Interference of PTK6/GAB1 signaling inhibits cell proliferation, invasion, and migration of cervical cancer cells

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Abstract. Protein tyrosine kinase 6 (PTK6) has shown important cancer-promoting effects in a variety of cancer types. Nonetheless, its vital role in cervical cancer has not been completely elucidated. The present study sought to address whether PTK6 is involved in the malignant progression of cervical cancer via its interaction with GRB2-associated binding 1 (GAB1). Western blotting was used to examine PTK6 and GAB1 expression levels. Cell Counting Kit-8, Transwell, wound healing, and terminal deoxynucleotidyl-transferase-mediated dUTP nick end labeling assays were performed to estimate the corresponding proliferative, migratory, invasive, and apoptotic abilities of the cells. Co-immunoprecipitation (Co-IP) assays confirmed binding of PTK6 to GAB1. The results revealed that the expression levels of PTK6 and GAB1 were markedly increased in cervical cancer cell lines compared with those noted in normal cervical epithelial cells. The cell proliferative, invasive, and migratory activities of cervical cancer cells were reduced in the absence of PTK6 expression, whereas the induction of apoptosis was aggravated under these conditions. The results of the Co-IP assay indicated that PTK6 expression was positively associated with GAB1. In addition, the suppressive effect of PTK6 silencing on the malignant phenotypes of cervical cancer cells was reversed following overexpression of GAB1. In summary, the present study indicated that knockdown of PTK6 expression protected against the malignant progression of cervical cancer, while overexpression of GAB1 counteracted the inhibitory effects of PTK6 knockdown on cervical cancer cells.

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Introduction

Cervical cancer is attributed to human papillomavirus (HPV) infection and is one of the most serious malignancies in the world affecting the female reproductive tract (1-3). Although effective early screening and vaccination against HPV have greatly reduced cervical cancer morbidity and mortality worldwide, the clinical signs and symptoms of cervical cancer remain insidious until the advanced stage of this disease, resulting in a poor therapeutic outcome (4,5). Therefore, the identification of novel therapeutic targets is of considerable importance for providing valuable strategies to be used in cervical cancer treatment. It can also improve the survival rate of these patients.

Protein tyrosine kinase 6 (PTK6) is an intracellular non-receptor tyrosine kinase, which belongs to the SRC family of proteins and was originally identified in metastatic breast tumors (6). PTK6 contributes to the differentiation of normal epithelial cells and exhibits elevated expression in breast, ovarian and lung cancer (7-9). It is noteworthy that PTK6 participates in the regulation of different signaling pathways in a variety of cancer types. Epithelial-mesenchymal transition plays a vital role in regulating the tumorigenic activity of PTK6 by directly affecting tumor metastasis and colonization (10). Ono *et al* (11) indicated that PTK6 was involved in the progression of pancreatic cancer via the ERK signaling pathway. An additional study demonstrated that knockdown of PTK6 expression could inhibit proliferation, invasion, migration, and malignant progression of hepatocellular carcinoma cells (12). In addition, Wang *et al* (5) demonstrated that PTK6 expression was upregulated in cervical cancer tissues; this phenomenon was associated with a poor disease prognosis, suggesting that overexpression of PTK6 was closely associated with cervical cancer prognosis (5). However, little is known regarding the current mechanism of PTK6 in cervical cancer. Therefore, the present study aimed to explore whether PTK6 was involved in the malignant progression of cervical cancer and discuss its potential mechanism.

GRB2-associated binding (GAB) 1 is a multi-site docking protein with multiple tyrosine residues (13). Following phosphorylation of the tyrosine residues, GAB1 provides binding sites for multiple effector proteins to induce a variety of

tyrosine kinase signaling events (14,15). A large number of studies have shown that increased expression of GAB2 is strongly associated with tumor proliferation and metastasis in breast, prostate and ovarian cancers (16-18). Recent research has suggested that GAB1, a downstream gene of c-Met, is negatively modulated by microRNA (miR)-23b-3p in cervical cancer, indicating that GAB1 also exhibits an essential role in cervical cancer (19). Subsequently, the Biogrid database (https://thebiogrid.org/) predicted that PTK6 could bind to GAB1. Therefore, it is reasonable to hypothesize that PTK6 may interact with GAB1 to participate in the malignant progression of cervical cancer. The present study elaborated the impact of PTK6 and GAB1 on the aggressive phenotype of cervical cancer cells and explored its underlying mechanisms to identify novel therapeutic strategies for cervical cancer.

Materials and methods

Cell culture. Immortalized human normal cervical epithelial cells (Ect1/E6E7, CVCL_3679) and cervical cancer cell lines (SiHa, CVCL_0032; HeLa, CVCL_0030; Ca-Ski, CVCL_1100; C-33A, CVCL_1094) were purchased from American Type Culture Collection and cultured in Dulbecco's modified Eagle's medium (DMEM) (Merck KGaA) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin solution (both from Beijing Solarbio Science & Technology Co., Ltd.) in a 5% CO_2 incubator at 37˚C.

Bioinformatics. BiogridDataBase (version 4.4; https://thebiogrid.org/) is a database that can be used to predict the interactions between proteins. The Protein-Protein Affinity Predictor (PPA-Pred2) database (https://www.iitm.ac.in/bioinfo/PPA_Pred/prediction.html#) was used to predict the binding of PTK6 and GAB1.

Cell transfection. C-33A cells were harvested in the logarithmic growth phase, transferred into 6-well plates, and subsequently transfected when they had reached 80% confluence. Small interfering RNA (siRNA) targeting PTK6-1 (si-PTK6-1; 5'-GCCATTAAGGTGATTTCTCGAGG-3') and PTK6-2 (si-PTK6-2; 5'-TTCAATTGCACCTATGTTTTTAC-3'), the corresponding empty vector [siRNA-negative control (NC); 5'-AAGACAUUGUGUGUCCGCCTT-3'], the overexpression plasmid targeting GAB1 (Ov-GAB1), and its empty vector (Ov-NC) were synthesized by Geneseed Biotech Co., Ltd. According to the manufacturer's instructions, Lipofectamine® 2000 (Invitrogen; Thermo Fisher Scientific, Inc., Shanghai, China), was used to transfect the aforementioned vectors into C-33A cells ($1x10^5$ cells/well) at a concentration of 50 ng/ml at 37˚C for 24 h. Following incubation, the medium was replaced with fresh DMEM and the cells were routinely cultured for 48 h. Subsequently, the cells were used to assess the transfection efficiency.

Cell counting kit (CCK)-8 assay. Following incubation of the transfected C-33A cells ($5x10^3$ cells/ml) in 96-well plates for 24, 48, and 72 h, 10 μl CCK-8 solution (Beyotime Institute of Biotechnology) was added and incubated with the cells for an additional 2 h. The OD values were measured using a microplate reader (BioTek™ ELx800; BioTek Instruments, Inc.) at 450 nm and the growth curve was plotted.

Wound healing assay. The C-33A cells were seeded at a density of $1x10^5$ cells/well into 12-well plates. When the cell confluence had reached 80%, serum-free DMEM was added to the cells, which were cultured at 37˚C, overnight. Subsequently, a 200-μl pipette tip was utilized to make scratches in the cell monolayer. Following 24 h of incubation, an inverted microscope (Olympus Corporation) was used to monitor the wounds. The migratory rate was calculated as the ratio of 0 h scratch width/24 h scratch width.

Transwell assay. Matrigel (BD Biosciences) was placed in 24-well Transwell plates with 8-μm pores at 37˚C for 30 min. The C-33A cells ($5x10^4$ cells/ml) in 200 μl serum-starved medium were plated into the upper chamber, and the lower chamber was filled with 600 μl DMEM supplemented with 10% FBS. Following 24 h of cell culture, sterile cotton swabs were used to remove non-invading cells, while the invading cells were immobilized using 4% formaldehyde for 15 min at room temperature. The invading cells were observed in 5 random fields using an inverted microscope following staining with 0.1% crystal violet solution at room temperature for 30 min.

Terminal deoxynucleotidyl-transferase-mediated dUTP nick end labeling (TUNEL) assay. The induction of apoptosis was detected using a TUNEL kit (EMD Millipore). The C-33A cells were fixed using 4% paraformaldehyde at room temperature for 15 min following washing with PBS. A total of 0.3% Triton-X 100/PBS was used to permeabilize the cells for 10 min, followed by rinsing with PBS. Subsequently, 3% H_2O_2 was added to inhibit endogenous peroxidation, after which was the incubation of C-33A cells with TUNEL regent for 60 min at 37˚C. 3,3-Diaminobenzidine was added to stain the cells according to the manufacturer's instructions. Finally, nuclei were stained with DAPI at room temperature for 10 min. 10 visual fields were randomly selected and an Olympus BX51 fluorescence microscope (Olympus Corporation) was used to determine the number of apoptotic cells.

Reverse transcription-quantitative PCR (RT-qPCR). C-33A cells that were in the logarithmic phase were collected and total RNA was extracted from these cells using TRIzol® reagent (Sigma-Aldrich; Merck KGaA) according to the manufacturer's instructions. cDNA was synthesized using the QuantiTect Reverse Transcription kit (Qiagen GmbH) according to the manufacturer's instructions. An ABI 7500 Real-Time PCR instrument (Applied Biosystems; Thermo Fisher Scientific, Inc.) was used to conduct PCR amplification according to the operating procedures of the SYBR Green PCR kit (Takara Bio, Inc.). The following thermocycling conditions were used: Initial denaturation at 95˚C for 8 min; followed by 35 cycles of denaturation at 95˚C for 30 sec and annealing at 60˚C for 1 min; and extension for 10 min at 72˚C. The nucleotide sequences used are listed as follows: PTK6 forward, 5'-TGCCCCATTGGGATGACT G-3' and reverse, 5'-GTACAGCGCCAGGATGTGTTT-3';

GAB1 forward, 5'-GATGGTTCGTGTTACGCAGTG-3' and reverse, 5'-CGCTGTCTGCTACCAAGTAGAA-3'; GAPDH forward, 5'-TGTGGGCATCAATGGATTTGG-3' and reverse, 5'-ACACCATGTATTCCGGGTCAAT-3'. The $2^{-\Delta\Delta Cq}$ method was used to measure the gene expression levels, which were normalized compared with those of GAPDH (20).

Western blot analysis. RIPA lysis buffer (Beyotime Institute of Biotechnology) was used to extract total protein from collected C-33A cells. The protein concentration was determined using a BCA assay and 20 μg of each protein sample was separated using 10% SDS-PAGE gel electrophoresis. The proteins were then transferred to a polyvinylidene fluoride membrane at room temperature (30-min transfer), after which 5% skim milk was used for blocking of the non-specific binding sites at room temperature for 1 h. The primary antibodies targeting PTK6 (product code ab233392; 1:1,000 dilution), Bcl-2 (product code ab32124; 1:1,000 dilution), baculoviral IAP repeat containing 5 (Birc5; product code ab76424; 1:5,000 dilution), Bax (product code ab32503; 1:1,000 dilution), matrix metalloproteinase (MMP)12 (product code ab52897; 1:1,000 dilution), MMP9 (product code ab76003; 1:1,000 dilution), GAB1 (product code ab133486; 1:1,000 dilution), and GAPDH (product code ab181602; 1:10,000 dilution) were purchased from Abcam and were incubated with the corresponding membranes at 4˚C overnight. HRP-conjugated secondary antibodies (product no. 7074; 1:5,000; Cell Signaling Technology, Inc.) were used to probe the membranes at room temperature for 2 h the following day. Following the addition of the ECL solution (Vazyme Biotech Co., Ltd.; Nanjing, China), a gel imager (C150 model; Azure Biosystems, Inc.) was used to achieve visualization of the protein bands. The gray values of the protein bands were analyzed using ImageJ (v1.51; National Institutes of Health) and the relative expression levels of each protein were calculated.

Co-immunoprecipitation (Co-IP) assay. For immunoprecipitation, C-33A cells were harvested and lysed in 300 μl Pierce™ IP lysis buffer (Thermo Fisher Scientific, Inc.). A total of 400 μg protein was incubated with the antibody solution containing 2 μg PTK6 (product code ab233392; 1:30 dilution), or GAB1 (product code ab133486; 1:100 dilution), and goat anti-rabbit IgG (HRP) (product code ab205718; 1:20 dilution; all from Abcam) overnight at 4˚C. Subsequently, the cell lysates were incubated for 3 h following the addition of 30 μl Protein G/A agarose beads (Invitrogen; Thermo Fisher Scientific, Inc.). Following 600 x g centrifugation at 4˚C for 10 min, the pellets were washed three times with 1 ml lysis buffer before resuspension, in resuspended in 2X SDS-PAGE loading buffer. Finally, immunoblotting was used to detect immunoprecipitation products.

Statistical analysis. GraphPad Prism 7.0 software (GraphPad Software, Inc.) was used to analyze the data. The experimental data are presented as the mean ± SD. The comparison between the two groups was performed by the unpaired Student's t-test, whereas the comparison among multiple groups was performed by one-way ANOVA followed by Tukey's post hoc test. Each experiment was repeated at least three times unless otherwise specified. $P<0.05$ was used to indicate a statistically significant difference.

Results

PTK6 expression is increased and PTK6 deficiency exacerbates cell apoptosis in cervical cancer. The expression levels of PTK6 were examined in human normal cervical epithelial cells (Ect1/E6E7) and in cervical cancer cell lines (SiHa, HeLa, Ca-Ski and C-33A) by RT-qPCR (Fig. 1A) and western blotting (Fig. 1B) analyses. PTK6 expression was significantly increased in cervical cancer cell lines compared with that noted in Ect1/E6E7 cells. C-33A cells were selected for subsequent experiments since they displayed the highest expression of PTK6 among SiHa, HeLa, Ca-Ski, and C-33A cells. Subsequently, PTK6 expression was knocked down and the transfection efficacy was assessed via RT-qPCR (Fig. 1C) and western blotting (Fig. 1D) analyses. siRNA-PTK6-1 was selected for subsequent experiments due to the most efficient knockdown of PTK6 expression noted in the siRNA-PTK6-1 group compared with that of the siRNA-PTK6-2 group. Following knockdown of PTK6 expression, reduced cell viability was observed compared with that of the siRNA-NC group (Fig. 1E). PTK6 depletion distinctly aggravated the induction of apoptosis in C-33A cells (Fig. 1F). In addition, western blot analysis demonstrated that the expression levels of the apoptosis-related proteins Bcl-2 and Birc5 were downregulated, while those of Bax were upregulated in C-33A cells following knockdown of PTK6 expression (Fig. 1G). These findings confirmed that the proliferation of PTK6-silenced C-33A cells was inhibited, while the induction of their apoptosis was promoted.

Lack of PTK6 expression mitigates cell invasion and migration in cervical cancer. To confirm the effects of siRNA-PTK6-1 on the invasive and migratory activities of cervical cancer cells, wound healing and Transwell assays were performed. The cell migratory (Fig. 2A) and invasive (Fig. 2B) capacities of the siRNA-PTK6 cell group prominently decreased compared with those of the siRNA-NC group. Furthermore, the expression levels of the migration-related proteins MMP12 and MMP9 were markedly reduced in the siRNA-PTK6 group compared with those noted in the siRNA-NC group (Fig. 2C and D). The results suggested that PTK6 reduction suppressed the invasive and migratory activities of C-33A cells.

Knockdown of PTK6 expression reduces GAB1 expression. To examine further the specific mechanism of action of PTK6, its potential binding to GAB1 was predicted via the Biogrid database. The predicted binding threshold of PTK6 and GAB1 was-16.55 kcal/mol (<-5) as determined by the PPA-Pred2 database (data not shown). RT-qPCR and western blot analyses demonstrated upregulated GAB1 expression in the cervical cancer cell lines SiHa, HeLa, Ca-Ski, and C-33A, which was consistent with the expression levels of PTK6 expression in cervical cancer cells (Fig. 3A and B). Co-IP assays indicated that in the IgG group, neither PTK6

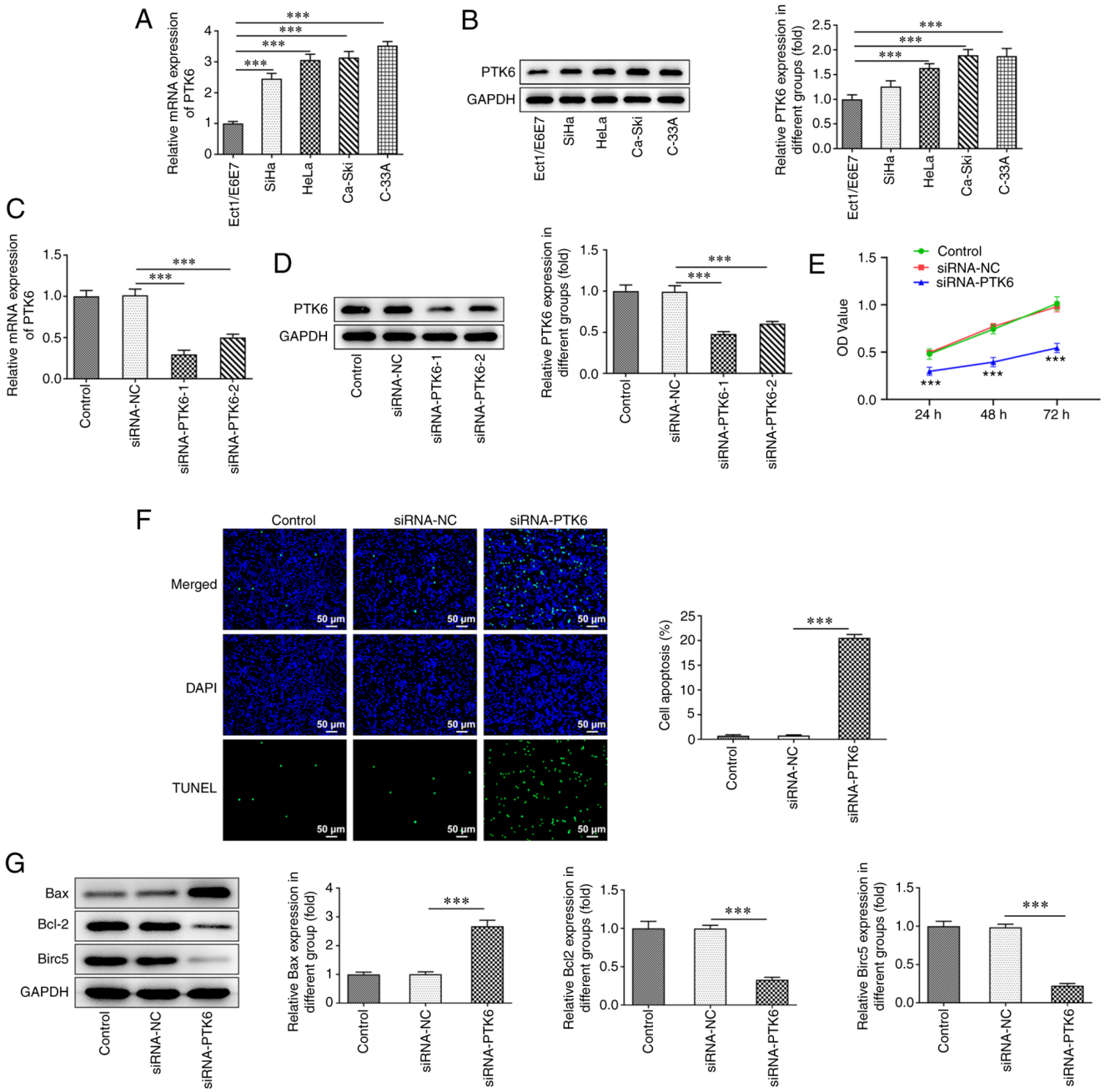

Figure 1. PTK6 expression is increased and PTK6 deficiency exacerbates cell apoptosis in cervical cancer. (A) RT-qPCR and (B) western blotting respectively assessed PTK6 expression in immortalized human normal cervical epithelial cells (Ect1/E6E7) and cervical cancer cell lines (SiHa, HeLa, Ca-Ski, C-33A). The interference effect of PTK6 was detected by (C) RT-qPCR and (D) western blotting, and the siRNA-PTK6-1 with more marked interference (as siRNA-PTK6) was selected for subsequent experiments. (E) C-33A cell proliferation was detected by CCK-8 assays. (F) TUNEL staining assays detected the apoptosis of C-33A cells. (G) Western blotting detected the protein levels of Bcl-2, Bax and Birc5 in C-33A cells. ***P<0.001 vs. Ect1/E6E7 or siRNA-NC. PTK6, protein tyrosine kinase 6; RT-qPCR, reverse transcription-quantitative PCR; siRNA, small interfering RNA; CCK-8, Cell Counting Kit-8; TUNEL, terminal deoxynucleotidyl-transferase-mediated dUTP nick end labeling; Birc5, baculoviral IAP repeat containing 5; NC, negative control.

nor GAB1 proteins were precipitated, indicating that these two proteins could not bind to IgG. However, in both the PTK6 and GAB1 groups, the GAB1 or PTK6 proteins were precipitated (Fig. 3C and D). These findings indicated a strong binding affinity between the PTK6 and GAB1 proteins. Furthermore, the protein and mRNA expression levels of GAB1 in the siRNA-PTK6 group were down-regulated compared with those in the siRNA-NC group, indicating a positive association between PTK6 and GAB1 (Fig. 3E and F).

Suppressive effects of knockdown of PTK6 expression on cervical cancer cell proliferation are reversed by elevation of GAB1 expression. To clarify whether PTK6 binds to GAB1 and to assess its involvement in the malignant progression of cervical cancer cells, an Ov-GAB1 plasmid was constructed and transfected into C-33A cells. GAB1 expression was successfully increased in the Ov-GAB1 group compared with that noted in the Ov-NC groups (Fig. 4A and B). In addition, following overexpression of GAB1, it was observed that C-33A cell viability (Fig. 4C)

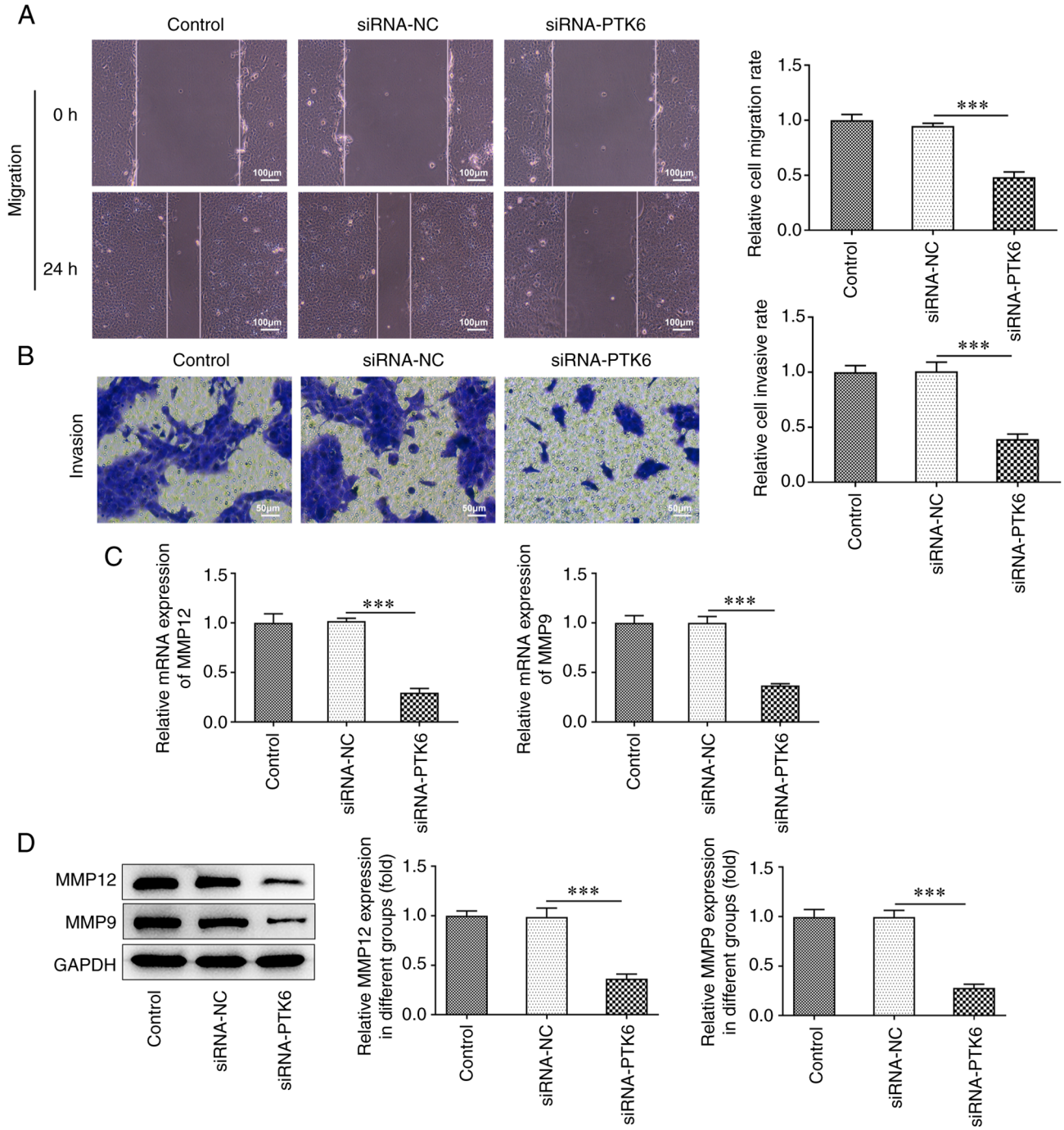

Figure 2. Silencing of PTK6 mitigates cervical cancer cell invasion and migration activities. (A) Representative images of wound healing assay (magnification, x100) and quantification of cell migration. (B) Representative images of Transwell assay (magnification, x100) and quantification of cell invasion. (C) RT-qPCR and (D) western blotting were used to assess MMP12 and MMP9 expression levels. ***P<0.001 vs. siRNA-NC. PTK6, protein tyrosine kinase 6; RT-qPCR, reverse transcription-quantitative PCR; MMP, matrix metalloproteinase; siRNA, small interfering RNA; NC, negative control.

was increased in the siRNA-PTK6 group, while the induction of apoptosis was reduced (Fig. 4D). Moreover, western blot analysis indicated an increase in the expression levels of Bcl-2 and Birc5 and a decrease in the expression levels of Bax in C-33A cells following GAB1 overexpression (compared with siRNA-PTK6 + Ov-NC; Fig. 4E).

Suppressive effects of the knockdown of PTK6 expression on cervical cancer cell migration and invasion are reversed following overexpression of GAB1 expression. Overexpression of GAB1 caused a partial reversal of the suppressive effects of PTK6 knockdown on the migration and invasion of C-33A cells (Fig. 5A and B). Moreover, the expression levels of MMP12 and MMP9 were increased in C-33A cells co-transfected with Ov-GAB1 and siRNA-PTK6 compared with those noted in the siRNA-PTK6 + Ov-NC group (Fig. 5C and D). In general, the overexpression of GAB1 could counteract the reduced migratory and invasive activities observed following knockdown of PTK6 expression in C-33A cells.

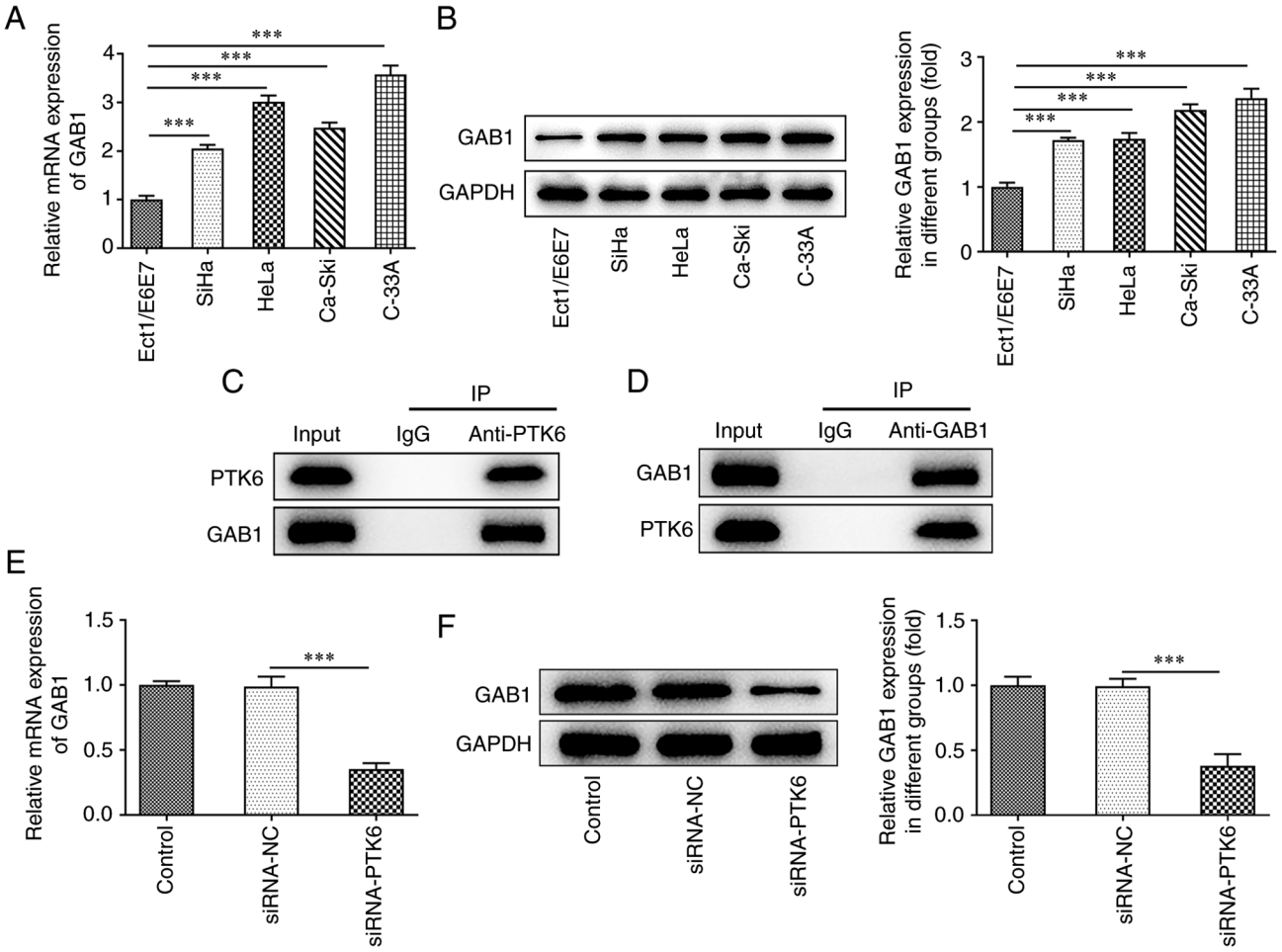

Figure 3. Silencing of PTK6 decreases GAB1 expression. (A) RT-qPCR and (B) western blotting, respectively, determined GAB1 expression in immortalized human normal cervical epithelial cells (Ect1/E6E7) and cervical cancer cell lines (SiHa, HeLa, Ca-Ski, C-33A). (C and D) Co-IP assay was performed using control IgG beads and immunoblotted for PTK6 and GAB1. The expression of GAB1 (E) mRNA and (F) protein levels following PTK6 interference in cervical cancer C-33A cells. ***P<0.001 vs. Ect1/E6E7 or siRNA-NC. PTK6, protein tyrosine kinase 6; GAB1, GRB2-associated binding 1; RT-qPCR, reverse transcription-quantitative PCR; Co-IP, co-immunoprecipitation; siRNA, small interfering RNA; NC, negative control.

Discussion

PTK6, a protein kinase, may act as an oncogene based on its high expression levels noted in a variety of cancer types, and has thus attracted considerable attention (21-23). Notably, strategies for pharmaceutical kinase inhibition have been clinically demonstrated and pursued in programs targeting some tumor types (24). Thus, in-depth studies of PTK6 signaling function would be critical for the future production or improvement of its inhibitors with clinical relevance. Increased expression of PTK6 has been reported to promote the development of prostate cancer through the activation of carcinogenic signaling pathways, which are involved in regulating cell growth, migration, and apoptosis (25). Dwyer *et al* (26) demonstrated that PTK6 was overexpressed in 86% of patients with breast cancer and correlated with tumor grade, while it was weakly expressed or undetectable in normal breast tissues (27). Wang *et al* (5) indicated that PTK6 was also overexpressed in cervical squamous cell carcinoma and was related to the short-term survival of these patients, implying that it may be associated with the prognosis of patients with cervical cancer. However, the specific mechanism by which PTK6 contributes to the development of cervical cancer has not yet been addressed. The present study revealed that PTK6 expression was increased in cervical cancer cells compared with that observed in human normal cervical epithelial cells (Ect1/E6E7). It should be noted that the study was selected in C-33A cells owing to the relatively high expression of PTK6 in C-33A cells. This was one of the limitations of the present study, and future studies will add cell lines for further validation. Moreover, in line with the results of Wang *et al* (5), the insufficient expression of PTK6 hindered C-33A cell proliferation, migration, and invasion. Therefore, it was tentatively hypothesized that targeting PTK6 may serve as a potential therapeutic strategy in the management of cervical cancer.

GAB1 is a multi-site docking protein containing multiple tyrosine residues (28); it can achieve binding to the non-receptor tyrosine kinase PTK6 and participate in PTK6 signal transduction events (14,15). Previous research on cervical cancer revealed that GAB1 expression was inhibited by the overexpression of the tumor suppressor gene miR-23b-3p, suggesting that this protein plays a similar role to PTK6 and that it is associated with poor prognosis of cervical cancer (19). Therefore, it was hypothesized that PTK6 may participate in the development of cervical cancer by binding to GAB1. In the present study, the Biogrid database (https://thebiogrid.

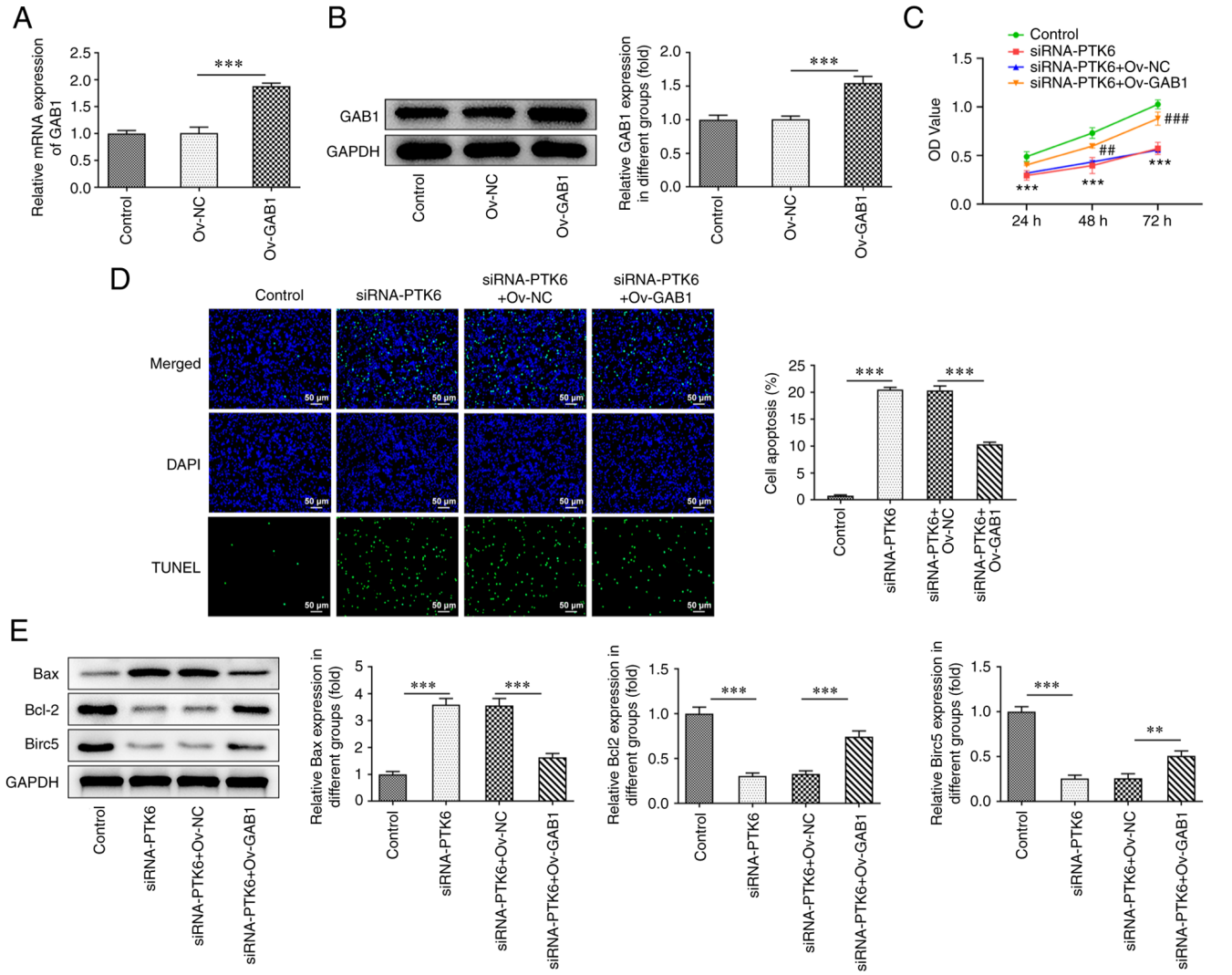

Figure 4. Overexpression of GAB1 reverses the inhibitory effect of PTK6 interference on the proliferation of cervical cancer cells. (A) RT-qPCR and (B) western blotting respectively, detected GAB1 expression in cervical cancer C-33A cells. ***P<0.001 vs. Ov-NC. (C) C-33A cell proliferation was detected by CCK-8 assays. ***P<0.001 vs. Control; ##P<0.01 and ###P<0.001 vs. siRNA-PTK6 + Ov-NC. (D) TUNEL staining assays detected the apoptosis of C-33A cells. (E) Western blotting was used to assess the levels of Bcl-2, Bax and Birc5 in C-33A cells. **P<0.01 and ***P<0.001 vs. Control or siRNA-PTK6 + Ov-NC. GAB1, GRB2-associated binding 1; PTK6, protein tyrosine kinase 6; RT-qPCR, reverse transcription-quantitative PCR; Ov, overexpression; NC, negative control; siRNA, small interfering RNA; TUNEL, terminal deoxynucleotidyl-transferase-mediated dUTP nick end labeling.

org/) predicted that PTK6 could bind to GAB1. Subsequently, *in vitro* cell experiments, demonstrated that depletion of PTK6 expression markedly ameliorated the proliferation, invasion, and migration of C-33A cells, while overexpression of GAB1 counteracted the PTK6-mediated inhibition of the aggressive phenotypes of C-33A cells, implying an indivisible association between them. Therefore, it was surmised that chemotherapeutic drugs would be an effective strategy to improve the treatment of cervical cancer by inhibiting the activation of PTK6 or GAB1 pathways. The present study proposed for the first time, to the best our knowledge, that the knockdown of PTK6 expression could promote the malignant phenotype of cervical cancer due to the activation of GAB1, providing a possible target for the clinical diagnosis and treatment of cervical cancer.

A previous study concerning hepatocellular carcinoma proposed that PSPC1 is responsible for the subcellular localization of PTK6/β-catenin tumor switch (29), and another study indicated that the ectopic expression of PTK6 facilitated the migration and metastasis of cancer cells via AKT activation (30). A main disadvantage of the present study is the lack of *in vivo* experiments that could verify the findings presented by the *in vitro* studies. Therefore, a mouse tumor-bearing model needs to be constructed to further investigate the effects of PTK6 on cervical cancer. This will be preceded by further clarification of the association between PTK6 expression and human papillomavirus.

In summary, PTK6 activated GAB1 to increase the proliferation, invasion, and migration of cervical cancer cells, which in turn promoted the malignant progression of cervical cancer. This suggests that PTK6 and GAB1 may be considered as prognostic or therapeutic markers for patients with cervical cancer. The identification of effective small molecule inhibitors for PTK6 and GAB1 is expected to improve the therapeutic efficacy of this treatment strategy against cervical cancer.

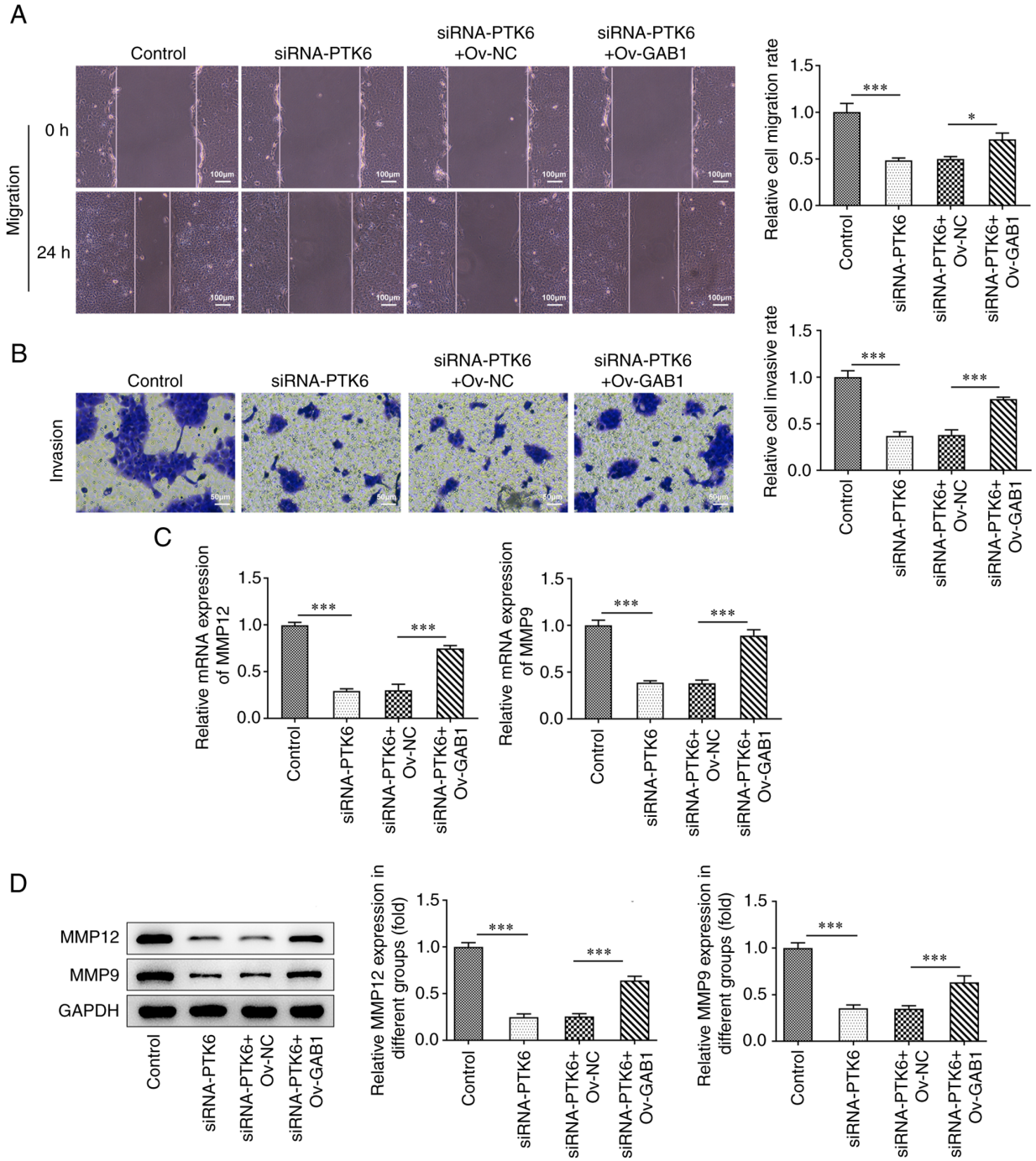

Figure 5. Overexpression of GAB1 reverses the inhibitory effect of PTK6 interference on the invasion and migration abilities of cervical cancer cells. (A) Representative images of wound healing assay (magnification, x100) and quantification of cell migration. (B) Representative images of Transwell assay (magnification, x100) and quantification of cell invasion. (C) RT-qPCR and (D) western blotting were used to assess MMP12 and MMP9 expression levels. *P<0.05 and ***P<0.001 vs. Control or siRNA-PTK6 + Ov-NC. GAB1, GRB2-associated binding 1; PTK6, protein tyrosine kinase 6; RT-qPCR, reverse transcription-quantitative PCR; MMP, matrix metalloproteinase; siRNA, small interfering RNA; Ov, overexpression; NC, negative control.

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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

JL, NY, XT, LO, MJ and SZ conceived and designed the study, as well as acquired and interpreted the data. JL was a

major contributor in writing the manuscript. JL, NY and SZ confirm the authenticity of all the raw data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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