Role and mechanism of PTEN in Burkitt's lymphoma

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Abstract. The aim of the present study was to explore the possible mechanisms of phosphatase and tensin homolog (PTEN) in the pathogenesis of Burkitt's lymphoma, and provide novel information that can be used in the targeted treatment of this disease. PTEN lentiviral overexpression vector and short-hairpin PTEN silencing vectors were constructed. The effect of PTEN on the growth and proliferation of CA46 and RAJI cells was analyzed using a Cell Counting Kit-8 assay. Apoptosis was detected by Hoechst 33342 and propidium iodide double staining. Flow cytometry was used to analyze the cell cycle. A Transwell chamber was used to detect cell migration and invasion abilities. Western blot analysis was used to detect related protein changes. The mechanism of the effect of PTEN on the biological characteristics of Burkitt's lymphoma cells was subsequently analyzed. The results revealed that PTEN inhibited the proliferation of CA46 and RAJI cells by downregulating the expression of p-AKT, It was indicated that the upregulation of proapoptotic proteins (including Bad and Bax) induced apoptosis, regulated cyclin (including P53, P21, CDK4, CDK6, cyclin D3 and cyclin H) to inhibit cell cycle progression, and mediated epithelial-mesenchymal transition-like cell markers (including E-cadherin, N-cadherin, β-catenin, TCF-8, vimentin, Slug and Snail) to inhibit cell migration and invasion. In conclusion, the tumor-suppressor gene PTEN inhibited the phosphoinositide 3-kinase/protein kinase B (PI3K/AKT) signaling pathway and inhibited the proliferation and migration of Burkitt's lymphoma cells, induced apoptosis and cell cycle arrest, thus playing a crucial role in the pathogenesis of Burkitt's lymphoma.

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Abbreviations: PTEN, phosphatase and tensin homolog; PI3K, phosphoinositide 3-kinase; AKT/PKB, protein kinase B; EBV, Epstein-Barr virus; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; PBS, phosphate-buffered saline; PI, propidium iodide; OD, optical density; CCK-8, Cell Counting Kit-8; DAPI, 4',6-diamidino-2-phenylindole; CDK, cyclin-dependent kinase; Bad, Bcl-2/Bcl-XL-associated death promoter; Bax, Bcl-2 associated X protein; TCF8/ZEB1, zinc finger E-box-binding protein 1; EMT, epithelial-mesenchymal transition

Key words: PTEN, Burkitt's lymphoma, cell proliferation, cell cycle, invasion

Introduction

Burkitt's lymphoma is a highly invasive cancer of the lymphatic system, particularly B lymphocytes found in the germinal center (1). In recent years, with the improvement of the chemotherapy regimen and the application of rituximab, the prognosis of children with Burkitt's lymphoma has significantly improved. The chance of successful treatment has been significantly increased by a short-course and high-dose combination chemotherapy (2,3). However, in adults (especially in patients aged 40 years) and patients with advanced disease, invasion to the central nervous system is often present. Chemotherapy presents with a number of side effects, and the prognosis for patients undergoing this treatment is poor. Therefore, the identification of a low-toxic and effective treatment method for Burkitt's lymphoma is urgently required (3,4). Targeted therapy is currently considered to be the best available treatment option. A previous study observed the abnormal activation of the phosphoinositide 3-kinase/protein kinase B (PI3K/AKT) pathway in Burkitt's lymphoma cells, and demonstrated that inhibition of PI3K/AKT pathway activation can inhibit the growth of Burkitt's lymphoma cells (5). Additionally, PTEN has been indicated to negatively regulate the PI3K/AKT signaling pathway. As a negative regulatory factor, PTEN exhibits mutations, deletions, methylation and abnormal activity in solid tumors (6-8). PTEN is also abnormally expressed in B-cell lymphomas, including diffuse large B-cell lymphoma (9), resulting in the aberrant activation of the PI3K/AKT pathway. In NK/T lymphoma cells, the expression level of PTEN is also decreased to activate the PI3K/AKT signaling pathway (10). Therefore, it is hypothesized that abnormal expression of PTEN may serve an essential role in the pathogenesis of Burkitt's lymphoma. In order to rule out the effect of lymphoma infection of Epstein-Barr virus (EBV) on the experimental results, EBV-positive and EBV-negative cells were used. In the present study, the effect of PTEN on the proliferation, apoptosis, cell cycle distribution and migration ability of Burkitt lymphoma cells (including CA46 and RAJI; CA46 is EBV negative; RAJI is EBV positive) was assessed. The mechanism of PTEN in Burkitt's lymphoma

was investigated by determining PTEN downstream-related proteins.

Materials and methods

Cell lines and plasmids. RAJI cells were purchased from Jiangsu Kaiji Bio-Technology Co., Ltd. and the CA46 cell strain was purchased from Guangzhou Jiniou Co., Ltd. The lentiviral packaging plasmids PLV-CMV-FLAG, pcDNA-VSVG, pCMV-Δ8/9 and the silencing vector plasmid PLKO.1-puro were purchased from Sigma-Aldrich; Merck KGaA. The overexpression vector plasmid GV358-PTEN and the vector plasmid GV358 were purchased from Shanghai Genechem Co., Ltd.

Lentiviral packaging and stable strain construction
Packaging of the overexpression lentiviral. Recombinant overexpression lentivirus (Lv-PTEN) and the negative control (Lv-NC) were constructed by a three-plasmid coinfection method. In regards to the system: 293FT cells were cotransfected with GV358/GV358-PTEN (15 μg), pCMV-Δ8/9 (15 μg) and pcDNA-VSVG (7.5 μg), and the virus suspension was collected after 48 h.

Packaging of the silencing lentiviral. First, the PTEN shRNA target sequence was designed. The PTEN-shRNA double-stranded hairpin structure was designed according to the target sequence (the primer was synthesized by Shanghai Biosynthesis) to synthesize two shPTEN oligonucleotide sequences (shPTEN#1 and shPTEN#2). The recombinant nucleotide sequence was ligated to the PLKO.1-puro vector according to the manufacturer's protocol, and the PTEN silencing vector plasmid was successfully constructed. The silencing lentivirus (shPTEN#1 and shPTEN#2) was also packaged using the three-plasmid cotransfection method. In regards to the system: 293FT cells were cotransfected with PLKO.1/PLKO.1-shPTEN#1/PLKO.1-shPTEN#2 (15 μg), pCMV-Δ8/9 (15 μg) and pcDNA-VSVG (7.5 μg), and then the virus suspension was collected after 48 h.

Finally, CA46 and RAJI cells were infected with the successfully packaged virus, and a stable cell line was selected by adding a final concentration of 1 μl/ml puromycin.

Cell proliferation. Cell proliferation was analyzed by the CCK-8 assay. The cell density was adjusted to $8x10^4$/ml and inoculated into 96-well plates; 100 μl of cell suspension was added to each well, and 5 replicate wells were set for each cell group, which included a blank group. The control group was tested at 0, 1, 2, 3 and 4 days after inoculation. CCK-8 (10 μl) was added to each well of a 96-well plate, and incubation was carried out for 2.5 h in a 37˚C incubator. The absorbance was determined using a microplate reader. The absorbance of each well was determined and a growth curve for each group was plotted.

Apoptosis. Hoechst 33342 and propidium iodide (PI) double staining were used to verify apoptosis. A total of ~10^6 cells were collected from each group. The cell pellet was resuspended in 0.8 ml cell staining buffer; and 5 μl Hoechst staining solution and 5 μl PI staining solution were added. Red fluorescence and blue fluorescence were detected using fluorescence microscopy. Multiple fields of view were randomly selected for each group of cells, and red and blue fluorescence images of the same field of view were synthesized and analyzed using PS software (Adobe Photoshop CS6; Adobe Systems, Inc).

Cell cycle distribution. The cell density was adjusted to ~10^6 cells/ml. The cells were mixed with 1 ml PBS and 3 ml absolute ethanol to avoid cell clumping and fixed at -20˚C overnight. The fixed cells were collected and suspended in 1 ml PBS buffer three times; and the supernatant was retained subsequently. The cells were incubated for 30 min in 1 ml PBS with 4 μl RNase (10 μg/μl) and 30 μl PI stain (1 mg/ml) at room temperature with protection from light. Cells were strained in 200-μm mesh sieves into a special flow cytometry centrifuge tube. The DNA content of each group of cells was determined using flow cytometry. FlowJo™ software (FlowJo 7.6.1; BD Biosciences) was used to calculate and analyze cell cycle distribution.

Cell migration ability. Cells were resuspended in RPMI-1640 medium at a cell density of 10^6 cells/ml. RPMI-1640 medium with 10% FBS (600 μl) was added to a 24-well plate and placed in a Transwell chamber with 200 μl of the cell suspension. For each group of cells, a total of three duplicate wells were incubated in 5% CO_2 at 37˚C for 18 h. Once the Transwell chamber was removed, each well was centrifuged at 100 x g and the supernatant was discarded. The remaining 100 μl of the liquid was pipetted, mixed, and inoculated into a 96-well plate. CCK-8 solution (10 μl) was added to each well, and the plate was subsequently incubated for 2 h. The absorbance of each well was measured at a wavelength of 450 nm using a microplate reader.

Cell invasion. The cell density was adjusted to 10^6 cells/ml in the upper chamber of a Transwell plate that was coated with Matrigel. The culture method was the same as that aforementioned in the migration experiment. The lower chamber was incubated with 4% paraformaldehyde and stained with 4',6-diamidino-2-phenylindole (DAPI). The cells were observed under fluorescence microscopy (magnification, x200). Three fields of view were randomly selected for imaging, and the number of cells was calculated for each group to perform statistical analysis.

Western blotting. Protein lysates were separated by SDS-PAGE, transferred to PVDF membranes and then incubated with primary antibodies (GAPDH, PTEN, AKT, pAKT, Bad, Bax, P53, P21, CDK4, CDK6, cyclin D3, cyclin H, E-cadherin, N-cadherin, β-catenin, TCF-8, vimentin, Slug and Snail). The membranes were then incubated with HRP-labeled secondary antibodies. Finally, the hybridization signal was detected using ECL, exposed and photographed with a gel imager. The protein extraction buffer was RIPA Lysis Buffer, which was purchased from Shanghai Biyuntian Institute of Biotechnology. The BCA kit was used for protein determination method, and the mass of protein loaded per lane was 15 μg. The percentage of separated gel was 15%, and the percentage of concentrated gel was 5%. Blocking reagent was 5% skim milk powder PBST solution at room temperature shock closure 2 h. The

primary antibodies used were rabbit anti-human antibodies. The secondary antibody was goat anti-rabbit IgG(H+L) HRP. All antibodies were diluted in PBST solution. The primary antibody was incubated for 12 h at a temperature of 4˚C, and the secondary antibody was incubated at room temperature for 2 h. All antibodies and kits were purchased from Cell Signaling Technology (CST). The catalog numbers of anti-GAPDH, anti-PTEN, anti-AKT, anti-pAKT, anti-Bad, anti-Bax, anti-P53, anti-P21, anti-CDK4 and anti-CDK6 were #5157, #9188, #4685, #4060, #9268, #5023, #2527, #2947, #12790, #13331, respectively. Anti-cyclin D3, and anti- H were included in the Cyclin Antibody Sampler Kit (#9869). Anti-E-cadherin, anti-N-cadherin, anti-β-catenin, anti-TCF-8, anti-vimentin, anti-Slug and anti-Snail were from the Epithelial-Mesenchymal Transition (EMT) Antibody Sampler Kit (#9782).

Statistical analysis. The experimental results in the present study are represented as images and graphs. The data are expressed as the mean ± standard deviation (trials for each experiment were three). Data analyses were performed using SPSS 22.0 (IBM Corp.). The statistical analysis methods used included independent sample's t-test, rank sum test and one-way analysis of variance (ANOVA). P<0.01 was considered to indicate a statistically significant result.

Results

Overexpression of PTEN inhibits the growth and proliferation of CA46 and RAJI cells and induces apoptosis. The growth rate of the Lv-PTEN cell group was significantly lower compared with that of the Lv-NC cell group (P<0.01) (Fig. 1A and B). In addition, the expression level of p-AKT (Ser473) in the Lv-PTEN cell group was lower when compared with the Lv-NC cell group in both the CA46 and RAJI cell lines (Fig. 1C and D), suggesting that PTEN inhibited the proliferation of Burkitt's lymphoma cells by inhibiting AKT phosphorylation. Fig. 2A and B demonstrated that the Lv-PTEN cell group exhibited weak red and strong blue fluorescence, which indicates the presence of apoptotic cells. No apoptotic cells were observed in the Lv-NC cell group. The expression levels of Bad and Bax in the PTEN cell group were higher than levels in the Lv-NC cell group in the CA46 and RAJI cell lines (Fig. 2C and D), suggesting that PTEN may promote the apoptosis of Burkitt's lymphoma cells by upregulating the expression of proapoptotic proteins Bad and Bax.

Overexpression of PTEN blocks the cell cycle progression of the CA46 and RAJI cells. As shown in Fig. 3, the percentage (%) of cells in the cell cycle distribution of the CA46-Lv-NC cell group are as follows: 53.19±0.99 in G1, 36.56±0.84 in S phase, and 8.09±0.61 in G2 phase. The percentage (%) of cells in the cell cycle distribution of the CA46-Lv-PTEN cell group are as follows: 67.06±0.63 in G1, 23.84±1.65 in S phase, and 6.05±0.32 in G2 phase. The percentage (%) of cells in the cell cycle distribution of the RAJI-Lv-NC cell group was as follows: 52.69±0.55 in G1, 35.89±1.47 in S phase, and 9.53±0.32 in G2 phase. The percentage (%) of cells in the cell cycle distribution of the RAJI-Lv-PTEN cell group was as follows: 65.45±1.31 in G1, 25.43±0.66 in S phase, and 5.99±0.58 in G2 phase. From the results of the data analysis, the proportion of G1 phase cells in the Lv-PTEN cell group was significantly higher compared with the Lv-NC cell group (P<0.01), while the proportion of S phase cells in the Lv-PTEN cell group was significantly lower compared with that in the Lv-NC cell group (P<0.01). As presented in Fig. 3C and D, the expression levels of P53 and P21 in the Lv-PTEN cell groups were higher compared with the Lv-NC cell groups. The expression levels of CDK4, CDK6, cyclin D3 and cyclin H in the Lv-PTEN cell groups were lower when compared with these levels in the Lv-NC cells, suggesting that PTEN overexpression inhibited the Burkitt's lymphoma cell cycle by promoting the expression of P53 and P21, and inhibiting the expression of CDK4, CDK6, cyclin D3 and cyclin H. In conclusion, the cell cycle was arrested at the G1 phase.

Overexpression of PTEN inhibits the migration and invasion of CA46 and RAJI cells. As shown in Fig. 4A and B, the optical density (OD) values of the cells in each group were 0.506±0.012 in the CA46-Lv-NC cell group, 0.194±0.024 in the CA46-Lv-PTEN cell group, and 0.403±0.015 in the RAJI-Lv-NC cell group. The OD value in the RAJI-Lv-PTEN cell group was 0.186±0.004. The numbers of migrated cells in the Lv-PTEN cell groups were significantly lower compared with these numbers in the Lv-NC cell groups (P<0.01). The results of the cell invasion into Matrigel for each group are presented in Fig. 4C and D. The number of cells per field in the CA46-Lv-NC, CA46-Lv-PTEN, RAJI-Lv-NC and RAJI-Lv-PTEN was 165±10.15, 93±3.51, 103.67±5.13 and 34.67±3.22, respectively. The number of invasive cells in the Lv-PTEN cell group was significantly lower compared with that in the Lv-NC group in both cell lines (P<0.01). As presented in Fig. 4E and F, the expression level of E-cadherin in the Lv-PTEN cell group was higher when compared with the Lv-NC cell group. The expression levels of N-cadherin, β-catenin, TCF-8, vimentin, Slug and Snail in the Lv-PTEN cell group were lower compared with the Lv-NC cell group in both cell lines, suggesting that PTEN upregulates E-cadherin and downregulates N-cadherin, β-catenin, TCF-8, vimentin, Slug, and Snail to inhibit the migration and invasion of Burkitt's lymphoma cells.

Silencing of PTEN promotes the growth, migration and invasion of CA46 and RAJI cells. To further verify the biological function of the silencing of PTEN on CA46 and RAJI cells, PTEN was silenced and cell function assays were performed. As expected, silencing of PTEN promoted the growth, migration and invasion of the two cell lines. As presented in Fig. 5A and B, the growth rate of the shPTEN cell groups was significantly higher compared with the shNC cell group in both cell lines (P<0.01). As indicated in Fig. 5C and D, the OD values were 0.515±0.006 for the CA46-shNC cell group, 1.116±0.042 for the CA46-shPTEN#1 cell group, 0.989±0.048 for the CA46-shPTEN#2 cell group, 0.434±0.013 for the RAJI-shNC cell group, 0.888±0.034 for the RAJI-shPTEN#1 cell group, and 0.986±0.005 for the RAJI-shPTEN#2 cell group. The numbers of migrated cells in the shPTEN cell groups were significantly higher compared with the shNC cell groups for both cell lines (P<0.01). As presented in Fig. 5E and F, the invasive number of cells per field

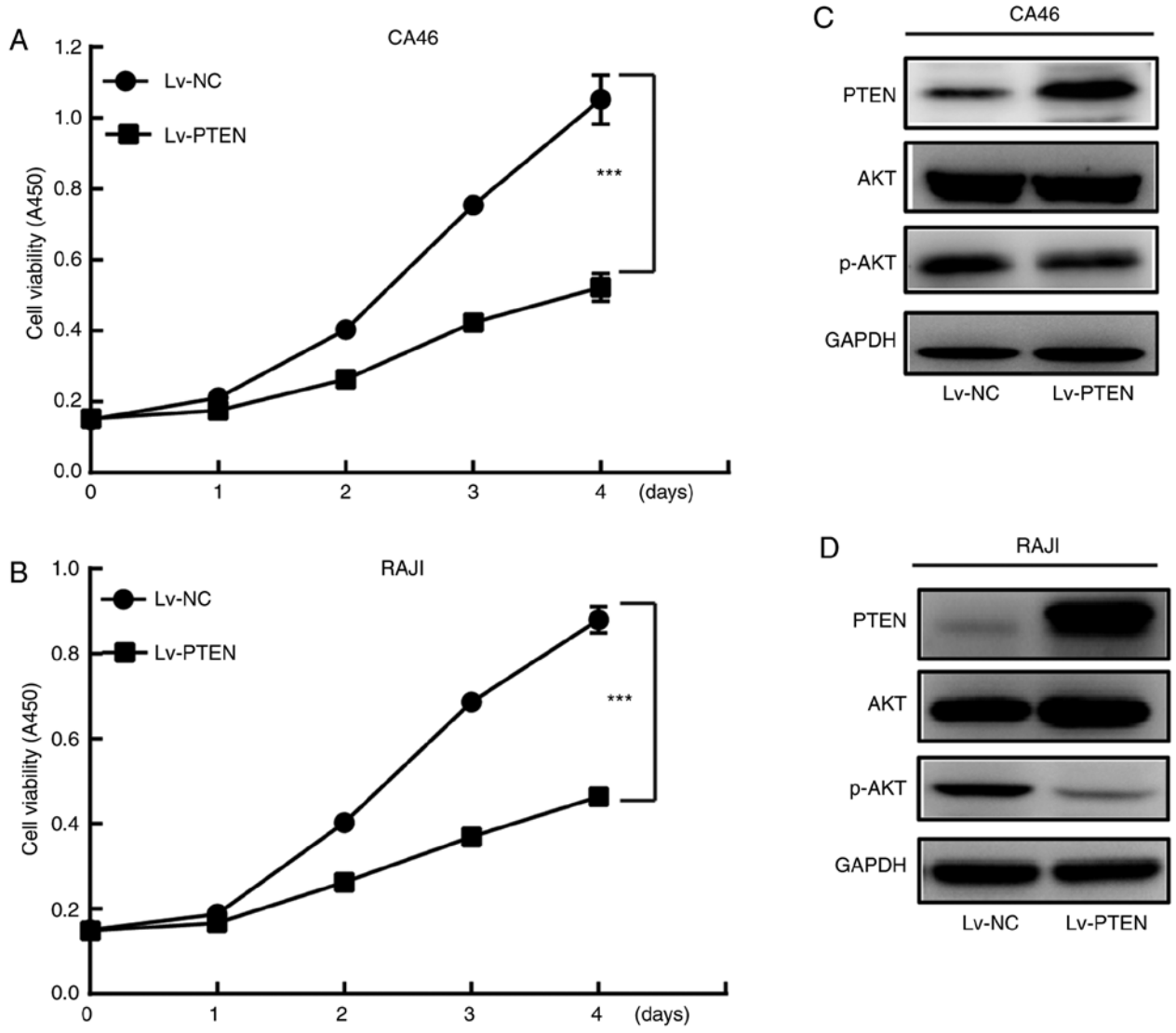

Figure 1. Overexpression of PTEN inhibits the growth and proliferation of CA46 and RAJI cells. (A and B) CCK-8 assay was conducted and plots of the growth curves of each group of cells are shown. (C and D) Western blot analysis of the expression of AKT and p-AKT (Ser473) in each group of cells. ***P<0.01 vs. the Lv-NC group. PTEN, phosphatase and tensin homolog; AKT/PKB, protein kinase B; Lv-PTEN, PTEN-overexpression group; Lv-NC, negative control group.

for each group was 154.33±6.11 for CA46-shNC, 440.67±7.77 for CA46-shPTEN#1, 426.33±9.45 for CA46-shPTEN#2; 95.00±6.25 for RAJI-shNC, 331.67±5.86 for RAJI-shPTEN#1, and 346.00±17.18 for RAJI-shPTEN#2. The numbers of invasive cells in the shPTEN cell groups were significantly higher compared with the shNC groups in both cell lines (P<0.01). These results demonstrated that the silencing of PTEN produced opposite results on cell behavior when compared with the overexpression of PTEN.

Discussion

Burkitt's lymphoma is a highly invasive cancer of the lymphatic system, particularly B lymphocytes found in the germinal center (11). A number of studies have investigated Burkitt's lymphoma; however, the specific pathogenesis of the disease is not fully understood. Our previous studies found that inhibition of the PI3K pathway can inhibit the proliferation of Burkitt's lymphoma, and abnormal expression of phosphatase and tensin homolog (PTEN) is common in solid tumors, including breast cancer, kidney cancer, esophageal cancer, endometrial cancer (6-8) and diffuse large B cell lymphoma (12). Therefore, a relationship between PTEN overexpression and the incidence of Burkitt's lymphoma has been indicated.

Research has demonstrated that increased expression levels of PTEN inhibits the growth of breast cancer cells (13). Zhao *et al* (14) used PTEN as a silencing target and performed PTEN knockdown in neuronal cells, which activated the PI3K/AKT signaling pathway and promoted cell growth and proliferation. The results of the present study indicated that PTEN overexpression downregulated the expression of p-AKT, inhibited the proliferation of Burkitt's lymphoma cells and induced apoptosis. Silencing of PTEN promoted the growth and proliferation of Burkitt's lymphoma cells. The results of the present study demonstrated that PTEN inhibited the PI3K/AKT signaling pathway, decreased the activity of AKT, upregulated the expression of proapoptotic proteins Bad and Bax, inhibited the proliferation of Burkitt's lymphoma cells and induced apoptosis.

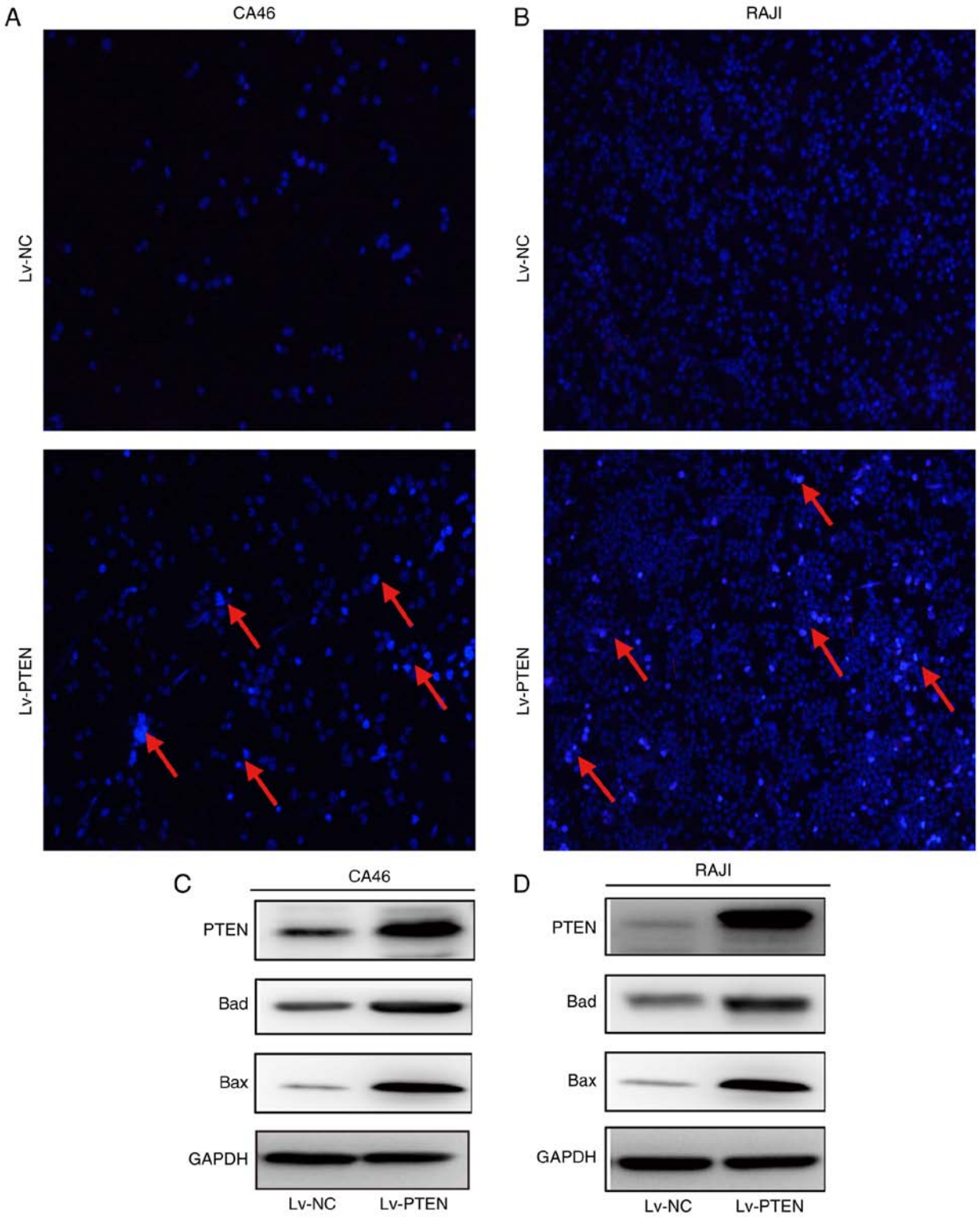

Figure 2. PTEN overexpression induces apoptosis of CA46 and RAJI cells. (A and B) Fluorescence images (magnification, x200) after double staining with Hoechst 33342 and propidium iodide (PI) in each group. (C and D) Western blot analysis of the expression of Bad and Bax in each group. PTEN, phosphatase and tensin homolog; Lv-PTEN, PTEN-overexpression group; Lv-NC, negative control group; Bad, Bcl-2/Bcl-XL-associated death promoter; Bax, Bcl-2 associated X protein.

The present study also indicated that overexpression of PTEN promoted the expression of P53 and P21 and inhibited the expression of CDK4, CDK6, cyclin D3 and cyclin H, which can arrest the Burkitt's lymphoma cell cycle at the G1 phase. G1/S phase checkpoints are critical for the regulation of the cell cycle (15). The overexpression of PTEN upregulated the expression of the tumor-suppressor gene P53 in Burkitt's lymphoma cells, thereby activating P21 transcription. P21 is an inhibitor of CDK, which can bind to CDK4/6, and inhibit its activity. The activity of CDK4/6 was decreased, which further affected the expression of cyclin D3 and cyclin H, and caused G1 phase arrest of Burkitt's lymphoma cells. Zheng *et al* (16)

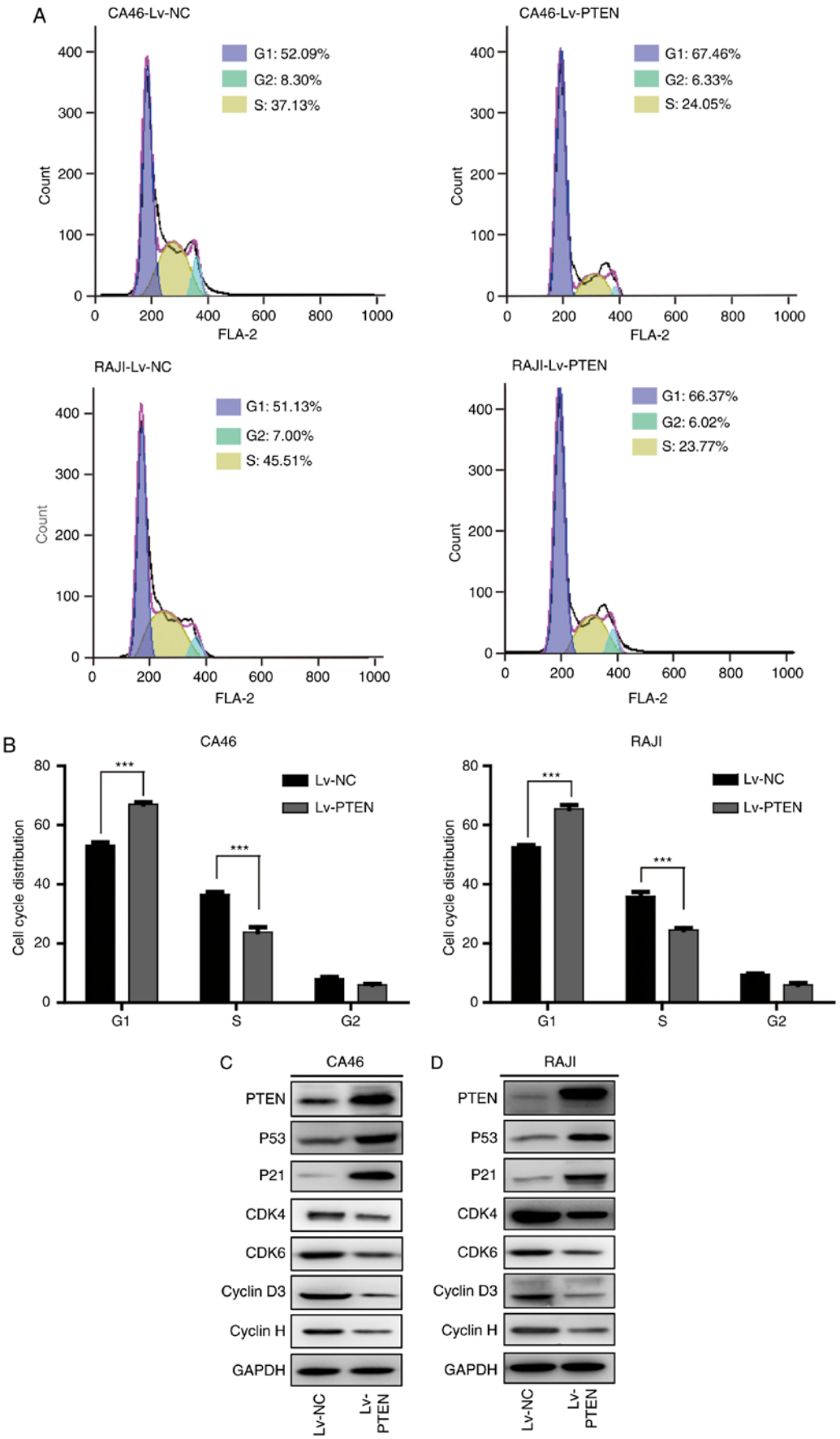

Figure 3. Overexpression of PTEN blocks CA46 and RAJI cell cycle progression. (A) Flow cytometric analysis was used to detect the cell cycle distribution of the Lv-PTEN and Lv-NC groups in the CA46 and RAJI cell lines. (B) Comparison of the proportion of cells at each stage of the cell cycle in the CA46 and RAJI cell lines. (C and D) Western blot analysis of cell cycle-associated proteins in the Lv-PTEN and Lv-NC groups in the CA46 and RAJI cell lines. ***P<0.01 vs. the Lv-NC group. PTEN, phosphatase and tensin homolog; CDK4, cyclin dependent kinase 4; CDK6, cyclin dependent kinase 6; Lv-PTEN, PTEN-overexpression group; Lv-NC, negative control group.

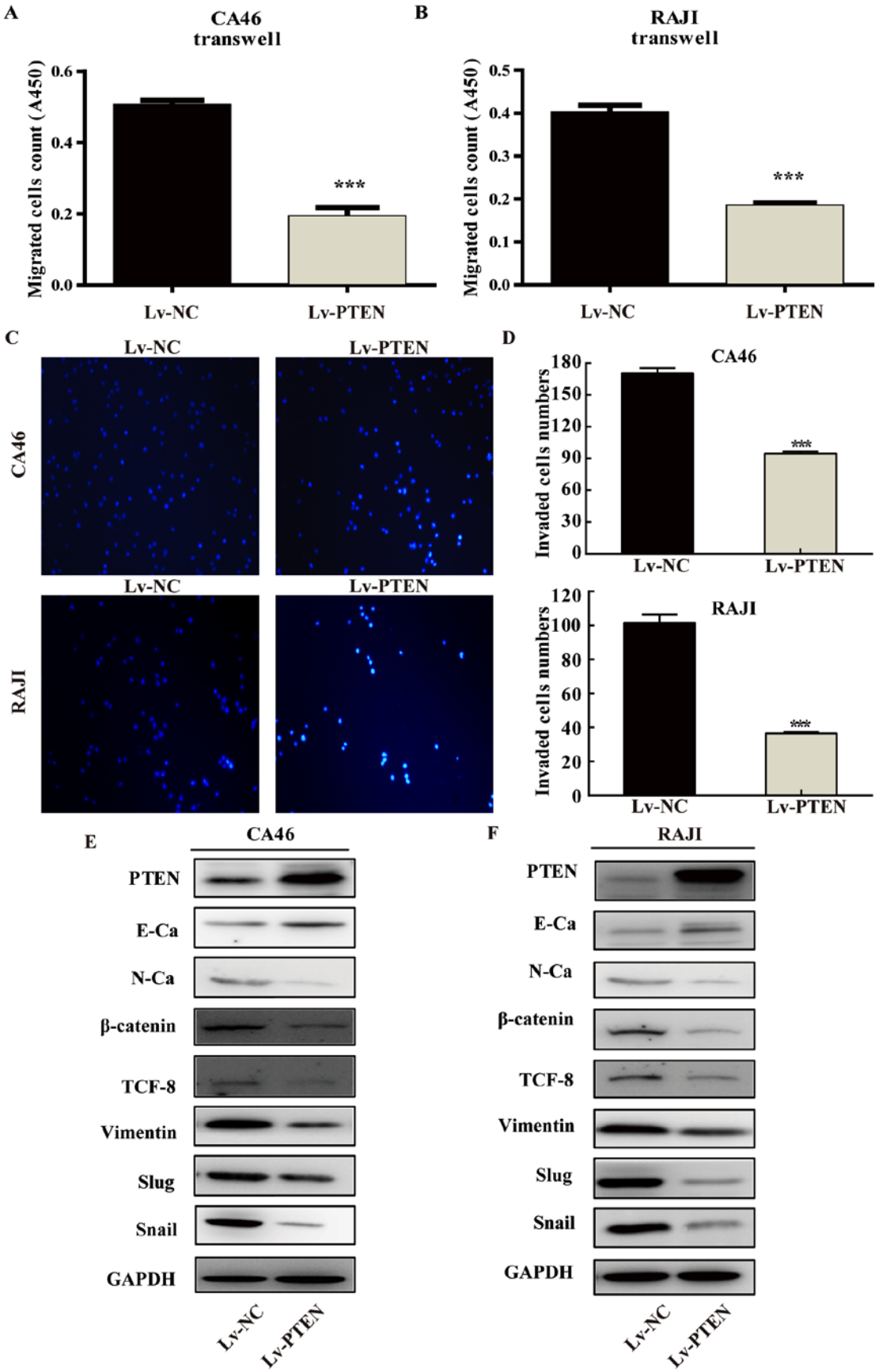

Figure 4. Overexpression of PTEN inhibits the migration and invasion of CA46 and RAJI cells. (A and B) The Transwell chamber analysis was used to determine the migration ability of the Lv-PTEN and Lv-NC groups in the CA46 and RAJI cell lines. (C) Matrigel Transwell assay was used to detect the invasive ability of the Lv-PTEN and Lv-NC groups in the CA46 and RAJI cell lines (magnification, x200). (D) Comparison of the invasive ability. (E and F) Western blot analysis was used to detect the expression of EMT-like cell markers in the Lv-PTEN and Lv-NC groups in the CA46 and RAJI cell lines. $^{***}P<0.01$ vs. control group. PTEN, phosphatase and tensin homolog; E-Ca, E-cadherin; N-Ca, N-cadherin; TCF-8 or ZEB1, zinc finger E-box-binding homeobox 1; Lv-PTEN, PTEN-overexpression group; Lv-NC, negative control group.

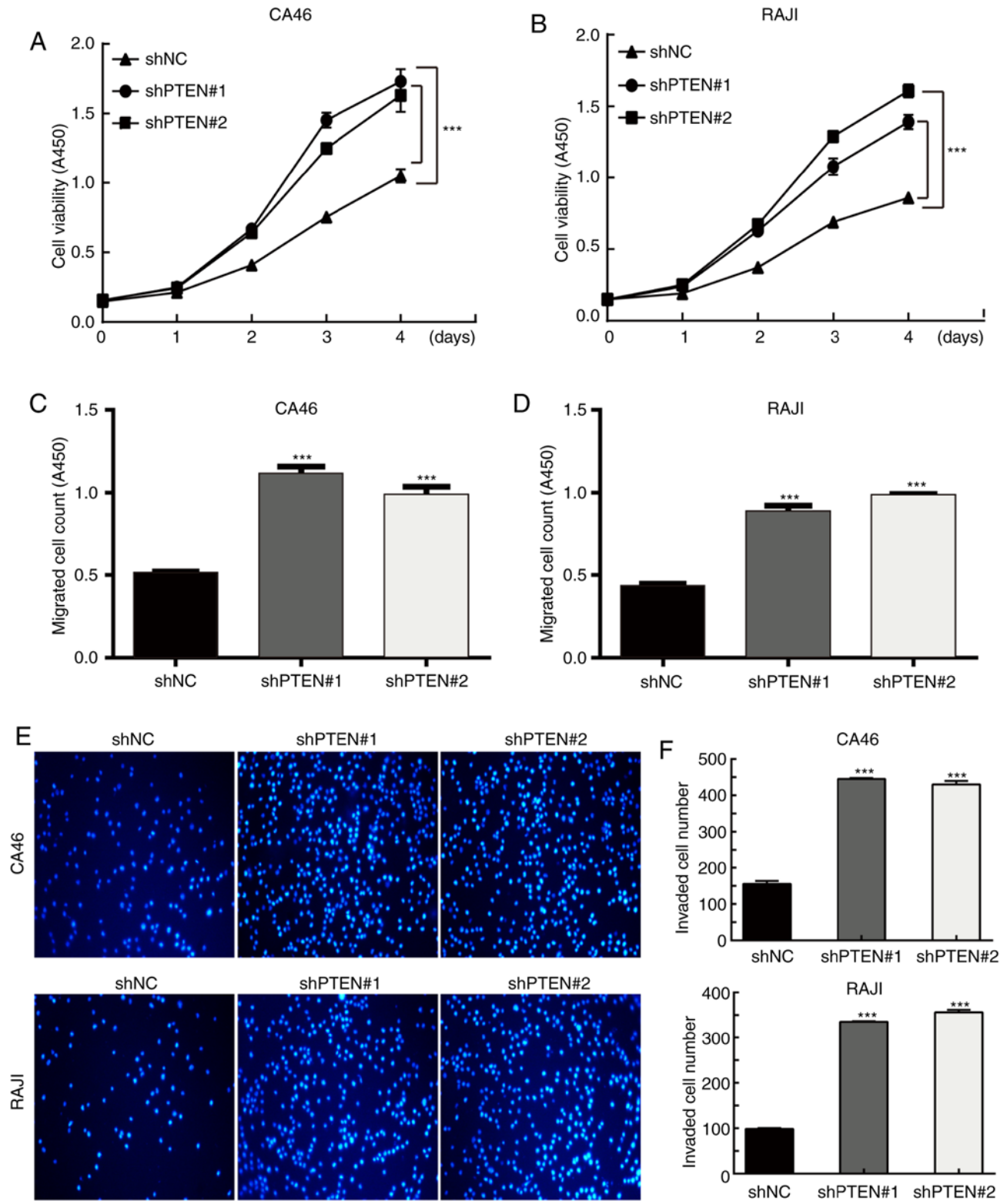

Figure 5. Silencing of PTEN promotes growth, migration and invasion of CA46 and RAJI cells. (A and B) CCK-8 assay was carried out to plot the growth curves in the shNC, shPTEN#1 and shPTEN#2 groups in the CA46 and RAJI cell lines. (C and D) Transwell chamber was used to detect the migration ability of the shNC, shPTEN#1 and shPTEN#2 groups in the CA46 and RAJI cell lines. (E) Matrigel Transwell assay was used to detect the cell invasion ability of the shNC, shPTEN#1 and shPTEN#2 groups in the CA46 and RAJI cell lines (magnification, x200). (F) Comparison of cell invasion ability of each group. ***P<0.01 vs. the shNC group. PTEN, phosphatase and tensin homolog; shPTEN#1 and shPTEN#2, PTEN-silenced groups, shNC, negative control group.

used PTEN as a therapeutic target and applied FTY720 immunosuppressive agents to gastric cancer cells. The inhibitors were found to suppress the PI3K/AKT signaling pathway and increase the expression of P53 and P21 by upregulating PTEN expression. The inhibition of CDK4/6 expression and cell cycle arrest at the G1 phase indicated that PTEN upregulation significantly inhibited the proliferation of gastric cancer cells. Moon *et al* (17) also demonstrated that PTEN

is associated with inhibition of the growth and regulation of vascular smooth muscle cells and regulation of the cell cycle, and this inhibition is associated with the downregulation of cyclins and CDKs and the upregulation of CDK inhibitors P21 and P27. Luo *et al* (18) transfected airway smooth muscle cells with a plasmid vector carrying PTEN and revealed that PTEN overexpression blocked the G1 phase of the airway smooth muscle cell cycle by inhibiting the expression of P21 and cyclin D. All of the aforementioned studies demonstrated that PTEN overexpression can inhibit cell growth by blocking the cell cycle progression of Burkitt's lymphoma cells.

The invasion and metastasis of malignant tumor cells are attributed to poor patient prognosis and mortality (19,20). Therefore, the prevention of tumor metastasis is essential. The present study demonstrated that PTEN overexpression significantly inhibited the migration and invasion of Burkitt's lymphoma cells. In contrast, silencing of PTEN was revealed to promote the migration and invasion of Burkitt's lymphoma cells. These results indicated that PTEN may be a potential target for preventing the invasion and metastasis of Burkitt's lymphoma cells. It still remains unclear as to which molecular mechanisms are involved in the inhibition of Burkitt's lymphoma progression by PTEN. The epithelial-mesenchymal transition (EMT) is an important process that is associated with tumor metastasis and invasion (21).Xu *et al* (22) demonstrated that treatment with doxorubicin can upregulate the expression of PTEN and inhibit the phosphorylation of AKT in gastric cancer cells, thereby inhibiting the metastasis and invasion of gastric cancer. Wang *et al* (23) demonstrated that knockdown of PTEN can promote the migration of nasopharyngeal carcinoma cells. Wang *et al* (24) demonstrated that when SYNJ2BP induced PTEN degradation in breast cancer tissues, PTEN expression levels were decreased, and the PI3K/AKT signaling pathway was activated to induce the invasion of breast cancer cells. In recent years, a number of studies have revealed that activation of AKT can promote the metastasis and invasion of tumor cells. Activated AKT binds to vimentin, which induces the metastasis of gastric cancer cells through the regulation of the E-cadherin/β-catenin complex (25,26). Additionally, activated AKT regulates EMT via the AKT/GSK-3β/β-catenin signaling pathway (27,28). Nuclear β-catenin not only regulates proteins that are associated with the EMT signaling pathway, but also induces transcriptional upregulation of TCF-8, which in turn regulates DNA damage. The aforementioned results demonstrate that activated AKT can promote EMT and induce tumor formation and metastasis. Jin *et al* (29) also indicated that inhibition of the PTEN/PI3K/AKT signaling pathway can enhance the sensitivity of esophageal cancer to radiotherapy and reverse the process of EMT. This studied showed that the upregulation of PTEN expression and inhibition of AKT phosphorylation can promote esophageal cancer cell apoptosis, enhance the expression of E-cadherin and downregulate the expression of N-cadherin and vimentin to inhibit cancer cell invasion and metastasis. The present study demonstrated that PTEN upregulates E-cadherin and downregulates the expression of N-cadherin, β-catenin, TCF-8, vimentin, Slug and Snail. β-catenin is an important molecule in the Wnt pathway, and abnormal expression of β-catenin can promote tumor metastasis and infiltration. PTEN overexpression downregulates the expression of zinc finger transcription factors TCF-8, Slug and Snail by reducing the accumulation of β-catenin in Burkitt's lymphoma cells, and consequently inhibits the transition from epithelial cadherin E-cadherin to neurokarin N-cadherin and the expression of vimentin, thereby inhibiting the migration and invasion of Burkitt's lymphoma cells. This process is consistent with previous reports (22,30). These findings indicate that PTEN overexpression can inhibit the migration and invasion of Burkitt's lymphoma cells. However, there is also a shortcoming in our study. We did not use normal cells as a control to detect the specificity of the effect of PTEN on Burkitt's lymphoma. This is a limitation to the present study. In conclusion, the present study demonstrated that the tumor-suppressor gene PTEN inhibited the growth and proliferation of Burkitt's lymphoma cells by inhibiting the PI3K/AKT signaling pathway during *in vitro* experiments; PTEN was indicated to induce apoptosis by upregulating proapoptotic proteins. PTEN was also demonstrated to arrest the cell cycle of Burkitt's lymphoma cells by regulating cell cycle-associated proteins. Additionally, PTEN was revealed to inhibit cell invasion by mediating EMT-like cell markers. PTEN serves an important role in the development of Burkitt's lymphoma and the present study provides insight into this potential clinical target for the treatment of Burkitt's lymphoma. We studied the mechanism of PTEN only in lymphoma, which confirms that it may become a target for the treatment of lymphoma. In the future, tumor cells should be induced to specifically express high PTEN using other methods, without affecting the expression level of PTEN in normal cells; thereby achieving the aim of treating lymphoma.

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Availability of data and materials

The datasets used during the present study are available from the corresponding author upon reasonable request.

Authors' contributions

CL participated in the design of this study. YX collected the data and performed the statistical analysis. PX carried out the study, together with YZ, and collected important background information. XZ drafted the manuscript. All authors read and approved the final manuscript and agree to be accountable for all aspects of the research in ensuring that the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Ethics approval and consent to participate

Not applicable as only cell lines were used in the study and no human or animal subjects were utilized in the study.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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