79 cells. (A) The cell proliferation of transfected HXO-RB44 and Y79 cells. (B) The cell clones number of transfected HXO-RB44 and Y79 cells. (C) The expression of PCNA in transfected HXO-RB44 and Y79 cells. \*\* $P < 0.01$ , vs. BLANK and pcDNA-Mock group; && $P < 0.01$ , vs. BLANK and si-NC group.



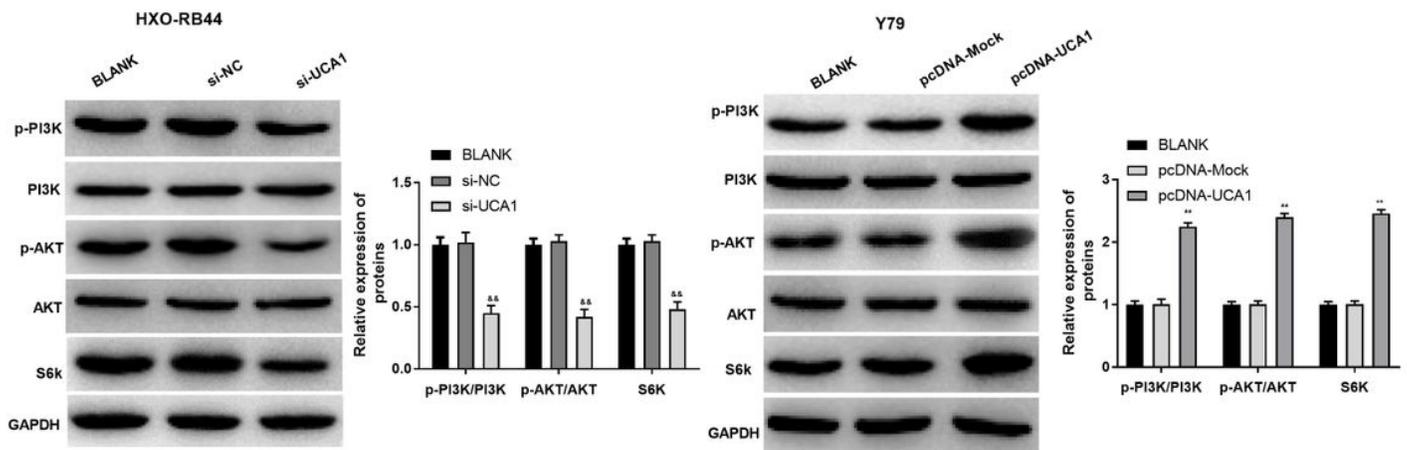
**Figure 3**

lncRNA-UCA1 inhibited cell apoptosis in HXO-RB44 and Y79 cells. (A) The apoptosis of transfected HXO-RB44 and Y79 cells. (B) The expressions of Caspase-3 and survivin in transfected HXO-RB44 and Y79 cells. <sup>\*\*</sup> $P < 0.01$ , vs. BLANK and pcDNA-Mock group; <sup>&&</sup> $P < 0.01$ , vs. BLANK and si-NC group.



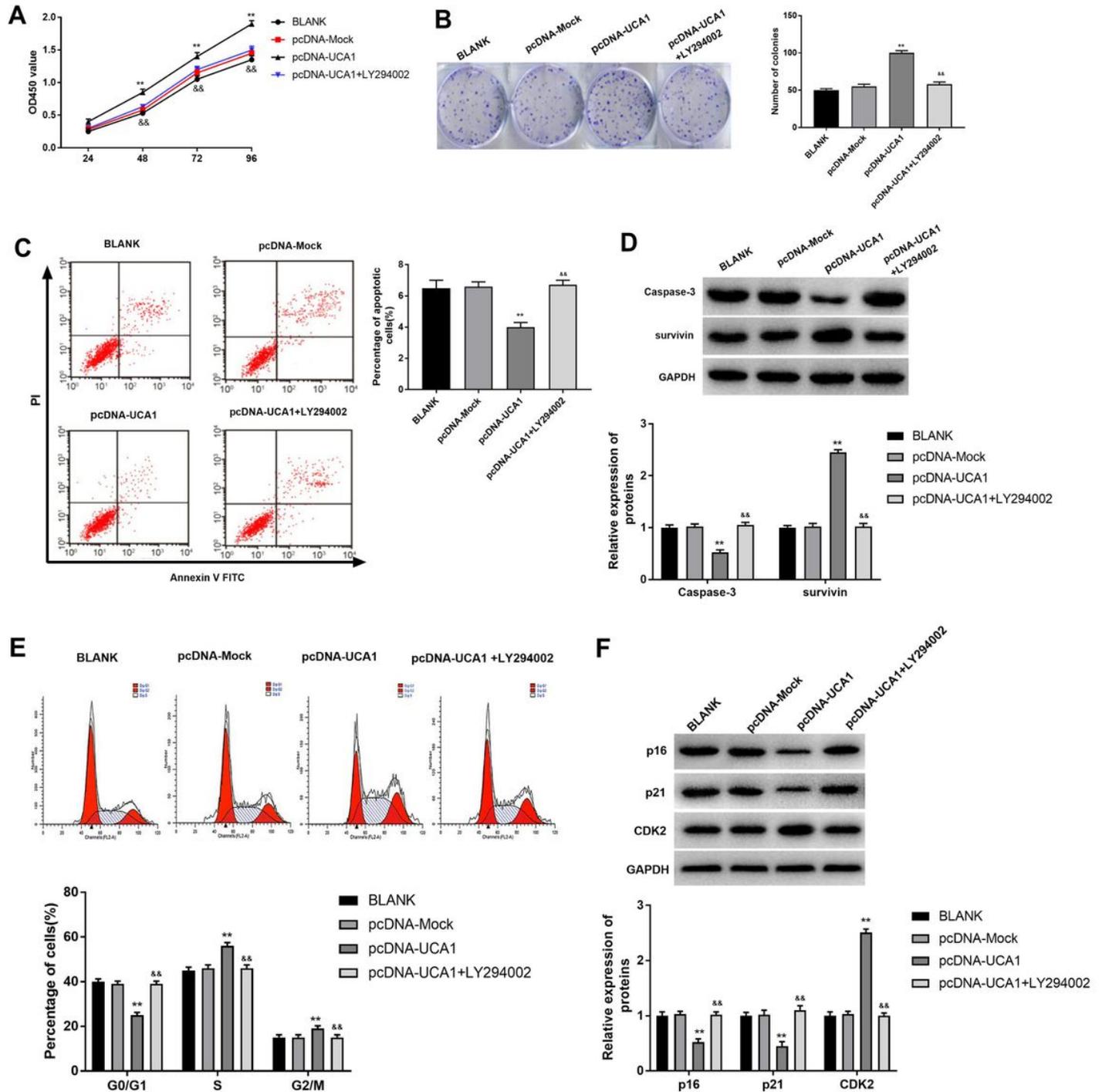
**Figure 4**

lncRNA-UCA1 promoted cell cycle progression in HXO-RB44 and Y79 cells. (A) The cell cycle distribution in transfected HXO-RB44 and Y79 cells. (B) The expressions of p16, p21 and CDK2 in transfected HXO-RB44 and Y79 cells. \*\* $P < 0.01$ , vs. BLANK and pcDNA-Mock group; && $P < 0.01$ , vs. BLANK and si-NC group.



**Figure 5**

lncRNA-UCA1 activated PI3K/Akt signaling pathway in HXO-RB44 and Y79 cells. \*\* $P < 0.01$ , vs. BLANK and pcDNA-Mock group; && $P < 0.01$ , vs. BLANK and si-NC group.



**Figure 6**

lncRNA-UCA1 promoted RB cell proliferation and cell cycle procession and inhibited cell apoptosis via activating PI3K/Akt pathway. (A) The cell proliferation of transfected Y79 cells. (B) The cell clones number of transfected Y79 cells. (C) The cell apoptosis of transfected Y79 cells. (D) The expressions of Caspase-3 and survivin in transfected Y79 cells. (E) The cell cycle distribution in transfected Y79 cells. (F)

The expressions of p16, p21 and CDK2 in transfected Y79 cells. \*\*P < 0.01, vs. BLANK and pcDNA-Mock group; &&P < 0.01, vs. pcDNA-UCA1 group.