

Effect of integrin $\alpha 7$ on cell proliferation, invasion, apoptosis and the PI3K/AKT pathway, and its association with clinicopathological features in endometrial cancer

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Abstract. Targeting integrin $\alpha 7$ (ITGA7) suppresses malignant progression of several types of cancer, including tongue squamous cell carcinoma, hepatocellular carcinoma and non-small cell lung cancer, while the effect of its knockdown on cell function and its association with clinicopathological features in endometrial cancer (EC) is unclear. The present study aimed to investigate this issue. ITGA7 was knocked down by short-interfering (si)RNA in Ishikawa and RL95-2 cells followed by western blotting and reverse transcription-quantitative PCR assays. Subsequently, cell proliferation, apoptosis, invasion and expression levels of PI3K, phosphorylated (p-) PI3K, AKT and p-AKT were determined using Cell Counting Kit-8, TUNEL, Transwell assays and western blotting. Moreover, ITGA7 in tumor and adjacent tissues from 50 patients with endometrial cancer was detected using immunohistochemical assay. ITGA7 expression was increased in EC cell lines (HEC-1A, RL95-2, Ishikawa and KLE) compared with telomerase-immortalized human endometrial stromal cells (THESCs). In both Ishikawa and RL95-2 cells, three ITGA7 siRNAs all demonstrated good efficiency on ITGA7 knockdown, amongst which the one with the highest efficiency was selected for the following experiments. ITGA7 knockdown reduced cell proliferation and invasion, while inducing apoptosis; moreover, it suppressed p-PI3K/PI3K and p-AKT/AKT ratios. In patients with EC, ITGA7 expression was increased in tumor tissues compared with adjacent tissues, and its lower tumor expression was associated with myometrial invasion ($<1/2$), non-lymphovascular invasion and decreased FIGO stage. In conclusion, ITGA7 knockdown repressed

proliferation, invasion and the PI3K/AKT pathway while inducing apoptosis in EC cell lines, and its insufficiency was associated with less advanced tumor features in EC patients. These results indicated that ITGA7 may be a potential target for the treatment of EC.

Introduction

Endometrial cancer (EC) is a common gynecological malignancy that endangers the lives of women worldwide (1,2). Meanwhile, the incidence of EC is increasing, not only in developed countries but also in developing ones, which renders EC an enormous threat to public health (3,4). It is proposed that obesity, diabetes, polycystic ovary syndrome and Lynch syndrome are risk factors for EC; molecular abnormalities such as PI3K/AKT and Wnt/ β -catenin pathway mutations are also highly associated with EC (5-8). Currently, treatment strategies for EC mainly include surgical resection, radiotherapy, hormone therapy and immunotherapy (9,10); while efforts have never stopped in the search for novel treatment targets of EC.

Integrins are a group of vital proteins that regulate cell adhesion and signaling transduction, among which integrin $\alpha 7$ (ITGA7) is involved in the pathogenesis and progression of several types of cancer (11,12). For example, ITGA7 knockdown suppresses cell proliferation, induces apoptosis, reduces CD44 and CD133 expression levels as well as decreases sensitivity to cisplatin in tongue squamous cell carcinoma cell lines (13). Another study revealed that ITGA7 modulates cell proliferation, apoptosis and stemness through the PI3K/AKT pathway in hepatocellular carcinoma (14). Moreover, a previous study also revealed how important the regulation of ITGA7 is for cell function in non-small cell lung cancer (15). However, regarding gynecological malignancy, only one previous study demonstrated that ITGA7 is downregulated in high grade serous ovarian cancer tissues compared with that of normal tissues (16). Based on the aforementioned information, it was hypothesized that ITGA7 may also be involved in the pathogenesis and progression of EC.

The present study aimed to investigate the effect of ITGA7 knockdown on cell proliferation, apoptosis, invasion and its potential downstream pathway in EC cell lines, then explore

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its association with clinicopathological features in patients with EC.

Materials and methods

Cell source and culture conditions. Telomerase-immortalized human endometrial stromal cells (THESCs) and human EC cell lines (including HEC-1A, RL95-2, Ishikawa and KLE) were purchased from the American Type Culture Collection or European Collection of Authenticated Cell Cultures. THESCs, RL95-2 and KLE cells were cultured in DMEM/F-12 (Gibco; Thermo Fisher Scientific, Inc.) containing 10% fetal bovine serum (FBS; Gibco; Thermo Fisher Scientific, Inc.). HEC-1A cells were cultured in Eagle's medium (Gibco; Thermo Fisher Scientific, Inc.) containing 15% FBS (Gibco; Thermo Fisher Scientific, Inc.). Ishikawa cells were cultured in MEM (MilliporeSigma) containing 5% FBS (Gibco; Thermo Fisher Scientific, Inc.). The incubation conditions were 5% CO₂, 37°C for all the cells.

Detection of ITGA7 expression in EC cell lines and THESCs. The ITGA7 mRNA and protein relative expression levels in THESCs and EC cell lines (including HEC-1A, RL95-2, Ishikawa and KLE) were analyzed using reverse transcription-quantitative PCR (RT-qPCR) and western blot assays, respectively, as described below.

Transfection of short interfering (si)RNAs. ITGA7 siRNAs (si-ITGA7) and scrambled siRNA as a negative control (si-NC) were purchased from Generay Biotech Co., Ltd. Ishikawa, RL95-2 cells and THESCs were transfected with 50 nM si-ITGA7 and 50 nM si-NC using HilyMAX reagent (Invitrogen; Thermo Fisher Scientific, Inc.), respectively, based on the manufacturer's protocol. The siRNA sense sequences were as follows: Si-ITGA7-1, 5'-CAGCUACUU UGGCUCUCUUU-3'; si-ITGA7-2, 5'-CAGCUACUUUGG CUUCUCUUU-3'; si-ITGA7-3, 5'-GGGUCUGUUUCAGCU ACAUUU-3'; si-NC, 5'-GAAUUAUUAAGAUGGCCCG UUGUACU-3'.

RT-qPCR. THESCs, Ishikawa and RL95-2 cells were harvested at 48 h after transfection. Total RNA of each group was extracted using PureZOL RNA isolation reagent (Bio-Rad Laboratories, Inc.). Qubit® 4 Fluorometer (Invitrogen; Thermo Fisher Scientific, Inc.) was used for analyzing the RNA concentration. Reverse transcription of RNA was performed using QuantiNova Reverse Transcription Kit in accordance with the manufacturer's protocol (Qiagen GmbH). qPCR was performed using QuantiNova SYBR® Green PCR kit (Qiagen GmbH), and the following thermal cycles were conducted: 95°C For 2 min, 1 cycle; 95°C for 5 sec; and 61°C for 30 sec, 40 cycles. Primers were obtained from Sangon Biotech Co., Ltd. The primer sequences were listed as follows: ITGA7 Forward, 5'-GCCACTCTGCCTGTCCAATG-3', and reverse, 5'-GGA GGTGCTAAGGATGAGGTAGA-3'. GAPDH forward, 5'-GAGTCCACTGGCGTCTTCAC-3', and reverse, 5'-ATC TTGAGGCTGTTGTCATACTTCT-3'. ITGA7 mRNA expression was analyzed using the 2^{-ΔΔC_q} calculation, with GAPDH as an internal control (17).

Western blotting. At 48 h after transfection, Ishiwaka and RL95-2 cells were harvested and lysed in RIPA lysis buffer containing Protease Inhibitor Cocktail at 1x10⁷ cells per 200 μ l (MilliporeSigma) for protein extraction, based on the manufacturer's protocol. A BCA protein concentration quantification kit (Beyotime Institute of Biotechnology) was used for measuring the protein concentration in each group. A total of 25 μ g proteins of each group were boiled at 98°C for 5 min and proteins were separated using 10% NuPAGE Bis-Tris Gels (Thermo Fisher Scientific, Inc.) and transferred into nitrocellulose membrane (Beijing Solarbio Science and Technology Co., Ltd.). The membranes were blocked with 5% BSA (Thermo Fisher Scientific, Inc.) for 1.5 h at 37°C, and then incubated with the ITGA7, AKT, p-AKT, PI3K, p-PI3K, cleaved-caspase 3 (C-caspase 3) and GAPDH primary antibodies for 1.5 h at 37°C, respectively. Subsequently, the membranes were incubated with the secondary antibodies for 50 min at 37°C. Finally, the membranes were reacted with Pierce™ ECL Plus Western Blotting Substrate Thermo Fisher Scientific, Inc. for chemiluminescence. The source and dilution of antibodies are presented in Table I.

Cell proliferation assay. Cell proliferation of Ishiwaka and RL95-2 cells after ITGA7 interference was performed using Cell Counting Kit-8 (CCK-8; Dojindo Laboratories, Inc.). In short, Ishiwaka and RL95-2 cells were seeded on a 96-well plate (4x10³ cells in 100 μ l medium) and incubated overnight. At 0, 24, 48 and 72 h after transfection, 10 μ l CCK-8 detection solution was added and incubated for 2 h at 37°C, respectively. Optical density (OD) value was measured using an Automated Enzyme Immunoassay Analyzer AIA-900 (Tosoh Corporation) at 450 nm.

Apoptosis assay. TUNEL apoptosis detection kit (Beyotime Institute of Biotechnology) was used for analyzing Ishiwaka and RL95-2 apoptosis after ITGA7 interference. In brief, at 48 h after transfection, Ishiwaka and RL95-2 cells were fixed with 4% paraformaldehyde fix solution (Beyotime Institute of Biotechnology) at 700 μ l/well for 0.5 h. Triton X-100 solution (Beyotime Institute of Biotechnology) was added to cells (200 μ l) for 10 min. Subsequently, cells were blocked in 5% BSA (Thermo Fisher Scientific) in TBST (0.05% Tween-20) for 0.5 h. Cells were incubated with TUNEL apoptosis detection solution (Beyotime Institute of Biotechnology) for 1 h. Antifade mountant (Beyotime Institute of Biotechnology) was used to reduce fluorescence quenching. All procedures of TUNEL assay were carried out at room temperature. Fluorescent images of three random fields were captured and analyzed using a fluorescence microscope (Olympus Corporation).

Cell invasion assay. Transwell assay was used for analyzing Ishiwaka and RL95-2 cell invasion after ITGA7 interference with Matrigel-plated Transwell insert (Corning, Inc.). The Matrigel-plated Transwell insert (Corning, Inc.) were precoated with Matrigel (BD Bioscience) at 37°C for 1 h. Briefly, at 48 h after transfection, 4x10⁴ Ishiwaka and RL95-2 cells in serum-free medium (Gibco; Thermo Fisher Scientific, Inc.) were added into the upper Matrigel-plated Transwell insert, and the lower wells contained 600 μ l corresponding

Table I. Antibodies applied in western blotting.

Antibody	Company	Cat. no.	Dilution
Primary antibodies			
ITGA7 mouse mAb	Santa Cruz Biotechnology, Inc.	sc-81807	1:800
AKT rabbit mAb	Cell Signaling Technology, Inc.	#4691	1:1,000
p-AKT rabbit mAb	Cell Signaling Technology, Inc.	#4060	1:1,000
PI3K rabbit mAb	Cell Signaling Technology, Inc.	#4257	1:1,000
p-PI3K rabbit mAb	Cell Signaling Technology, Inc.	#17366	1:1,000
Cleaved caspase 3 rabbit mAb	Cell Signaling Technology, Inc.	#9664	1:1,000
GAPDH mouse mAb	Abcam	ab9484	1:2,000
Secondary antibodies			
Goat anti-mouse IgG-HRP	Abcam	ab6789	1:4,000
Goat anti-rabbit IgG H&L (HRP)	Cell Signaling Technology, Inc.	#7074	1:4,000

p-, phosphorylated; HRP, horseradish peroxidase; ITGA7, integrin $\alpha 7$.

complete medium [MEM (MilliporeSigma) containing 5% FBS for Ishiwaka cells or DMEM/F-12 (Gibco; Thermo Fisher Scientific, Inc.) containing 10% FBS for RL95-2 cells]. After 24 h at 37°C, the non-migrated cells were gently removed. Subsequently, migrated cells were fixed with 20% methanol for 20 min at room temperature, followed by staining with crystal violet staining solution (Beyotime Institute of Biotechnology) for 5 min at room temperature. Images of stained cells were captured and cells were counted by investigators using an inverted fluorescence microscope (Olympus Corporation).

EC tissue sample detection. To further confirm the association of ITGA7 with EC tumor features, 50 female patients (mean age, 63.9±10.1 years; median age, 64.5 years; age range, 39-80 years) with primary EC who underwent resection between November 2019 and June 2021 were retrospectively analyzed after ethical approval by Union Hospital, Tongji Medical College of Huazhong University of Science and Technology (Wuhan, China). The inclusion criteria were: i) Pathologically diagnosed as primary EC; ii) received tumor resection; and iii) tumor tissue was accessible for immunohistochemistry (IHC) assay. The exclusion criteria were: i) History or complicated with other primary cancers; ii) received neoadjuvant therapy; and iii) pregnant or lactating women. Written informed consents were received from all the patients/guardians. Myometrial invasion $\geq 1/2$ or $<1/2$ indicated the depth of myometrial invasion (10). Paraffin-embedded tumor tissues and adjacent tissues (within 2-cm next to tumor tissues) were acquired and IHC was used to detect ITGA7 expression, and the ITGA7 IHC score was calculated. The IHC assay and scoring method referred to a previous study (13). In brief, the tissues were fixed by 4% paraformaldehyde (Sangon Biotech Co., Ltd.) for 24 h at 4°C. The tissues were then embedded in paraffin (Sangon Biotech Co., Ltd.) and cut into 4- μ m sections. The sections were then deparaffined in xylene, rehydrated in descending alcohol series and antigen retrieved in 98°C citric acid buffer for 3 min. The sections were subsequently blocked by 5% BSA (Sangon Biotech Co., Ltd.) at 37°C for 20 min after incubating in 3% H₂O₂ for 15 min. Next, the

sections were incubated with ITGA7 antibody (dilution rate, 1:150; cat. no. ab203254; Abcam) at 4°C overnight followed by incubating with goat anti-rabbit IgG H&L (HRP; dilution rate, 1,000; cat. no. #7074; Cell Signaling Technology, Inc.) at 37°C for 1 h. The sections were stained by 3,3'-Diaminobenzidine (Sangon Biotech Co., Ltd.) for 10 min and counterstained by hematoxylin (Sangon Biotech Co., Ltd.) for 2 min, at last, at room temperature. The images with a magnification of x200 were captured using a light microscope (Motic China Group Co., Ltd.) and analyzed by two independent pathologists. The total score method was calculated by multiplying by intensity score and percentage score of stained cells. The intensity score was as follows: 0, No staining; 1, weak staining, light yellow; 2, moderate staining, yellow brown; and 3, strong staining, brown. The percentage score of stained cells was as below: 0, 0; 1, 1-25; 2, 26-50; 3, 51-75; and 4, 76-100% positive cells.

Statistical analysis. GraphPad Prism Software (version 7.0; GraphPad Software, Inc.) was used for statistical analysis and graph plotting in all assays. For cell experiments, data were presented as mean with standard deviation (SD); multigroup comparison was analyzed using one-way ANOVA followed by Dunnett's or Tukey's multiple comparisons test. For clinical investigation, data were presented as median with interquartile range (IQR); two-group comparison was analyzed using Mann-Whitney U test or Wilcoxon signed-rank test. $P<0.05$ was considered to indicate a statistically significant difference.

Results

ITGA7 is overexpressed in EC cell lines. The mRNA and protein levels of ITGA7 in THESCs and EC cell lines were detected using RT-qPCR and western blotting, respectively. Data demonstrated that ITGA7 mRNA expression was significantly enhanced in EC cell lines (including HEC-1A, RL95-2, Ishikawa and KLE) compared with THESCs (all $P<0.01$; Fig. 1A); meanwhile, the ITGA7 protein expression was also significantly increased in EC cell lines compared with THESCs (all $P<0.05$; Fig. 1B and C). RL95-2 and Ishikawa

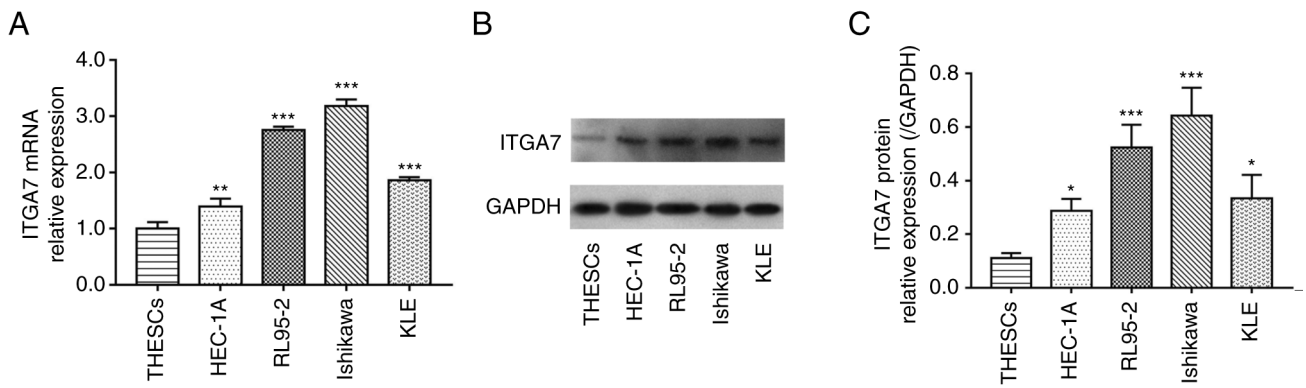


Figure 1. ITGA7 expression in THESCs and EC cell lines. (A) ITGA7 mRNA level; (B) Representative images of ITGA7 protein levels as detected by western blot analysis; (C) Grayscale analyses of quantified blots of ITGA7 protein level. * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$ compared with THESCs. ITGA7, integrin $\alpha 7$; EC, endometrial cancer; THESCs, telomerase-immortalized human endometrial stromal cells.

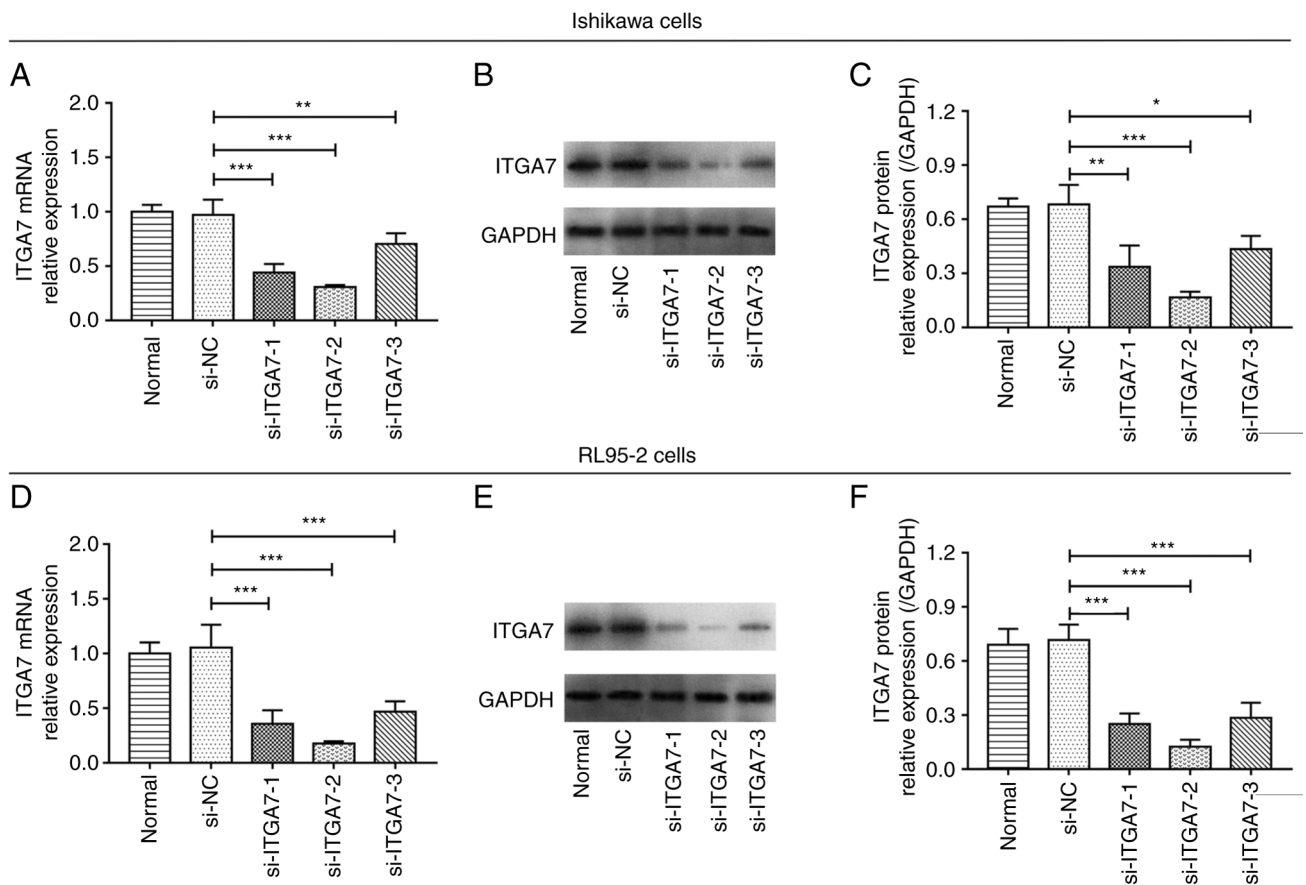


Figure 2. ITGA7 expression after transfection in Ishikawa and RL95-2 cells. (A) ITGA7 mRNA level after transfection in Ishikawa cells. (B) Representative images of ITGA7 protein level detection by western blot after transfection in Ishikawa cells. (C) Grayscale analyses of blots of quantified ITGA7 protein levels after transfection in Ishikawa cells. (D) ITGA7 mRNA level after transfection in RL95-2 cells. (E) Representative images of ITGA7 protein level detection by western blot after transfection in RL95-2 cells. (F) Grayscale analyses of blots of quantified ITGA7 protein levels after transfection in RL95-2 cells. * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$. ITGA7, integrin $\alpha 7$; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; si-, short interfering; NC, negative control.

cell lines were selected for further knockdown experiments due to the fact that ITGA7 was most significantly increased in these two cell lines. These data suggested that ITGA7 was highly expressed in EC cells.

ITGA7 knockdown efficiency. Subsequently, in order to explore the effect of ITGA7 on EC cell function and its downstream pathways, siRNA transfection was conducted.

In both Ishikawa and RL95-2 cell lines, it was revealed that all three si-ITGA7 plasmids significantly suppressed ITGA7 mRNA and protein levels compared with the si-NC (all $P < 0.05$; Fig. 2A-F). Furthermore, the expression of ITGA7 in cells transfected with si-ITGA7-2 had the lowest ITGA7 level, indicating that si-ITGA7-2 presented the most effective knockdown efficiency. Therefore, it was used in further experiments.

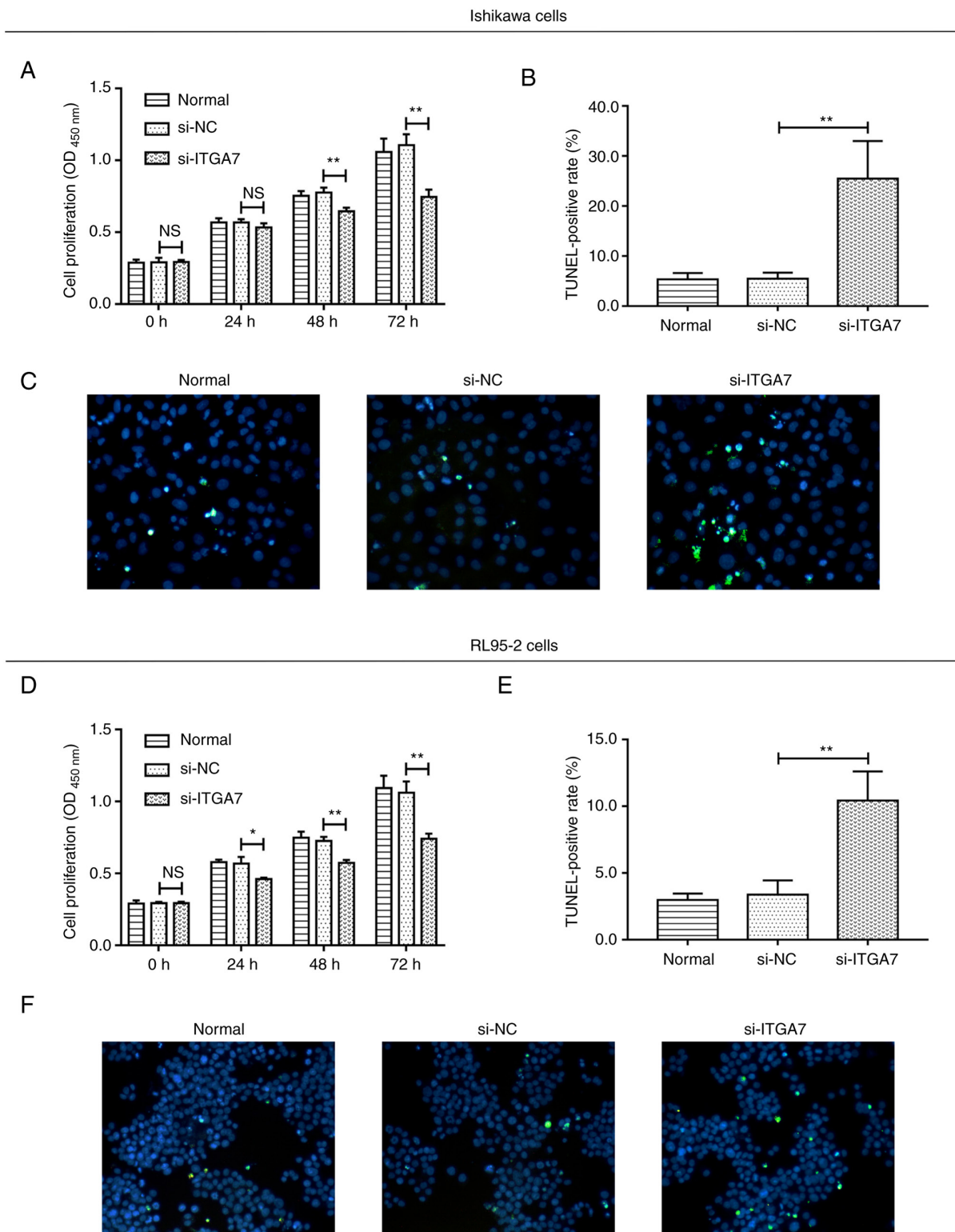


Figure 3. Proliferation and apoptosis after transfection in Ishikawa and RL95-2 cells. (A) Proliferation after transfection in Ishikawa cells. (B) TUNEL-positive rate after 48 h of transfection in Ishikawa cells (magnification, x200). (C) Representative images of TUNEL analysis after transfection in Ishikawa cells. (D) Proliferation after transfection in RL95-2 cells; (E) TUNEL-positive rate after transfection in RL95-2 cells. (F) Representative images of TUNEL analysis after 48 h of transfection in RL95-2 cells (magnification, x200). * $P < 0.05$ and ** $P < 0.01$. ITGA7, integrin $\alpha 7$; NC, negative control; NS, not significant; si-, short interfering; OD, optical density.

Si-ITGA7 suppresses proliferation and invasion but promotes apoptosis in EC cell lines. The effect of ITGA7 knockdown on EC cell functions, including proliferation, apoptosis and invasion, were investigated. In Ishikawa cells,

compared with the si-NC group, si-ITGA7 significantly suppressed cell proliferation at 48 and 72 h (both $P < 0.01$; Fig. 3A) and invasion ($P < 0.05$; Fig. 4A and B), while it promoted apoptosis ($P < 0.01$; Fig. 3B and C). Meanwhile,

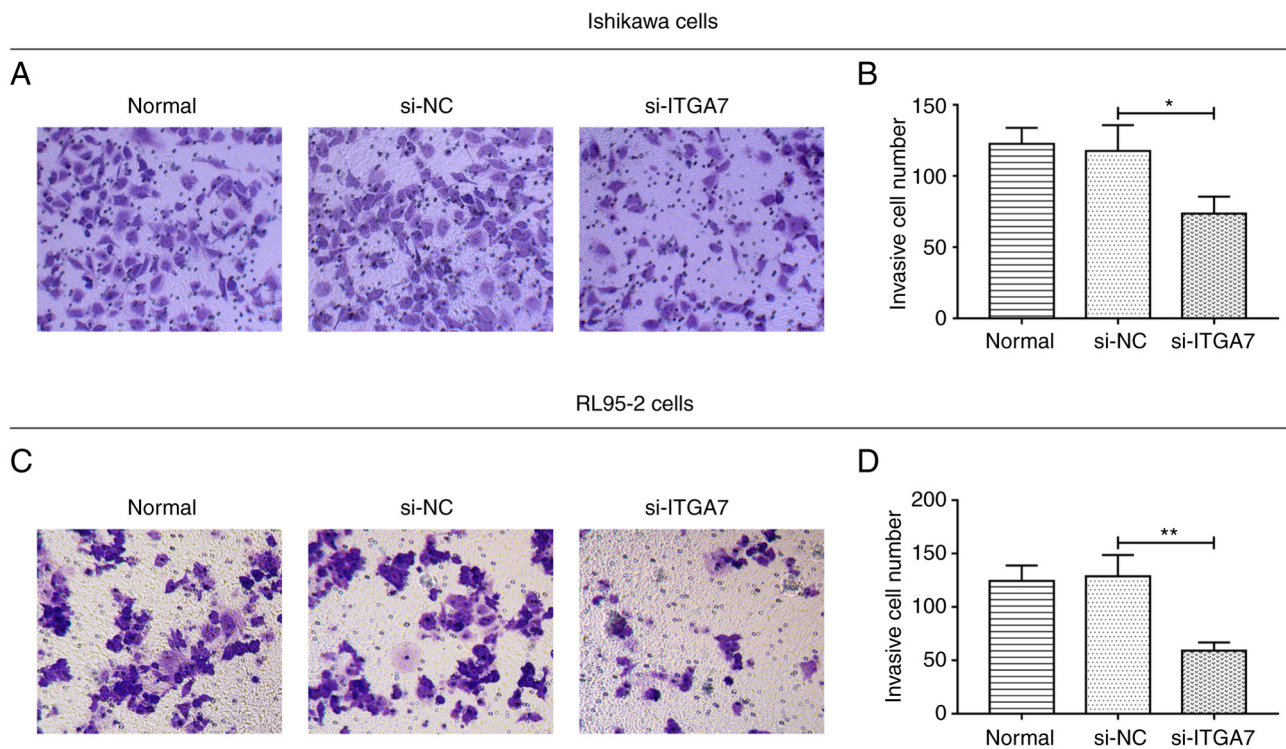


Figure 4. Invasion after transfection in Ishikawa and RL95-2 cells. (A) Representative images of invasion assay after transfection in Ishikawa cells (magnification, x200). (B) Invasive cell number after transfection in Ishikawa cells. (C) Representative images of invasion assay after transfection in RL95-2 cells (magnification, x200). (D) Invasive cell number after transfection in RL95-2 cells. * $P < 0.05$ and ** $P < 0.01$. ITGA7, integrin $\alpha 7$; NC, negative control; si-, short interfering.

in RL95-2 cells, si-ITGA7 also significantly inhibited cell proliferation at 24, 48 and 72 h (all $P < 0.05$; Fig. 3D) as well as invasion ($P < 0.01$; Fig. 4C and D), but it significantly increased apoptosis ($P < 0.01$; Fig. 3E and F). C-caspase 3 determined by western blotting also confirmed that si-ITGA7 elevated apoptosis compared with si-NC in Ishikawa (grayscale density, 0.556 ± 0.072 vs. 0.386 ± 0.049) and RL95-2 (grayscale density, 0.552 ± 0.049 vs. 0.344 ± 0.050) cells (Fig. S1A and B). These data revealed that knockdown of ITGA7 suppressed proliferation and invasion but promoted apoptosis in EC cells.

Additionally, si-ITGA7 was transfected into THESCs, in which transfection efficiency was demonstrated using RT-qPCR and western blotting (Fig. S2A-C). Subsequently, CCK-8, TUNEL and Transwell assays indicated that si-ITGA7 had a decreased effect on proliferation, apoptosis and invasion in THESCs, while these effects were minor and not significant (Fig. S2D-G).

Si-ITGA7 inactivates PI3K/AKT pathway. In addition, the implication of ITGA7 knockdown on the PI3K/AKT pathway in EC cell lines was also assessed. In both Ishikawa and RL95-2 cells, p-PI3K and p-AKT were reduced by si-ITGA7; however, PI3K and AKT were not influenced by si-ITGA7 (Fig. 5A). Further grayscale analyses demonstrated that the ratio of p-PI3K to PI3K and that of p-AKT to AKT were both significantly reduced by si-ITGA7 compared with the si-NC group in Ishikawa and RL95-2 cells (all $P < 0.01$; Fig. 5B). These data illustrated that ITGA7 knockdown inactivated the PI3K/AKT pathway in EC cells.

Reduced tumor ITGA7 is associated with less advanced tumor features in patients with EC. In order to further verify the aforementioned data in clinical setting, 50 patients with EC were enrolled. The patients had a mean age of 63.9 ± 10.1 years; 32% patients had myometrial invasion $> 1/2$, 20% patients had cervical invasion and 26% patients had lymphovascular invasion. Regarding the International Federation of Gynecology and Obstetrics (FIGO) stage, 60, 14, 22 and 4% of patients were of stage I, II, III and IV, respectively. ITGA7 levels in tumor and adjacent tissues from these patients with EC were detected using IHC. Data demonstrated that ITGA7 was elevated in tumor tissues compared with adjacent tissues ($P < 0.001$; Fig. 6A). Moreover, a lower tumor ITGA7 IHC score was associated with the absence of myometrial invasion $\geq 1/2$ ($P < 0.05$; Fig. 6B) and lymphovascular invasion ($P < 0.01$; Fig. 6D), as well as FIGO stage I/II vs. III/IV ($P < 0.01$; Fig. 6E), but not stromal invasion ($P > 0.05$; Fig. 6C). These data suggested that lower level of tumor ITGA7 was associated with less advanced tumor burden in patients with EC.

Discussion

ITGA7 is regarded as an important regulator for carcinogenesis and progression, and its dysregulation has been revealed in several types of cancer. For example, previous studies have revealed that ITGA7 is increased in colorectal cancer cell lines, hepatocellular carcinoma cell lines and non-small cell cancer cell lines (14,15,18). However, to the best of our knowledge, it has not yet been reported whether ITGA7 is also upregulated in EC cell lines. Therefore, the present study was conducted and

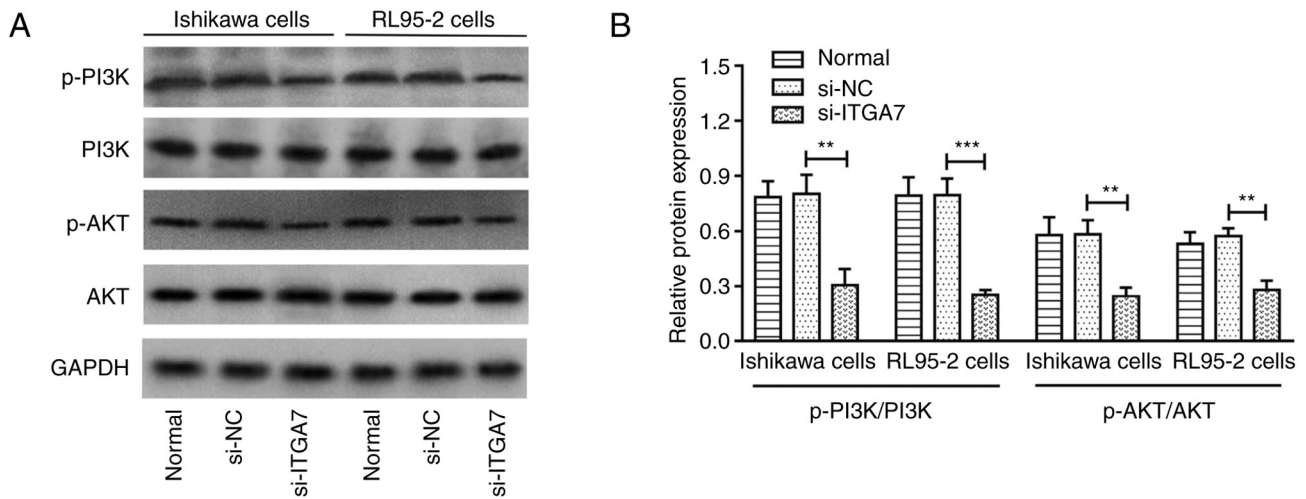


Figure 5. PI3K/AKT pathway after transfection in Ishikawa and RL95-2 cells. (A) Representative images of PI3K, p-PI3K, AKT, p-AKT detection by western blot analysis after transfection. (B) Grayscale analyses of blots of PI3K, p-PI3K, AKT, p-AKT levels after transfection. ** $P < 0.01$ and *** $P < 0.001$. ITGA7, integrin $\alpha 7$; NC, negative control; PI3K, phosphatidylinositol 3-kinase; AKT, protein kinase B; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; si-, short interfering; p-, phosphorylated.

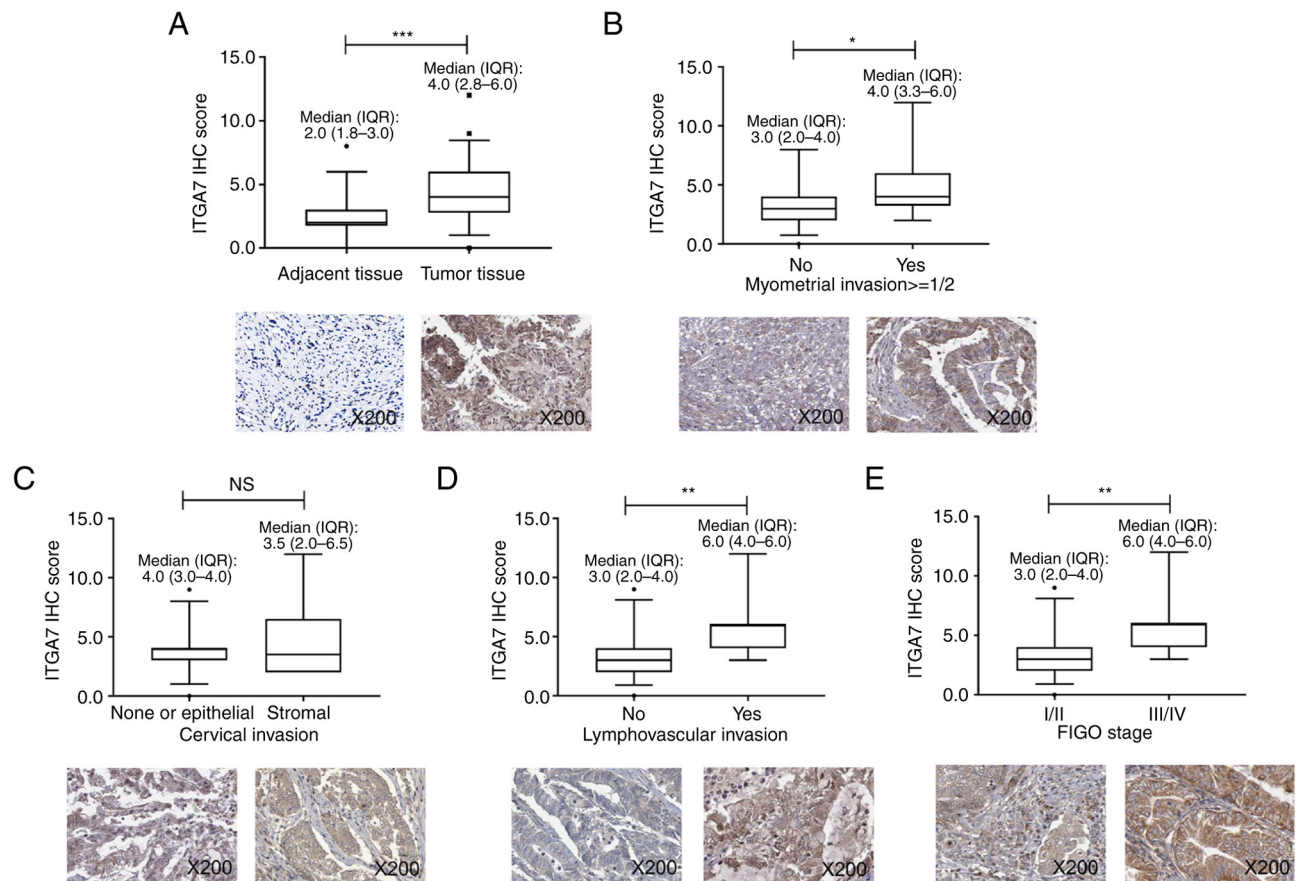


Figure 6. ITGA7 expression and its association with clinical characteristics in patients with EC. (A) Comparison of ITGA7 IHC score in tumor and adjacent tissues. Comparison of ITGA7 IHC score (B) in patients with myometrial invasion $\geq 1/2$ vs. those with myometrial invasion $< 1/2$, (C) in patients with cervical invasion vs. those without that, (D) in patients with lymphovascular invasion vs. those without that and (E) among patients with different FIGO stage. All images were captured with x200 of magnification. * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$. ITGA7, integrin $\alpha 7$; EC, endometrial cancer; IHC, immunohistochemical; FIGO, International Federation of Gynecology and Obstetrics.

revealed that ITGA7 expression was significantly enhanced in EC cell lines (including HEC-1A, RL95-2, Ishikawa and KLE) compared with THESCs. A possible explanation for these data

might be that high levels of ITGA7 could alter the activation of several signaling pathways that are associated with tumorigenesis, such as the PI3K/AKT, Wnt/ β -catenin and Ras

pathways (18-20), to increase the malignant proliferation of endometrial stromal cells and increase EC genesis. Therefore, ITGA7 upregulation was noted in EC cell lines compared with THESCs.

According to previous studies, ITGA7 modulates cell function, including proliferation, apoptosis, stemness and chemosensitivity, in several types of cancer, such as tongue squamous cell carcinoma, hepatocellular carcinoma and non-small cell lung cancer (13-15). The effect of ITGA7 on EC cell function has yet to be elucidated. The present study discovered that ITGA7 knockdown reduced cell proliferation and invasion, but enhanced apoptosis in EC cell lines Ishikawa and RL95-2. Possible explanations for these data may be: i) Low ITGA7 expression can regulate the activation of several proliferation/apoptosis-related pathways, such as the PI3K/AKT pathways (as shown by western blotting) and Ras pathway [as in colorectal cancer (18)]. Therefore, ITGA7 knockdown suppressed proliferation while promoting apoptosis in EC cell lines Ishikawa and RL95-2. Or ii) low ITGA7 expression may suppress several invasion-related mechanisms including the Wnt/ β -catenin pathway and epithelial-mesenchymal transition [as in hepatocellular carcinoma (21)]. Therefore, ITGA7 knockdown repressed invasion in EC cell lines Ishikawa and RL95-2.

The PI3K/AKT pathway is highly involved in cell proliferation, survival and invasion (22). However, dysregulation of the PI3K/AKT pathway is also a common phenomenon in human cancer (23). Meanwhile, it is also proposed that the PI3K/AKT pathway is one of the most common pathways that is altered in EC: The molecular spectrum of EC suggests that the mutation rates of PI3KCA, PIK3R1 and AKT1 are 59.7, 33 and 3.2 % respectively in all cases (22,24). Previous studies illustrate that inhibiting the PI3K/AKT pathway greatly suppresses the proliferation, migration and invasion of EC cell lines (25,26). Moreover, it is reported that ITGA7 regulates PI3K/AKT (14). Therefore, the effect of ITGA7 on the PI3K/AKT pathway in EC cell lines was investigated in the present study. The data demonstrated that ITGA7 knockdown inhibited the phosphorylation of PI3K and AKT. These data were in line with a previous study, suggesting that inhibiting ITGA7 also represses phosphorylation of the PI3K/AKT pathway in hepatocellular carcinoma cell lines (14). Although the present study discovered some notable findings, it is necessary to conduct *in vivo* experiments in the future to further investigate the effect of ITGA7 on EC progression using xenograft mice.

In order to further verify the *in vitro* findings, the present study enrolled patients with EC and detected ITGA7 in their tumor and adjacent tissues. Data demonstrated that the expression levels of ITGA7 were higher in tumor tissues compared with in adjacent tissues, which was in line with the aforementioned data that ITGA7 was elevated in EC cell lines compared with THESCs. Moreover, the present study revealed that tumor ITGA7 was negatively associated with myometrial invasion ($\geq 1/2$), lymphovascular invasion and FIGO stage. These data could be explained by: i) ITGA7 Knockdown reduced invasion of EC cells, and thus directly decreased myometrial and lymphovascular invasion; or ii) ITGA7 knockdown might inactivate several pathways including the PI3K/AKT and Wnt/ β -catenin pathways (14,20) to decrease proliferation and invasion of EC cells, which could indirectly result in lower

FIGO stage. ITGA7 was not associated with cervical invasion, which could be explained by low statistical power due to the small sample size (N=50). However, the prognostic value of ITGA7 should be investigated in the future.

Collectively, ITGA7 knockdown represses proliferation, invasion and the PI3K/AKT pathway while inducing apoptosis in EC cell lines. Its insufficiency is associated with less advanced tumor features in patients with EC. These indicate that ITGA7 may be a potential target for the treatment of EC.

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Availability of data and materials

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

Authors' contributions

JM contributed to the conception and design of the study. ML contributed to performing the experiments. CL contributed to data acquisition and analysis. TL and SG contributed to the analysis and interpretation of data. JM and ML 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

This study was approved by the ethics approval of Union Hospital, Tongji Medical College of Huazhong University of Science and Technology. Written informed consents were received from all the patients.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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