Lycorine upregulates the expression of RMB10, promotes apoptosis and inhibits the proliferation and migration of cervical cancer cells

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Abstract. Although there are numerous treatment strategies, including surgery and chemotherapy, the prognosis of cervical cancer remains far from satisfactory. There is an urgent need to develop more effective, more tolerable and safer therapeutics for the treatment of cervical cancer. Lycorine is a natural plantextract that has been previously found to confer anti-tumor activities. Therefore, in the present study, the effects of lycorine and its possible mechanism of action in cervical cancer were investigated. Cell Counting Kit-8, wound healing and Transwell assays were used to verify the proliferation and migration of HeLa cells following lycorine intervention. The results demonstrated that lycorine significantly inhibited the proliferation and migration of HeLa cells. RNA binding motif 10 (RBM10) is a protein associated with apoptosis. It has been suggested that lycorine can affect the expression of RBM10. Flow cytometry demonstrated that lycorine may inhibit the initiation and progression of cervical cancer by promoting apoptosis, which may be mediated through the upregulation of RBM10 expression and increasing TNF-α levels. Xenograft mouse experiments indicated that when lycorine was injected through the tail vein, HeLa tumor growth was inhibited. Mechanistically, western blotting demonstrated that lycorine significantly inhibited the activation of the Akt signaling pathway and potentially reversed epithelial-mesenchymal transition, which was also mediated by RBM10. Furthermore, following RBM10 knockdown with small interfering-RNA, the inhibitory effects of lycorine on cervical cancer was significantly abrogated. Overall, results of the present study suggest that lycorine can upregulate the expression of RBM10 and inhibit the proliferation and migration of cervical cancer cells.

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Introduction

Cervical cancer is the fourth most common cancer in the world among women (1). Although the etiology of cervical cancer has not been fully clarified, human papillomavirus has been identified as the main causative agent of cervical cancer (2). However, smoking can also promote the occurrence and development of cervical cancer because tobacco can promote the epithelial-mesenchymal transition of cervical epithelial cells (3). The cause of the majority of the mortality cases from cervical cancer is cancer metastasis (1-3). At present, surgery, radiotherapy and chemotherapy remain to be the main treatment approaches for cervical cancer and immunotherapy is also used (4,5). However, these existing therapeutic approaches have limitations. Radiotherapy and chemotherapy can cause serious side effects, such as leucopenia and hemolysis (6). Therefore, it is important to develop more effective and safer therapeutics for the treatment of cervical cancer.

Previous studies have demonstrated that traditional Chinese medicine may be a promising approach, since they appear to have fewer side effects compared to chemically synthesized drugs for the treatment of tumors (7-10). Lycorine is an alkaloid that can be found in Lycoris bulbs and is mainly used as an emetic in the clinic (7). Furthermore, lycorine has been reported to inhibit the proliferation and migration of a variety of tumors, such as colorectal cancer, breast cancer, gastric cancer and liver cancer (8-11). It has also been previously demonstrated that lycorine can inhibit the initiation and progression of cervical cancer. However, its underlying mechanism has not been determined (12-14).

In the present study, the effect of lycorine on human cervical cancer cells was investigated and its possible underlying mechanism of action was explored. It is hoped that the present study will provide a novel approach for the treatment

of cervical cancer and reduce the disease burden of patients with cervical cancer.

Materials and methods

Cell culture. HeLa cells were purchased from The Cell Bank of Type Culture Collection of The Chinese Academy of Sciences. The cells were cultured in Dulbecco's modified Eagle's medium (DMEM; Hyclone; Cytiva) containing 10% fetal bovine serum (FBS; Gibco; Thermo Fisher Scientific, Inc.) and 1% penicillin-streptomycin. The cells were cultured in a 1% humidified atmosphere of 5% CO_2 and 95% air at 37˚C.

Bioinformatics analysis. Gene Expression Profiling Interactive Analysis (GEPIA; http://gepia.cancer-pku.cn/) was used to analyze the data from The Cancer Genome Atlas (TCGA; https://www.cancer.gov/about-nci/organization/ccg/research/structural-genomics/tcga) to determine the mRNA expression levels and the effects of RBM10 on the survival rates of patients with cervical cancer. The expression of RBM10 in numerous types of cancer was analyzed using The Human Protein Atlas (https://www.proteinatlas.org/).

Antibodies. Rabbit monoclonal phosphorylated (p)-Akt and rabbit monoclonal Akt antibodies were purchased from Affinity Biosciences (both 1:1,000; cat. no. af0016 and af0836, respectively). Rabbit monoclonal E-cadherin and N-cadherin antibodies were purchased from Boster Biological Technology (both 1:500; cat. no. BA0415 and BA0673, respectively). Rabbit monoclonal cyclin D1 and polyclonal β-catenin antibodies were purchased from Wuhan Servicebio Technology Co., Ltd. (all 1:1,000; cat. no. gb111372 and gb11015, respectively). A goat anti-rabbit IgG HRP-binding secondary antibody was purchased from Wuhan Servicebio Technology Co., Ltd. (1:1,000; cat. no. gb23303). Rabbit anti-GAPDH primary antibody was obtained from Wuhan Servicebio Technology Co., Ltd. (1:500; cat. no. gb11002).

Small interfering (si)RNA transfection. The siRNAs for RBM10 (siRNA-RBM10-1 and siRNA-RBM10-2) or the scrambled siRNA negative control (NC; siRNA-NC) were synthesized via Guangzhou RiboBio Co., Ltd. siRNA (20 nM) transfection into HeLa cells was performed using Lipofectamine® 2000 reagent (Invitrogen; Thermo Fisher Scientific, Inc.) at room temperature for 20 min. Cells were harvested at 48 h after transfection for the subsequent experiments. The siRNA sequences used are presented in Table SI.

Colony formation assay and crystal violet staining. HeLa cells were seeded in 6-well dishes at a density of $5x10^2$ cells/dish and cultured at 37˚C in DMEM with or without 5 μg lycorine (cat. no. HY-N0288; MedChemExpress) for 1 week. Subsequently, the cells were washed with PBS, fixed with 4% paraformaldehyde for 30 min at room temperature and stained with 0.1% crystal violet for 15 min at room temperature. The number of colonies (>50 cells) formed was then quantified manually. For quantitative crystal violet staining, the absorbance of each well was measured at 595 nm using a multifunctional enzyme microplate reader (iMark; Bio-Rad Laboratories, Inc.).

Wound healing assay. Cells from different treatment groups, with or without 10 μM lycorine ($5x10^5$ cells/well) were seeded into a six-well plate and DMEM/F12 (Gibco; Thermo Fisher Scientific, Inc.) supplemented with 10% FBS, respectively, allowed to adhere to the bottom of the well. Cells were cultured to 80% confluency at 37˚C. Subsequently, a scratch wound was made using a 200 μl pipette tip and the wells were washed twice with PBS to remove all floating cells. The cells in each well were then exposed to serum-free DMEM/F12 with 3 μg lycorine for 24 and 48 h at 37˚C. In the NC group, the same amount of PBS was added as the control. Images were captured using an optical light microscope (magnification, x200). The distance of the scratch was measured using ImageJ (version 1.53e, National Institutes of Health) at 0, 24 and 48 h following incubation and relative invasion distance was compared with the NC group (without lycorine group). The invasiveness is calculated by the following formula: Healing area ratio=healing area/original area. Healing area was represented by the difference between the initial scratch area and the area at the indicated time points; whereas the original area was defined by that originally made using the 200-μl pipette tip).

Transwell invasion assay. HeLa cells ($2x10^4$ cells/0.4 ml) were seeded into Transwell inserts (pore size 8 μm) containing Matrigel (Corning, Inc.) and the upper chamber was filled with DMEM (containing 1% FBS) with 10 μM lycorine or with 10 μM PBS. DMEM containing 10% FBS was placed in the lower chamber. The cells were incubated at 37˚C for 48 h. Subsequently, the cells were fixed with 4% formaldehyde for 15 min at 37˚C and stained with 0.1% crystal violet in 0.01 M PBS for 15 min at room temperature. The number of cells penetrating the membrane was quantified according to a previously reported method (15). Images were captured using an optical light microscope (magnification, x200). The images of HeLa cells that invaded through the Transwell membrane following different treatments were analyzed using Image-Pro Plus 6.0 software (Media Cyberkinetics, Inc.).

Cytotoxicity assay. The Cell Counting Kit-8 (CCK-8; Thermo Fisher Scientific, Inc.) assay was used to assess the cytotoxicity of HeLa cells. In brief, cells were seeded into 96-well plates at a density of $3x10^3$ cells/well. In total, 10 μl CCK-8 reagent and lycorine (0, 2 or 4 μg) were then added to each well and the cells were cultured for 12, 24 and 48 h at 37˚C. In the gene knockdown experiment, 5 μg lycorine was added into each well and observed at 0, 24, 48 and 72 h. Cell cytotoxicity was determined by assessing the optical density of each well at a wavelength of 450 nm using a microplate reader (Thermo Fisher Scientific, Inc.).

Flow cytometry analysis. HeLa cells ($1x10^6$ cells/well) were pre-seeded into 10 cm plates. The HeLa cells were treated with 5 μg lycorine for 48 h at 37˚C, according to the manufacturer's instructions. The HeLa cells were then collected and centrifuged at 140 x g for 4 min at room temperature. Annexin V-FITC Apoptosis Detection Kit (MilliporeSigma) was used to determine cell apoptosis at 37˚C, according to the manufacturer's protocols. Cells were fixed with cold 70% ethanol for 1 h before being centrifuged (1,000 x g, 37˚C and 5 min) and washed twice using cold PBS. A total of $1x10^6$ cells were then incubated with

Annexin V (1X) and propidium iodide (1X) for 30 min in the dark at 37˚C. Samples were analyzed using a Beckman MoFlo Astrios EQs Flow Cytometer (Beckman Coulter, Inc.). Data were analyzed using FlowJo V10 software (FlowJo LLC).

ELISA. ELISA was performed using a Human TNF-α Single Step ELISA Kit (cat. no. ab181421; Abcam) according to the manufacturer's instructions. The cell culture supernatant was extracted and passed through a 0.45 μM filtering device (MilliporeSigma). The 450 nm absorbance of the sample was assessed using an ELISA reader and the standard curve.

RNA extraction and reverse transcription-quantitative (RT-q)PCR. Total RNA was extracted using the EZ-press RNA Purification kit (cat. no. B0004DP; EZBioscience). A total of 1 μg RNA was used for complementary (c)DNA synthesis using the First Strand cDNA Synthesis kit ReverTra Ace™ qPCR RT Master Mix (cat. no. FSQ-201; Toyobo Life Science) at 37˚C for 10 min and 50˚C for 5 min. The reaction was terminated by incubating the samples at 95˚C for 3 min. qPCR was performed using the MonAmp SYBR Green qPCR Mix (cat. no. RN04006M; Monad Biotech Co., Ltd.) with a RT fluorescence quantitative PCR system (Light Cycler 96 SW 1.1; Roche Diagnostics) according to the manufacturer's protocols. β-actin was used as the internal reference gene. The PCR system included, 5 μl PCR mix, 0.2 μl upstream primer, 0.2 μl downstream primer, 2.6 μl RNase-free double-distilled H_2O and 2 μl cDNA template. qPCR was performed for the initial activation at 95˚C for 20 sec, followed by 40 cycles at 95˚C for 10 sec, 63˚C for 30 sec, and 70˚C for 30 sec. mRNA expression levels were analyzed using the $2^{-\Delta\Delta Cq}$ method and were normalized using the *ACTB* gene (16). The primer sequences used are presented in Table SII.

Xenograft mouse model of cervical cancer. All animal experimental procedures were performed in accordance with the guidelines provided by the National Institutes of Health Guide for the Care and Use of Laboratory Animals (17). The present study was approved by the Ethics Committee of The School of Stomatology of Shandong University. BALB/C nude mice (age, 4 weeks; weight, 30 g) were used in the present study and were purchased from Beijing Huafukang Biotechnology Co., Ltd. The mice were housed in accordance with animal welfare regulations (17), under specific-pathogen-free conditions at 25˚C, 50% humidity and a 12-h light/dark cycle. The animals also had free access to food and water. In total, 20 female nude mice were randomly divided into the experimental group and control group. HeLa cells ($1x10^6$ cells/100 μl PBS) were injected into the groin of nude mice. At 1, 2 and 3 weeks, 100 μl lycorine or PBS was injected into the tail vein of the nude mice once a week. The health and behavior of the animals were monitored every 2 days. Tumor mass and volume (V) were checked. The tumor was almost spherical with a radius of L and the following formula was used: $V=4/3\pi L^3$. The tumor diameter was assessed every 5 days (on day 1, 6, 11, 16 and 21) and the tumor volume was determined according to the formula. Subsequently, 21 days following cell injection, the mice were sacrificed via cervical dislocation. The humane endpoints were as follows: A marked reduction in food or water intake, labored breathing, the inability to stand and no response to external stimuli. No abnormal signs that signified the humane endpoints of the experiment were observed in any of the mice during the experiment. When it was confirmed that the experimental animals had no heartbeat or breathing, the tumors were isolated and weighed (18).

Western blotting. Cells were collected and total protein was extracted using RIPA lysis buffer (Beyotime Institute of Biotechnology). Protein concentration was determined using the BCA method. Total protein (10 μg/lane) was separated on a 10% gel using SDS-PAGE and the separated proteins were transferred to a PVDF membrane (MilliporeSigma). After blocking with 5% fat-free milk powder in Tris-buffered saline with 0.1% Tween-20 (TBST) for 1 h at room temperature, the membrane was incubated with primary antibody at 37˚C for 45 min. After washing with TBST, the membrane was sealed with 5% fat-free milk powder overnight at 4˚C and incubated with HRP-bound secondary antibody (1:1,000) in the dark for 1 h at 25˚C. Finally, the protein bands were visualized using the Common ECL chemiluminescence detection kit (cat. no. PK10001; ProteinTech Group, Inc.) and were analyzed using Gel Pro Analyzer 4.0 software (Media Cybernetics, Inc.). The Gel Pro Analyzer 4.0 software was used to quantify the western blotting bands, assess the gray values of the different bands, list the gray values obtained and determine the protein expression levels using histograms (19).

Statistical analysis. All statistical analysis was performed using GraphPad Prism 8.0 software (GraphPad Software, Inc.). Two-tailed unpaired Student's t-tests were used to analyze two groups. One-way ANOVA was used to analyze multiple groups. The Tukey's post-hoc test was used following ANOVA. All experiments are repeated at least three times unless otherwise stated. All data are presented as the mean ± SEM. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Lycorine inhibits the proliferation and migration of cervical cancer cells. To assess the effect of lycorine on the initiation and progression of cervical cancer, the subsequent experiments were performed. The results of the CCK-8 assay demonstrated that compared with NC group, lycorine (Fig. 1A) significantly inhibited the proliferation of cervical cancer cells and this inhibition significantly intensified in a dose- and time-dependent manner (Fig. 1B). The subsequent plate cloning experiments confirmed these results (Fig. 1C). Furthermore, to verify the effect of lycorine on the migration of cervical cancer cells, the wound healing assay was performed. The results demonstrated that at 24 and 48 h, the lycorine treatment significantly inhibited cell migration compared with the NC. These results therefore indicated that lycorine can potentially inhibit the migration of cervical cancer cells (Fig. 1C). Moreover, in the Transwell experiment, lycorine treatment significantly reduced the number of cells migrating to the bottom of the insert after 48 h compared with the NC, which suggested that lycorine can potentially inhibit the invasion ability of cervical cancer cells (Fig. 1D). These data therefore indicated that lycorine can potentially inhibit the proliferation and migration of cervical cancer cells.

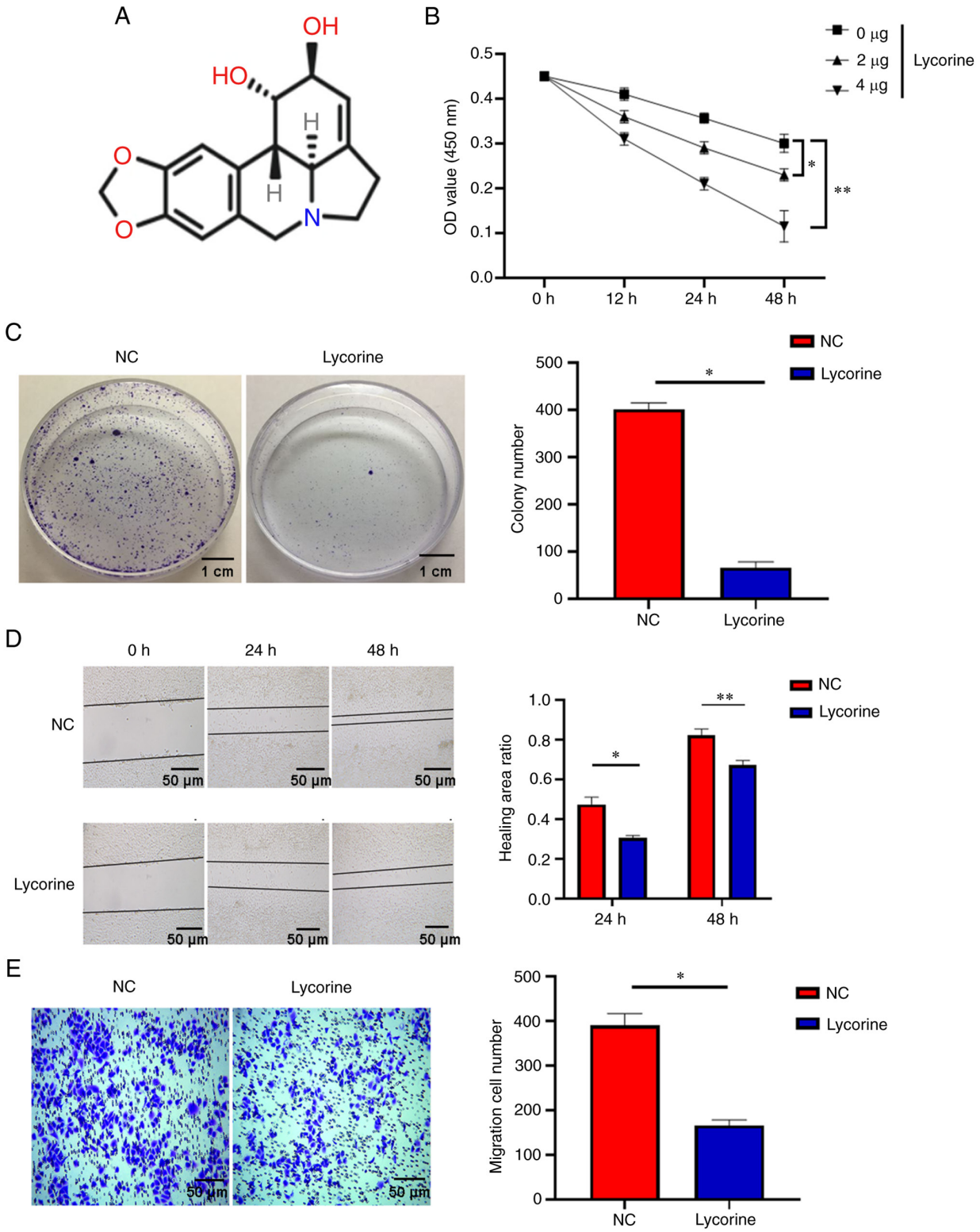

Figure 1. Lycorine inhibits the proliferation and migration of cervical cancer. (A) Chemical structure of lycorine. (B) Inhibitory effect of lycorine on the cytotoxicity of cervical cancer was time- and dose-dependent. (C) Lycorine inhibited the production of cervical cancer colonies (scale bar, 1 cm). (D) Lycorine delayed the healing rate of scratches (scale bar, 50 μm). (E) Lycorine significantly inhibited the migration of cervical cancer cells (scale bar, 50 μm). *P<0.05 and **P<0.01. NC, negative control; OD, optical density.

Lycorine upregulates the expression of RBM10 and promotes apoptosis. RBM10 is involved in a variety of inflammatory processes and is closely associated with apoptosis (20-22). An imbalance of RBM10 expression is linked to the initiation and progression of a variety of tumors (23,24). Therefore, in the present study the expression of RBM10 in tumor tissues was examined using the TCGA database. The results demonstrated that the mRNA expression levels of RBM10 in tumor tissues were significantly increased compared with adjacent tissues (Fig. 2A). Subsequently, it was demonstrated that patients with

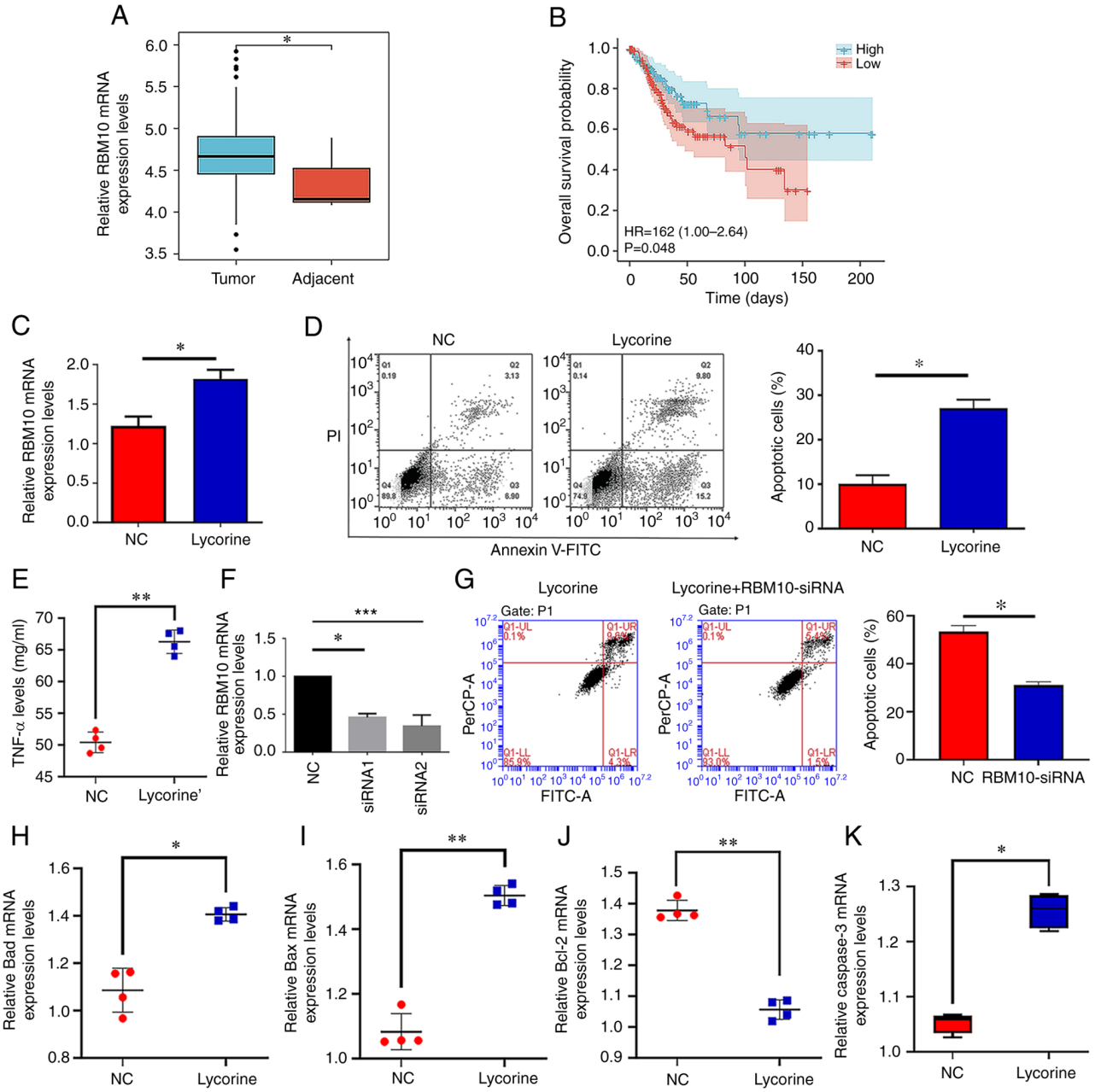

Figure 2. RBM10 mediates the antitumor effect of lycorine. (A) Compared with the adjacent tissues, the mRNA expression levels of RBM10 in cervical cancer were significantly decreased. (B) Low mRNA expression levels of RBM10 are associated with a poor prognosis in patients with cervical cancer. (C) Lycorine induced a significant increase in RBM10 mRNA expression levels in cervical carcinoma. (D) Lycorine significantly promoted the apoptosis of cervical cancer cells. (E) Lycorine significantly increased TNF-α levels. (F) siRNA knockdown of RBM10 expression. (G) RBM10 knockdown significantly reduced the promoting effect of lycorine on apoptosis. (H-K) Changes in the expression levels of apoptosis markers in cervical cancer after lycorine treatment. *P<0.05, **P<0.01 and ***P<0.001. RBM10, RNA binding motif 10; NC, negative control; HR, hazard ratio; PerCP-A, peridinin-chlorophyll-A; siRNA, small interfering RNA.

high RBM10 mRNA expression levels had significantly longer survival rates compared with patients with low RBM10 expression (Fig. 2B). Therefore, low RBM10 expression levels may be associated with a poor prognosis in patients with cervical cancer. Furthermore, the mRNA expression levels of RBM10 significantly increased after the addition of lycorine compared with those in the NC group (Fig. 2C). In the subsequent flow cytometry results, it was demonstrated that the percentage of apoptotic cells significantly increased following lycorine treatment compared with the NC (Fig. 2D). As TNF-α is closely associated with apoptosis (25), the level of TNF-α in the two groups was investigated. The results demonstrated that the expression level of TNF-α significantly increased following lycorine treatment compared with the NC (Fig. 2E). Moreover, when the expression of RBM10 was knocked-down using siRNA, the results demonstrated that siRNA significantly reduced the mRNA expression levels of RBM10. (Fig. 2F), the level of apoptosis was also significantly decreased compared with the NC (Fig. 2G). Consistent with the aforementioned results, the results demonstrated that the mRNA expression levels of the proapoptotic factors Bax and Bad were significantly increased (Fig. 2H and I) and the Mrna expression levels of the antiapoptotic factor Bcl-2 were significantly decreased (Fig. 2J) when cells were treated with lycorine, compared

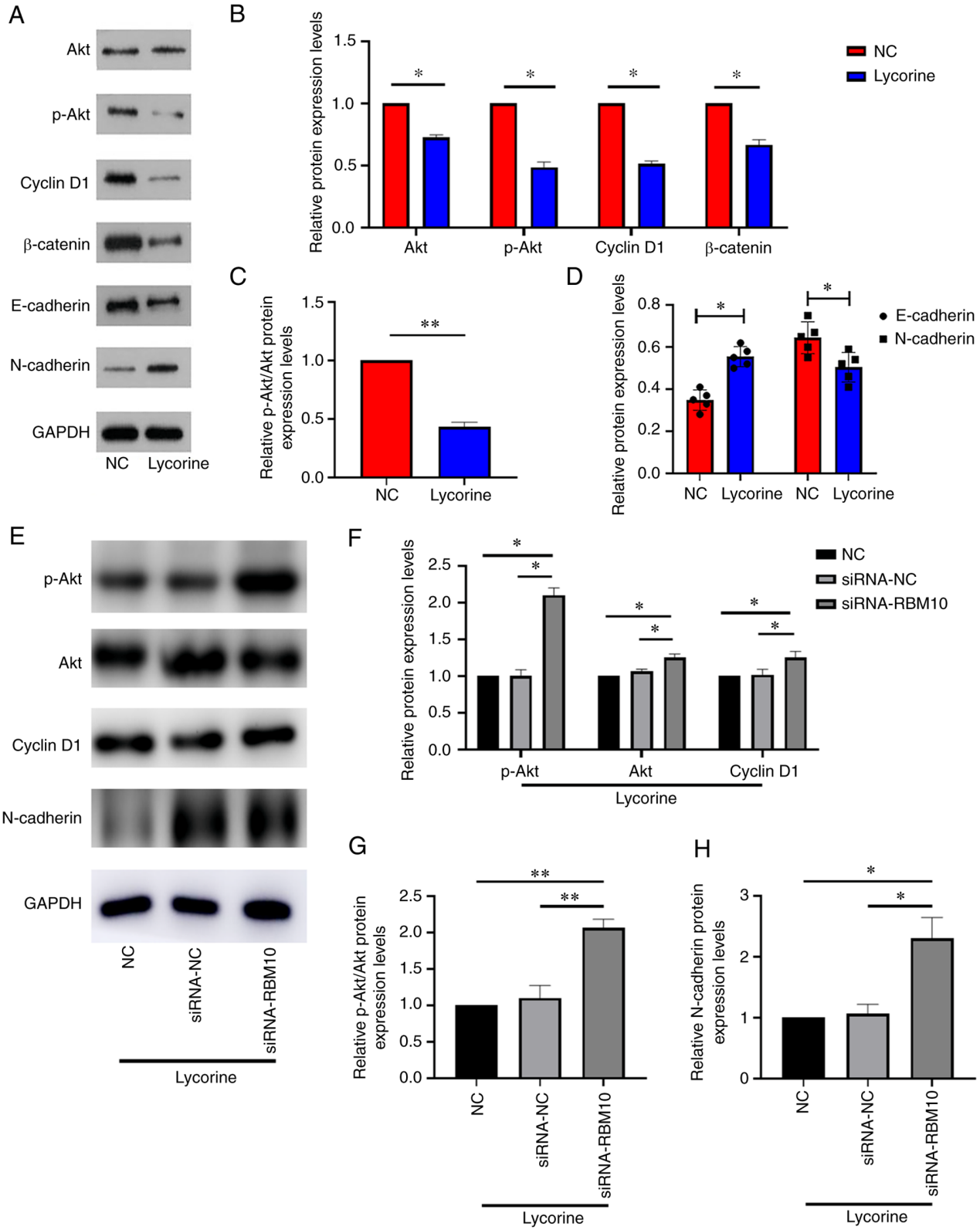

Figure 3. Lycorine changes the activation level of the Akt signaling pathway via RBM10. (A-C) Lycorine significantly decreased the activation level of the Akt signaling pathway. (D) Lycorine significantly increased the protein expression levels of E-cadherin and significantly reduced the protein expression levels of E-cadherin. (E-G) Despite the presence of lycorine, the inhibition of RBM10 significantly increased the activation level of the Akt signaling pathway. (H) Decreasing RBM10 expression induced a significant increase in N-cadherin protein expression. *P<0.05 and **P<0.01. RBM10, RNA binding motif 10; NC, negative control; p, phosphorylated; siRNA, small interfering RNA.

with the NC. Furthermore, the mRNA expression level of the apoptotic protein caspase-3 significantly increased compared with the NC (Fig. 2K). These data indicated that lycorine may potentially promote the apoptosis of cervical cancer cells and that this mechanism may be mediated by the upregulation of RBM10 expression.

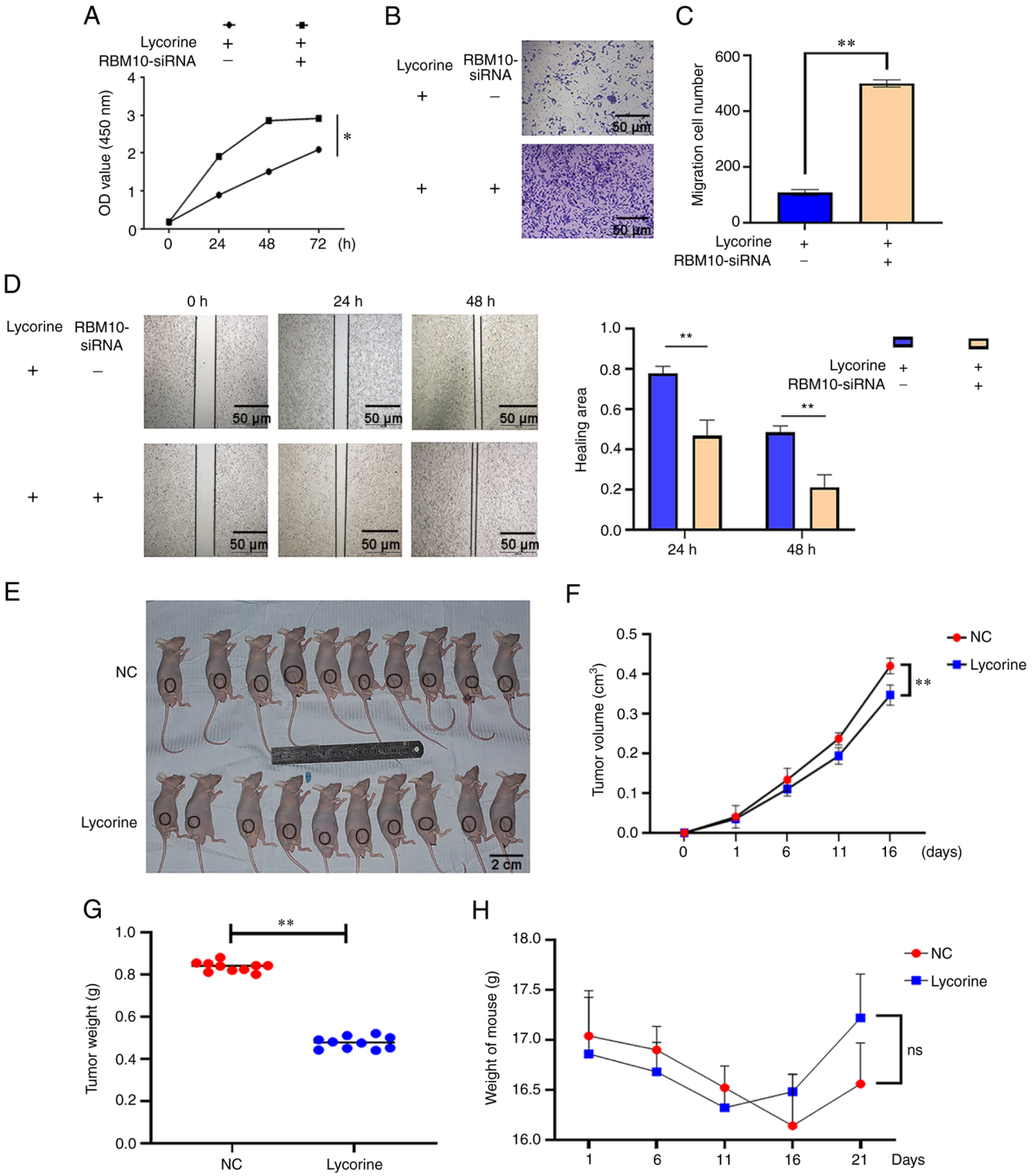

Figure 4. RBM10 knockdown reverses the inhibitory effect of lycorine on cervical cancer and lycorine inhibits the development of cervical cancer *in vivo*. (A-D) Despite the presence of lycorine, the proliferation and migration of cervical cancer was significantly enhanced when the expression of RBM10 was reduced (scale bar, 50 μm). (E) Animal model of cervical cancer. The tumor is indicated by the black circle (scale bar, 2 cm). (F) Lycorine significantly reduced the tumor volume in mice. (G) Lycorine significantly reduced tumor weight in mice. (H) From the weight of the experimental animals it was indicated that lycorine had no toxic effect on the mice. *P<0.05 and **P<0.01. RBM10, RNA binding motif 10; siRNA, small interfering RNA; NC, negative control; OD, optical density; ns, not significant.

Elevated expression of RBM10 reduces the activation level of the AKT signaling pathway. RBM10 interacts with key proteins in a variety of Akt signaling pathways (26), which are involved in the initiation and progression of a variety of malignancies (27,28). Moreover, the Akt signaling pathway serves a key role in numerous types of tumor (29). As shown in Fig. 2C, lycorine treatment was able to increase the expression of RBM10. To explore the relationship between RBM10 and the Akt signaling pathway, western blotting was performed to assess the activation level of the Akt signaling pathway when RBM10 expression was increased via lycorine treatment. The results demonstrated that the increased

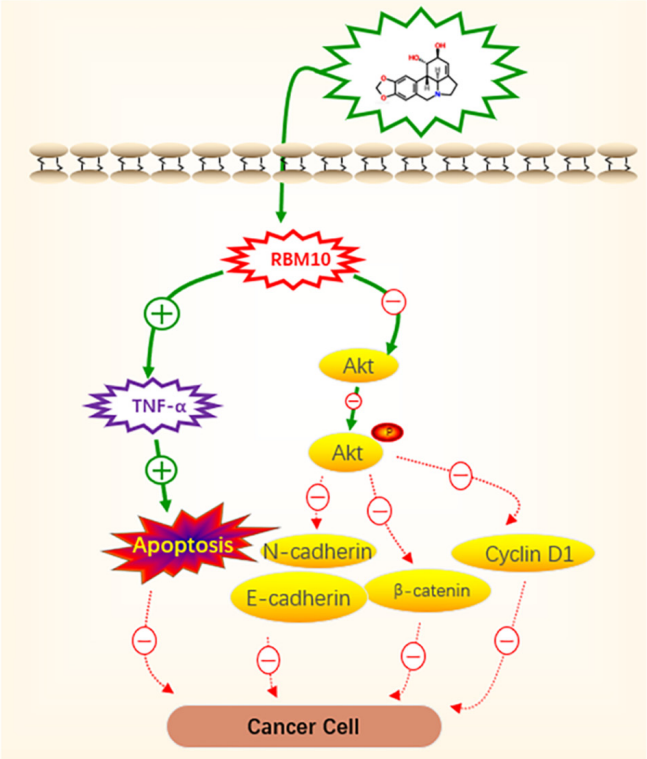

Figure 5. Summary schematic of the present study. Lycorine increases the expression of RBM10. Moreover, RBM10 may increase the expression level of TNF-α, which thereby promotes cell apoptosis, and reduce the activation level of Akt signaling pathway, which thereby inhibits the occurrence and development of cervical cancer. +, promoting expression; -, inhibiting expression. RBM10, RNA binding motif 10; p, phosphate.

expression levels of RBM10 resulted in changes in the protein expression levels of Akt and p-Akt (Fig. 3A and B). The ratio of p-Akt/Akt significantly decreased (Fig. 3C) following lycorine treatment compared with the NC, which indicated that the phosphorylation of Akt was potentially inhibited. To further test the hypothesis that lycorine will reduce the activation level of the Akt signaling pathway, other proteins of the Akt signaling pathway were investigated, including cyclin D1 and β-catenin. The protein expression levels of both proteins were significantly downregulated via lycorine treatment compared with the NC. However, following lycorine treatment, the expression of E-cadherin, a well-known epithelial marker, was significantly increased and the expression of N-cadherin was significantly decreased compared with the NC (Fig. 3D). These results therefore suggested that lycorine may potentially reverse the EMT process via RBM10. To further support this conclusion, siRNA was used to knockdown the expression of RBM10 and the activation level of the Akt signaling pathway was assessed (Fig. 3E). It was demonstrated that the decreased protein expression levels of RBM10 significantly improved the decreased protein expression levels of Akt, p-Akt, cyclin D1 and β-catenin caused by lycorine compared with the NC and siRNA-NC (Fig. 3F and G). Similarly, RBM10-siRNA potentially improved the decreased expression of E-cadherin (Fig. 3H), which was opposite to the effect of lycorine. These results indicated that lycorine may reduce the activation level of the Akt signaling pathway, which is related to RBM10.

RBM10 knockdown can reduce the therapeutic effect of lycorine. It was previous demonstrated in the present study that lycorine inhibited the proliferation and migration of cervical cancer cells and that RBM10 may be mediating this process. To verify this hypothesis, RBM10 mRNA expression levels were knocked-down using siRNA targeting RBM10. In the CCK-8, wound healing and Transwell assays, the results demonstrated that a reduction in RBM10 mRNA expression levels significantly increased the proliferation and migration of cervical cancer cells compared with the lycorine only group (Fig. 4A-D). Combined with the aforementioned experimental results, these data indicated that lycorine may potentially inhibit the proliferation and migration of cervical cancer cells, and that this effect is mediated via apoptosis caused by the increased expression of RBM10.

Lycorine can inhibit cervical cancer in vivo. Subsequently, animal experiments were performed (Fig. 4E). The results demonstrated that the tumor volume and mass of the lycorine treatment group were significantly smaller compared with the NC group (Fig. 4F and G). The largest tumor diameter observed was 5.31 mm and the largest tumor weight observed was 0.87 g. To assess the safety of lycorine, the weight changes of nude mice in the experimental group and the control group were also assessed at various time points. The results demonstrated that the weight of mice did not significantly change regardless of treatment (Fig. 4H), which indicated that lycorine potentially had no obvious toxic effect on nude mice. These results indicated that lycorine may potentially inhibit cervical cancer *in vivo* and this inhibition will not cause obvious damage to the health of experimental animals.

Discussion

Cervical cancer is one of the most common malignant tumors of the female reproductive system, as it is prone to lymph node and blood-borne metastases (30). Therefore, even with systematic treatment, the prognosis of patients is still poor (30). Developing more effective treatment methods is an important issue in the field of cervical cancer research. In the present study, the results demonstrated that lycorine, a commonly used emetic, had an inhibitory effect on cervical cancer cells. The present study reported that lycorine significantly inhibited the proliferation and migration of human cervical cancer cells in a time- and dose-dependent manner. The therapeutic effect of lycorine was verified using *in vivo* experiments. Lycorine was demonstrated to significantly reduce the volume of the tumor in the experimental animals. Therefore, it can be hypothesized that lycorine may act as a potential therapeutic for the treatment of cervical cancer. Furthermore, the results demonstrated that the therapeutic effect of lycorine was potentially mediated via RBM10. When RBM10 expression was knocked-down using siRNA, cervical cancer initiation and progression were restored.

A loss of cell proliferation regulation is considered to be an important mechanism for the initiation and progression of malignant tumors, and the induction of apoptosis is considered to be an effective factor to inhibit the growth of malignant tumors (31,32). RBM10 is closely related to the level of apoptosis (33). RBM10 is considered to promote apoptosis via the

high expression of TNF-α (33). In the present study, the results demonstrated that the expression of RBM10 was significantly decreased in tumor tissues, which may be related to the poor prognosis of patients. It was further demonstrated that lycorine can significantly induce high mRNA expression levels of RBM10 and that the high expression of RBM10 can potentially significantly elevate TNF-α levels and promote apoptosis. Bcl-2 family proteins are key regulators of apoptosis. In the Bcl-2 family, Bcl-2 is an antiapoptotic factor, whereas Bad and Bax are proapoptotic factors (34). Moreover, it is well established that caspase-3 serves a crucial role in apoptosis and is considered to act as a marker that can indicate the level of apoptosis (30). In the present study, the flow cytometry results demonstrated that lycorine significantly increased the level of apoptosis. To determine the specific underlying mechanism, the mRNA expression levels of Bcl-2, Bax, Bad and caspase-3 were assessed. It was demonstrated that after lycorine treatment, the mRNA expression levels of the antiapoptotic factor Bcl-2 significantly decreased, whereas the mRNA expression levels of the proapoptotic factors Bax and Bad significantly increased. These data indicated that lycorine potentially promoted the apoptosis of human cervical cancer cells, which was supported by the subsequent increase in caspase-3 mRNA expression levels.

The regulation of intracellular signaling is closely associated with the initiation and progression of malignant tumors (35). The Akt signaling pathway is considered to be closely linked to the initiation and progression of cervical cancer (36). Akt signaling pathway upregulation promotes the proliferation and migration of cervical cancer cells (35,36). Furthermore, the loss of epithelial characteristics is an important feature of tumor progression (33). In the present study, lycorine significantly downregulated the Akt signaling pathway. Moreover, after knocking down RBM10 expression it was demonstrated that the activation level of the Akt signaling pathway significantly increased. It can therefore be hypothesized that the lycorine-induced downregulation of the Akt signaling pathway is potentially dependent on RBM10. Furthermore, RBM10 potentially downregulated the Akt signaling pathway and reversed the EMT of cervical cancer cells, which was demonstrated by the significantly increased expression of the epithelial marker E-cadherin and the significantly reduced expression of the mesenchymal marker N-cadherin.

The present study also had certain limitations. For example, the experiments were not repeated with other cell lines to verify the conclusion. In the future, close attention will be given to the research progress of treatment strategies for cervical cancer and future research will continue to explore other effective therapeutic approaches for the treatment of cervical cancer. Moreover, MMPs are thought to be related to cancer migration (28) and therefore MMPs should be investigated in future studies.

In conclusion, lycorine was demonstrated to potentially inhibit the proliferation and migration of cervical cancer cells. The results indicated that this inhibition was potentially achieved via the induction of high RBM10 mRNA expression levels and the promotion of apoptosis. Furthermore, the data indicated that RBM10 potentially induces TNF-α, which is the cause for the observed increase in apoptosis. Mechanistically, the results indicated that the promotion of apoptosis and the inhibition of the initiation and progression of cervical cancer by RBM10 may potentially be accompanied by the downregulation of the AKT signaling pathway (Fig. 5). However, the mechanism of lycorine activity in cervical cancer still remains to be elucidated. The study of the cell-cell interaction between lycorine and cervical cancer cells may be of great significance for the development of novel therapeutic strategies for cervical cancer, which should be the focus of future research.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

ZL, QZ and GL guided the project, analyzed the data and wrote the manuscript. YL and XL conceived the technical details and designed the experiments. ZL, QZ and XF performed the experiments. QZ, GL and ZL confirm the authenticity of all the raw data. All authors read and approved the final version of the manuscript.

Ethics approval and consent to participate

Experiments were performed under a project license (approval no. 20201002) granted by the Institutional Ethics Board of the Stomatological Hospital of Shandong University (Jinan, China), in compliance with Chinese national or institutional guidelines for the care and use of animals.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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