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LIMA1 inhibits cisplatin resistance and malignant biological behavior of bladder cancer cells by suppressing the Wnt/β-catenin pathway

Zhong Lv[1,2,3†], Suchen Zhao[1,2,3†] and Haoran Wu[1,2,3*]

Abstract

Objective This study aimed to explore the effect of LIM domain and actin binding 1 (LIMA1) on bladder cancer (BCa) cells and to investigate its underlying molecular mechanisms.

Methods The expression of LIMA1 gene in clinical BCa tissue samples and BCa cell models was detected using real-time quantitative PCR and western blot. Subsequently, LIMA1 knockdown experiments were performed exclusively in the BCa J82 cell line, while LIMA1 overexpression was conducted only in the cisplatin-resistant J82/CR cell line. The proliferation of the cells was assessed by colony formation assay. Cisplatin resistance was evaluated by MTT assay. Migration and invasion of the cells were tested by Transwell assay. Additionally, the levels of key proteins in the Wnt/β-catenin signaling pathway were examined by western blotting.

Results We found that LIMA1 was underexpressed in BCa tissues and cells ($P < 0.01$). Overexpression of LIMA1 inhibited the proliferation, migration, invasion, and epithelial-mesenchymal transition of BCa cells ($P < 0.01$) and improved their cisplatin resistance ($P < 0.01$), whereas knocking down LIMA1 produced opposite results ($P < 0.01$). Furthermore, overexpression of LIMA1 could suppress the Wnt/β-catenin signaling pathway in BCa cells ($P < 0.01$), and activation of this pathway partially reversed the anti-tumor effects produced by overexpression of LIMA1 ($P < 0.01$).

Conclusion LIMA1 could inhibit the malignant biological behavior of BCa cells and weaken their cisplatin resistance by negatively regulating the Wnt/β-catenin signaling pathway. Our findings provide new insights for the clinical treatment of BCa.

Keywords Bladder cancer, Cisplatin resistance, LIMA1, Wnt/β-catenin signaling pathway

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Introduction

Bladder cancer (BCa) is the most common malignant tumor in the urinary system and ranks as the tenth most common malignancy globally according to Global Cancer Statistics 2020 data released by the International Agency for Research on Cancer [1]. Approximately 500,000 people are diagnosed with BCa for the first time each year, with more than 200,000 deaths attributed to the disease [2]. Currently, the main clinical treatments for BCa include surgery, radiotherapy, chemotherapy, immunotherapy, and bladder instillation [3]. Among them, chemotherapy based on cisplatin remains the frontline treatment strategy for BCa, and the combination of cisplatin and gemcitabine has become the recognized standard chemotherapy regimen for BCa [4]. Unfortunately, the median overall survival time is only 14 months for patients receiving cisplatin combined with gemcitabine or cisplatin alone as adjuvant chemotherapy before and after bladder removal surgery, which may be related to acquired resistance to cisplatin [5]. Moreover, patients with BCa are associated with low 5-year survival rate and high risk of tumor recurrence after surgery, which may be attributed to the tendency of BCa to infiltrate into adjacent muscles and metastasize to distant sites [6]. Particularly, the prognosis for invasive and metastatic BCa is poor, with a 5-year survival rate of less than 5% [7, 8]. Therefore, finding new therapeutic targets to prevent BCa cells from invading the muscular layer and metastasizing to distant sites, and to reduce their resistance to cisplatin will be of significant importance in saving patients' lives.

LIM domain and actin binding 1 (LIMA1) gene is located on human chromosome 12q13.12, with a sequence length of 107,733 bp, capable of encoding two isoforms, namely LIMA1-α containing 600 amino acids and LIMA1-β containing 759 amino acids [9]. LIMA1 protein, also known as epithelial protein lost in neoplasms (EPLIN) and sterol regulatory element-binding protein 3 (SREBP3), is an important actin cytoskeletal regulator, playing a vital role in cell adhesion and migration [10]. In recent years, the relationship between the LIMA1 gene and cancer has gradually become a research focus. On one hand, clinical studies have found that the expression levels of the LIMA1-α gene are significantly downregulated in various tumor tissues, such as esophageal cancer [11], gastric cancer, and prostate cancer [12]. On the other hand, multiple basic studies using cell models have also confirmed that the expression level of LIMA1 is closely related to tumor cell proliferation, apoptosis, migration, invasion, and drug resistance [13–15]. These evidence suggest that LIMA1 holds promise as a new therapeutic target for malignant tumors. However, to date, few studies have explored the role of LIMA1 in the development and progression of BCa.

We therefore designed this study based on the previous findings and the issues that have not yet been clarified. This study evaluated the inhibitory effects of LIMA1 overexpression on BCa cells and explore its potential molecular mechanisms, providing new insights for the development of BCa treatment strategies.

Materials and methods

Collection of clinical tissue samples

This study included a total of three patients diagnosed with BCa and underwent partial cystectomy or radical cystectomy in Changzhou Wujin People's Hospital. During the surgery, the tumor tissue and a suitable amount of normal tissue adjacent to the tumor were removed. The removed tissues were then subjected to rapid frozen section. All tumor tissues exhibited typical tumor cell morphology under a microscope, whereas no cancer cells were found in all adjacent tissues under the microscope. After that, the tumor tissues were included in the BCa group and the adjacent tissues in the Adjacent tissue group, both stored at -80 °C for subsequent experimental use. This study was reviewed and approved by the Ethics Committee of Changzhou Wujin People's Hospital (Ethics number: 2024-SR-016), and all patients signed an informed consent form before surgery.

Cell culture, purchase of cisplatin, and induction of cisplatin resistance

This study used the immortalized human urothelial cell line SV-HUC-1 (CRL-9520) and human bladder transitional cell carcinoma cell line J82 (HTB-1) as cell models, both acquired from the American Type Cell Culture Collection. The cells were cultured in RPMI-1640 medium (Servicebio, China) with 10% fetal bovine serum (FBS) (Sigma-Aldrich, USA), 1% penicillin/streptomycin (Servicebio, China), and 1% L-glutamine (Sigma-Aldrich, USA). The culture conditions were maintained at a temperature of 37 °C and a CO_2 concentration of 5%.

Cisplatin (Topscience, USA) was dissolved in an appropriate amount of phosphate buffered saline (PBS) (Servicebio, China) to avoid hydrolysis and inactivation. Then, following the methods proposed by Gao et al. [16] and Elahi et al., [17] J82 cells were subjected to long-term, continuous, and gradual exposure to cisplatin to induce cisplatin resistance. In short, the induction process lasted for six months with each cycle lasting two weeks, and J82 cells were exposed to cisplatin (0.2–4.0 μM, concentration increased with cycles) for four hours on the first and third day of each cycle. After six months, J82 cells that had developed resistance to cisplatin, referred to as J82/CR cells, were collected and cultured under standard conditions in RPMI-1640 medium.

Cell transfection

In this study, small interfering RNA (siRNA) was used to knock down LIMA1, and the pcDNA3.1 vector was used to overexpress LIMA1. GenePharma company (Shanghai, China) designed and constructed siRNA targeting LIMA1, negative control siRNA not targeting any genes, pcDNA3.1 plasmid for overexpressing LIMA1, and negative control pcDNA3.1 plasmid not carrying any exogenous genes.

When J82 and J82/CR cells reached 70–80% confluence under standard culture conditions, they were transfected with the above-mentioned siRNAs and pcDNA3.1 plasmids using Lipofectamine 3000 (Invitrogen, USA) according to the reagent kit instructions. After transfection, the cells were further cultured for 48 h and then collected for subsequent experiments. Additionally, following the study by Bao et al., [18] 10 nM LiCl was added to a group of J82/CR cells transfected with pcDNA3.1-LIMA1 plasmid and cultured for 48 h to activate the Wnt/β-catenin signaling pathway in these cells.

J82 cells and J82/CR cells were finally divided into the following groups: (1) siRNA group: J82 cells transfected with negative siRNA; (2) si-LIMA1 group: J82 cells transfected with siRNA knocking down LIMA1; (3) vector group: J82/CR cells transfected with negative overexpression vector pcDNA3.1; (4) LIMA1 group: J82/CR cells transfected with LIMA1 overexpression vector pcDNA3.1-LIMA1; (5) LIMA1 + LiCl group: J82/CR cells transfected with pcDNA3.1-LIMA1 overexpression vector and then treated with LiCl (10 nM).

Real-time quantitative PCR (RT-qPCR)

Total RNA was extracted from clinical tissue samples and three cell lines using the Fast Cell/Tissue Total RNA Isolation Kit (Vazyme, China). Subsequently, total RNA was reverse-transcribed into cDNA using the Strand cDNA Synthesis Kit (Vazyme, China). RT-qPCR analysis was then performed on the Applied Biosystems 7500 Real-Time PCR system (Applied Biosystems, USA) using the SYBR Green PCR kit (Vazyme, China). GAPDH was used as an internal reference to normalize the expression levels of LIMA1, and the $2^{-\Delta\Delta Ct}$ method was employed for quantitative calculation. Each sample was tested for three times, and the average value was taken as the final result. Primers used in RT-qPCR are listed in Table 1.

Table 1 RT-qPCR primers

RNA	Sequences (5′ to 3′)
LIMA1	5′-GACGGCAATGGACCTCACTA-3′ (forward) 5′-TCCTCACCGTCACTTCTCTTC-3′ (reverse)
GAPDH	5′-TGGCTGGCTCAGAAAAAGGG-3′ (forward) 5′-GGGAGATTCAGTGTGGTGGG-3′ (reverse)

Colony formation assay

J82 cells and J82/CR cells were seeded in 6-well plates at a density of 300 cells/well and cultured for 12 days in RPMI-1640 medium, with the medium changed every 3 days. After culturing, cells were fixed with 4% paraformaldehyde and stained with 1% crystal violet (Sangon Biotech, China). Colonies, defined as clusters of more than 50 cells, were observed and recorded under a microscope. Each cell group underwent three parallel experiments, and the average value was taken as the final result.

MTT assay

J82 cells and J82/CR cells were seeded in 96-well plates at a cell density of 3×10^3/well, with 200 μL of RPMI-1640 medium added to each well. Subsequently, different concentrations of cisplatin solution (0, 1, 2, 4, 8, 16 μM) prepared in PBS buffer were added to the culture medium and incubated for 48 h. After 48 h, 20 μL of 0.5 mg/mL MTT solution (Sigma-Aldrich, USA) was added to each well and incubated for an additional four hours. Then 150 μL of DMSO was added to dissolve the MTT, and the absorbance at 570 nm was measured using the Multiskan SkyHigh microplate reader (Thermo Fisher Scientific, USA). Furthermore, to calculate the half-maximal inhibitory concentration (IC50) value, an online IC50 calculator developed by AAT Bioquest (https://www.aatbio.com/tools/ic50-calculator) was used to assess the survival ability of each cell group under cisplatin treatment.

Transwell assay

The migration and invasion capability of J82 cells and J82/CR cells were assessed using the Transwell assay, following the method described by Justus et al. [19] Prior to the Transwell assay, J82 cells and J82/CR cells were cultured in RPMI-1640 medium without FBS for 12 h to eliminate the interference of serum on the experimental results. The cells were then resuspended and adjusted to a density of 1×10^6 cells/mL. During the Transwell assay, 500 μL of the cell suspension was added to the upper chamber of the Transwell, with matrix gel covering the bottom of the upper chamber when assessing cell invasion capability or without matrix gel when assessing cell migration capability. A 600 μL of RPMI-1640 medium containing 15% FBS was added to the lower chamber. After 24 h of culture, the medium in the lower chamber was discarded, and cells were washed with calcium-free PBS, fixed with 4% paraformaldehyde, and stained with 0.1% crystal violet. Cells were observed and counted in five random fields under a microscope. Each cell group underwent three parallel experiments, and the average value was taken as the final result.

Western blot

Total protein was extracted from J82 and J82/CR cells using the Total Protein Extraction Kit (Sangon, China) containing protease and phosphatase inhibitors. Protein concentration was determined using the BCA Protein Assay Kit (Abcam, UK). After boiling, protein samples were separated by 6–10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (Servicebio, China) and then transferred to polyvinylidene difluoride (PVDF) membranes (Servicebio, China). The PVDF membrane was cut into sections prior to hybridization with antibodies to focus on specific protein regions of interest. The membranes were blocked with 5% skim milk as blocking buffer at room temperature for one hour. The membranes were then incubated overnight at 4 °C with primary antibodies as follows: LIMA1 (1:1,000, #PA5-54904, Invitrogen, USA), E-cadherin (1:2,000, #PA5-32178, Invitrogen, USA), N-cadherin (1:1,000, #PA5-19486, Invitrogen, USA), Snail (1:1,000, #MA5-14801, Invitrogen, USA), Wnt3a (1:1,000, #PA5-44946, Invitrogen, USA), β-catenin (1:1,000, #MA5-34961, Invitrogen, USA), C-myc (1:2,000, #700648, Invitrogen, USA), and β-actin (1:2,000, #PA1-183, Invitrogen, USA). The next day, the membranes were washed three times with PBS with Tween 20, each time for 10 min, and then incubated for one hour at room temperature with HRP-conjugated Goat Anti-Rabbit IgG H&L secondary antibody (1:5,000, #ab6721, Abcam, UK). The membranes were washed three times with PBS with Tween 20 again, each time for 10 min. Subsequently, detection was performed using the electrochemiluminescence method, and the membranes were imaged on a gel imaging analysis system (Tanon, China). Protein expression levels were semi-quantitatively analyzed using Image J software (NIH, USA), with β-actin protein as the internal reference. Each cell group underwent three parallel experiments, and the average value was taken as the final result.

Statistical analysis

The normality of data distribution was assessed using the Shapiro–Wilk test, and since the *P*-values in the Shapiro–Wilk test for all groups were greater than 0.05, the data were considered to follow a normal distribution. Hence, comparisons between two groups were conducted using the independent samples t-test; differences among multiple groups were analyzed using one-way analysis of variance, with Tukey post hoc test for pairwise comparisons between groups. Data are presented as mean ± standard deviation. Statistical analysis was carried out using SPSS 22.0 software (IBM Corp., USA), with $P < 0.05$ considered statistically significant.

Results

LIMA1 expression is downregulated in BCa tissues and cells

First, we verified the successful construction of J82/CR cells using the MTT assay. The results showed that the IC50 value and cell survival rate of J82/CR cells were higher than those of J82 cells ($P < 0.01$), indicating the successful construction of cisplatin resistant J82/CR cells (Fig. 1A).

The expression of LIMA1 in BCa was then examined. RT-qPCR results showed that the LIMA1 mRNA levels in tumor tissues of patients with BCa were significantly lower than those in their adjacent normal tissues ($P < 0.01$) (Fig. 1B). In the cell models, RT-qPCR and western blot results showed that LIMA1 mRNA and protein levels were lower in J82 and J82/CR cells compared to SV-HUC-1 cells ($P < 0.01$). Compared with J82 cells, J82/CR cells had lower LIMA1 mRNA and protein levels ($P < 0.01$) (Figs. 1C, D). These results indicated that LIMA1 was downregulated in both BCa tissue samples and cell models, and its decreased expression level may be associated with cisplatin resistance in BCa cells.

Overexpression of LIMA1 inhibits proliferation and cisplatin resistance of BCa cells

To assess the impact of LIMA1 expression levels on the proliferation capability and cisplatin resistance of BCa cells, this study utilized siRNA to knock down LIMA1 in J82 cells and the pcDNA3.1 plasmid to overexpress LIMA1 in J82/CR cells. RT-qPCR demonstrated that LIMA1 mRNA level was significantly lower in the si-LIMA1 group than in the siRNA group ($P < 0.01$) (Fig. 2A), and higher in the LIMA1 group than in the vector group ($P < 0.01$). These results were further confirmed by Western blot analysis (Fig. 2B). These results indicate successful knockdown or overexpression of LIMA1 expression by transfection.

Cell proliferation and cisplatin resistance of the aforementioned four cell groups were then assessed. Colony formation assay results demonstrated that the si-LIMA1 group had significantly more colonies than the siRNA group ($P < 0.01$) (Fig. 2C). In contrast, fewer colonies were observed in the LIMA1 group compared to the vector group ($P < 0.01$) (Fig. 2D). These results indicated that overexpression of LIMA1 inhibited the proliferation of BCa cells.

MTT assay was conducted to further evaluate the survival of each cell group under different concentration gradients of cisplatin solutions. The results showed that the IC50 and cell survival rate were higher in the si-LIMA1 group than in the siRNA group ($P < 0.01$) (Fig. 2E), and lower in the LIMA1 group than in the vector group ($P < 0.01$) (Fig. 2F). These results suggested LIMA1 could inhibit cisplatin resistance in BCa cells.

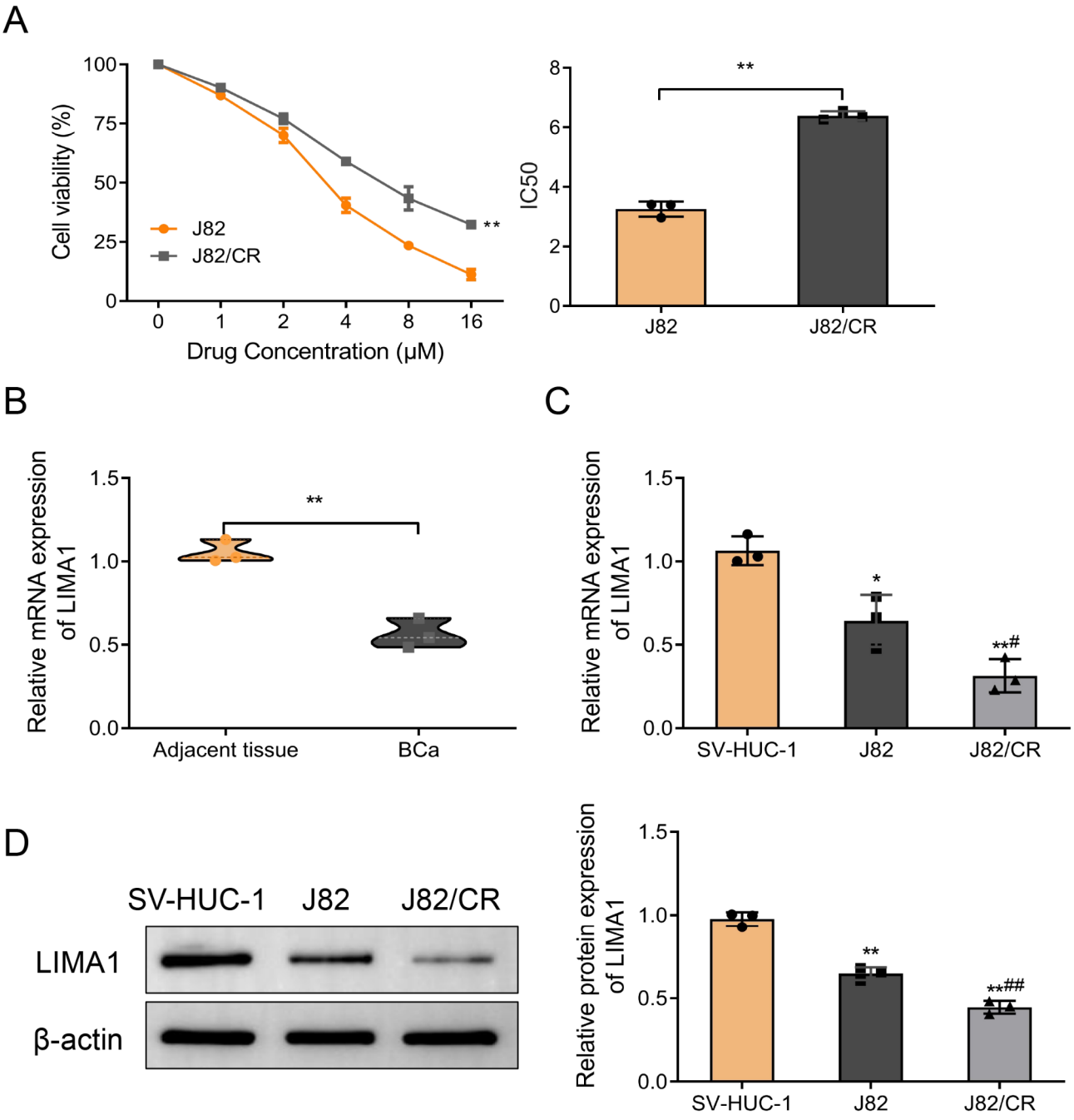

Fig. 1 LIMA1 expression is downregulated in BCa tissues and cells. (**A**) MTT assay was used to detect cell survival of J82 and J82/CR cells treated with different concentration gradients of cisplatin solutions (0, 1, 2, 4, 8, 16 μM), and IC50 was calculated, $^{**}P < 0.01$; (**B**) RT-qPCR was used to detect LIMA1 mRNA levels in tumour tissues and adjacent normal tissues, $^{**}P < 0.01$; (**C**) RT-qPCR was used to detect LIMA1 mRNA levels in SV-HUC-1, J82 and J82/CR cells; (**D**) Western blot was used to detect LIMA1 protein levels in SV-HUC-1, J82 and J82/CR cells; $N = 3$; $^{*}P < 0.05$ and $^{**}P < 0.01$ vs. SV-HUC-1 group; $\#P < 0.05$ and $\#\#P < 0.01$, vs. J82 group

These results collectively demonstrated that decreasing LIMA1 expression enhanced BCa cell proliferation and cisplatin resistance, whereas increasing LIMA1 expression had the opposite effects.

Overexpression of LIMA1 inhibits the migration and invasion of BCa cells

The tendency to infiltrate adjacent tissues and metastasize to distant organs is a significant reason for the high malignancy of BCa, leading to poor prognosis in many patients [20]. Therefore, we explored the impact of

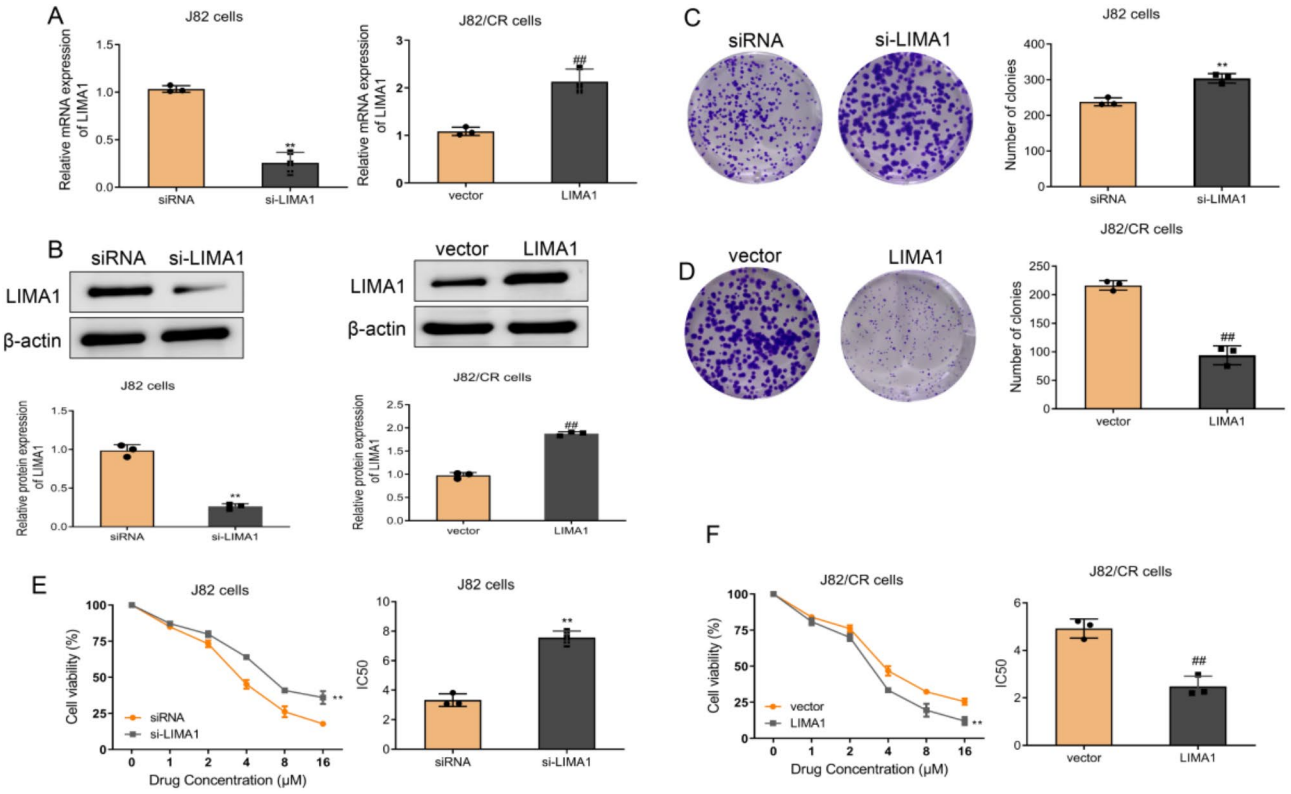

Fig. 2 LIMA1 overexpression inhibits proliferation and improves cisplatin resistance in BCa cells. **A**. RT-qPCR was used to detect LIMA1 mRNA levels in J82 cells of siRNA and si-LIMA1 groups; **B**. Western blot was used to detect LIMA1 protein levels in J82 cells of siRNA and si-LIMA1 groups; **C**. Colony formation assay was used to measure the proliferation of J82 cells of siRNA and si-LIMA1 groups; **D**. Colony formation assay was used to measure the proliferation ability of J82/CR cells in vector and LIMA1 groups; **E**. MTT assay was used to detect the cell survival rate of J82 cells in siRNA and si-LIMA1 groups treated with different concentration gradients of cisplatin solutions (0, 1, 2, 4, 8, and 16 μM), and the IC50 was calculated; **F**. MTT assay was used to detect the cell survival rate of J82/CR cells in vector and LIMA1 groups treated with different concentration gradients of cisplatin solutions (0, 1, 2, 4, 8, 16 μM), and IC50 was calculated. $N = 3$; $^{**}P < 0.01$ vs. siRNA group; $^{\#\#}P < 0.01$ vs. vector group

LIMA1 expression levels on the migration and invasion of BCa cells.

Transwell assay results showed that cell migration and invasion ability was significantly stronger in the si-LIMA1 group than in the siRNA group ($P < 0.01$) (Figs. 3A, C), and weaker in the LIMA1 group than in the vector group ($P < 0.01$) (Figs. 3B, D). Western blot further examined the changes in key protein levels during epithelial-to-mesenchymal transition (EMT) process. The results revealed that the si-LIMA1 group had lower E-cadherin protein level and higher level of N-cadherin and Snail proteins compared to the siRNA group ($P < 0.01$) (Fig. 3E). Additionally, the LIMA1 group had significantly higher E-cadherin protein level and lower level of N-cadherin and Snail proteins compared with the vector group ($P < 0.01$) (Fig. 3F). Collectively, lowering LIMA1 expression boosted BCa cell migration and invasion, whereas increasing LIMA1 reduced these abilities.

Overexpression of LIMA1 downregulates the Wnt/β-catenin signaling pathway in BCa cells

Subsequently, we delved into LIMA1's mechanism against BCa. Western blotting for the key proteins of the Wnt/β-catenin signaling pathway revealed that the levels of Wnt3a, β-catenin, and C-myc proteins were significantly higher in the si-LIMA1 group J82 cells than in the siRNA group ($P < 0.01$) (Figs. 4A, B), and lower in the LIMA1 group than in the vector group ($P < 0.01$) (Figs. 4C, D).

These results indicated that LIMA1 regulated the Wnt/β-catenin signaling pathway in BCa cells, with decreased LIMA1 expression activating this pathway and increased expression inhibiting it.

Wnt/β-catenin pathway activation partially counteracts the impact of LIMA1 on BCa cells

J82/CR cells were co-cultured with the LIMA1 overexpression plasmid and LiCl, a known Wnt/β-catenin pathway activator to explore whether LIMA1 influences the malignant behaviors of BCa cells through this pathway. The MTT assay results indicated that the cell survival

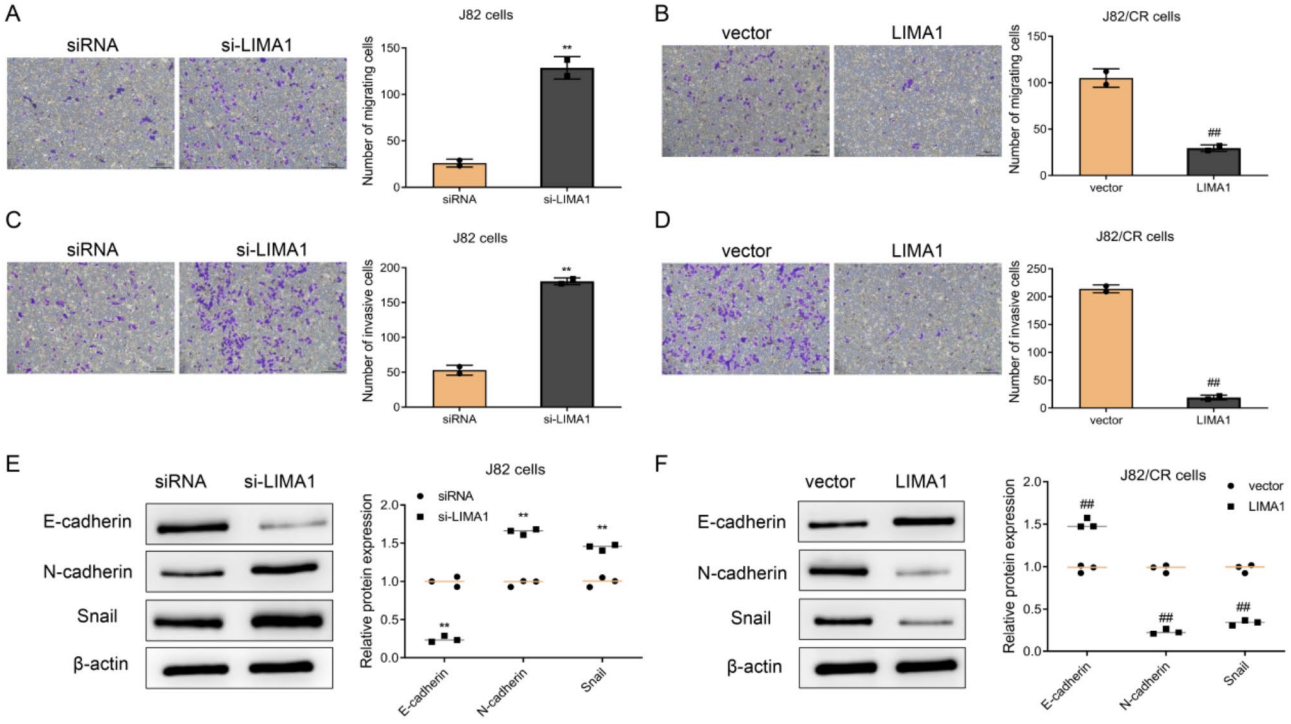

Fig. 3 LIMA1 overexpression inhibits the migration and invasion ability of BCa cells. (**A**) Transwell assay was used to detect the migration ability of J82 cells in siRNA and si-LIMA1 groups; (**B**) Transwell assay was used to detect the migration ability of J82/CR cells in vector and LIMA1 groups; (**C**) Transwell assay was used to detect the invasion ability of J82 cells in siRNA and si-LIMA1 groups; (**D**) Transwell assay was used to detect the invasion ability of J82/CR cells in vector and LIMA1 groups; (**E**) Western blot assay was used to detect protein expression levels of E-cadherin, N-cadherin, SNail in J82 cells of siRNA and si-LIMA1 groups, and quantitative analysis of the related protein levels was conducted; (**F**) Western blot assay was used to detect protein expression levels of E-cadherin, N-cadherin, Snail in J82/CR cells of vector and LIMA1 groups, and quantitative analysis of related protein levels was conducted. $N = 3$; $^{**}P < 0.01$ vs. siRNA group; $\#\#P < 0.01$ vs. vector group

rates in all groups decreased with increasing concentrations of cisplatin. However, compared to the LIMA1 group, the LiCl group partially reversed the inhibitory effects of LIMA1 on BCa cell survival and cisplatin resistance, while the LiCl + LIMA1 group significantly counteracted this inhibitory effect ($P < 0.01$) (Fig. 5A).

Moreover, colony formation and Transwell migration and invasion assay results showed a similar trend. The LiCl group enhanced cell proliferation, migration, and invasion compared to the LIMA1 group, while the LiCl + LIMA1 group exhibited significantly higher proliferation, migration, and invasion capabilities than the LIMA1 group alone ($P < 0.01$) (Fig. 5B-D). This suggests that LiCl partially reversed the inhibitory effects of LIMA1, especially in the LiCl + LIMA1 group. Western blot results further confirmed this trend: LIMA1 overexpression significantly upregulated E-cadherin expression and downregulated N-cadherin and Snail expression, while the LiCl group partially reversed these changes. Particularly in the LiCl + LIMA1 group, the upregulation of E-cadherin and the downregulation of N-cadherin and Snail were more pronounced ($P < 0.01$) (Fig. 5E **and F**). In conclusion, the activation of the Wnt/β-catenin pathway by LiCl partially counteracted the effects of LIMA1 on BCa cells, especially in the LiCl + LIMA1 group, suggesting that LIMA1 may exert antitumor effects in vitro on BCa cells at least partially by regulating the Wnt/β-catenin signaling pathway.

Discussion

Although cisplatin-based combination chemotherapy or perioperative adjuvant chemotherapy remains the frontline regimen for treating BCa, resistance to cisplatin develops easily during the course of treatment in patients [21]. A significant innovation of this study is the use of both conventional J82 cells and cisplatin-resistant J82/CR cells as in vitro BCa models. We believe that validating the antitumor effect of LIMA1 in both cisplatin-sensitive (simulating the situation of BCa patients who have not started or just started cisplatin chemotherapy) and cisplatin-resistant cell models (simulating the situation of BCa patients who have developed resistance to cisplatin due to long-term chemotherapy) can more accurately reflect the progression of the disease. Clinically, the standard dose of cisplatin for treating BCa is about 70 mg/m^2, with an appropriate dose determined by calculating the body surface area. Generally, in vivo concentration of cisplatin does not exceed 6 μM [16], and four hours after

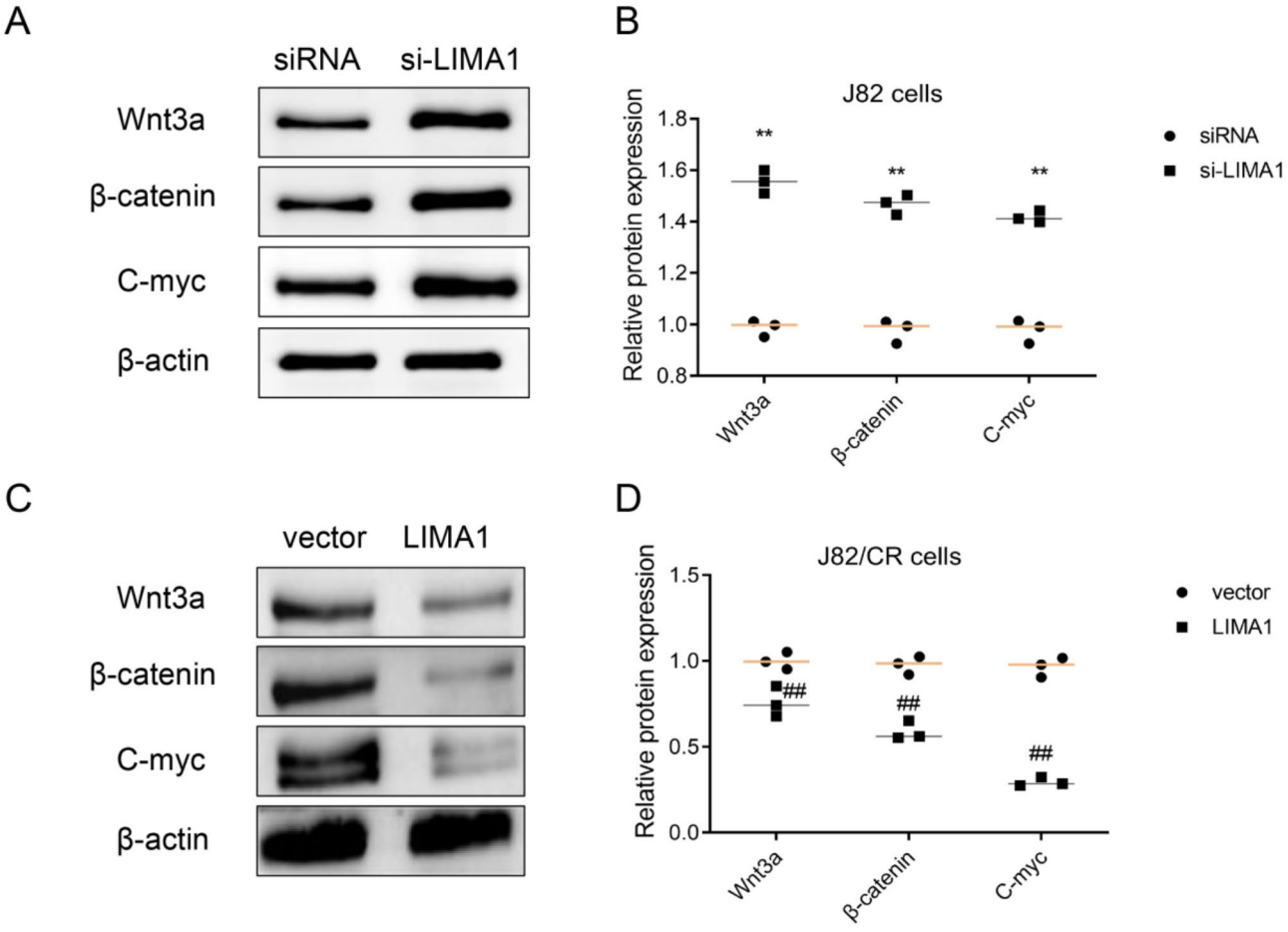

Fig. 4 LIMA1 overexpression downregulates the Wnt/β-catenin signalling pathway in BCa cells. **A**. Western blot was used to detect protein expression levels of Wnt3a, β-catenin, and C-myc in J82 cells of siRNA and si-LIMA1 groups, with representative bands provided; **B**. Results of statistical analysis of Wnt3a, β-catenin, and C-myc protein levels in J82 cells of siRNA and si-LIMA1 groups; **C**. Western blot was used to detect protein expression levels of Wnt3a, β-catenin, and C-myc in J82/CR cells of vector and LIMA1 groups, with representative bands provided; **D**. Results of statistical analysis of Wnt3a, β-catenin, and C-myc protein levels in J82/CR cells of vectora and LIMA1 groups. $N=3$; $^{**}P<0.01$ vs. siRNA group; $\#\#P<0.01$ vs. vector group

intravenous administration, cisplatin concentration in the blood drops almost to zero [22]. Based on these studies, we gradually exposed J82 cells to 0.2–4.0 μM of cisplatin continuously and gradually for a long time to induce resistance. The IC50 values of J82/CR cells in various concentration gradients of cisplatin solutions were significantly higher than those of their parent J82 cells, proving that the induction of resistance was successful. In this study, we found: (i) LIMA1 was downregulated in BCa tissues and cells; (ii) overexpression of LIMA1 inhibited the proliferation, migration, invasion, and EMT of BCa cells, reducing their cisplatin resistance, whereas knocking down LIMA1 produced the opposite results; (iii) activation of the Wnt/β-catenin signaling pathway could inhibit the antitumor effect of LIMA1 overexpression.

LIMA1 protein has multiple biological functions, including stabilizing the actin filament network to control actin, regulating cell adhesion, participating in epithelial defense against cancer, and inhibiting angiogenesis [23]. However, the impact of LIMA1 on the biological characteristics of BCa cells has not yet been clarified. In this study, we first explored the effects of LIMA1 expression levels on the proliferation capability and cisplatin resistance of BCa cells. Abnormal proliferation is a significant characteristic distinguishing tumor cells from normal cells [24]. We found that knocking down LIMA1 increased the capability of BCa cells to form colonies in vitro, whereas overexpressing LIMA1 had the opposite effect, proving that LIMA1 has a strong antiproliferative effect on BCa cells. On the other hand, since cisplatin resistance in BCa cells can lead to chemotherapy failure [5], enhancing the sensitivity of BCa cells to cisplatin is an important indicator for evaluating the clinical application prospects of LIMA1. We found that knocking down LIMA1 increased the IC50 value of BCa cells exposed to cisplatin, whereas overexpressing LIMA1 had the opposite effect, strongly proving that LIMA1 can effectively weaken cisplatin resistance in BCa cells.

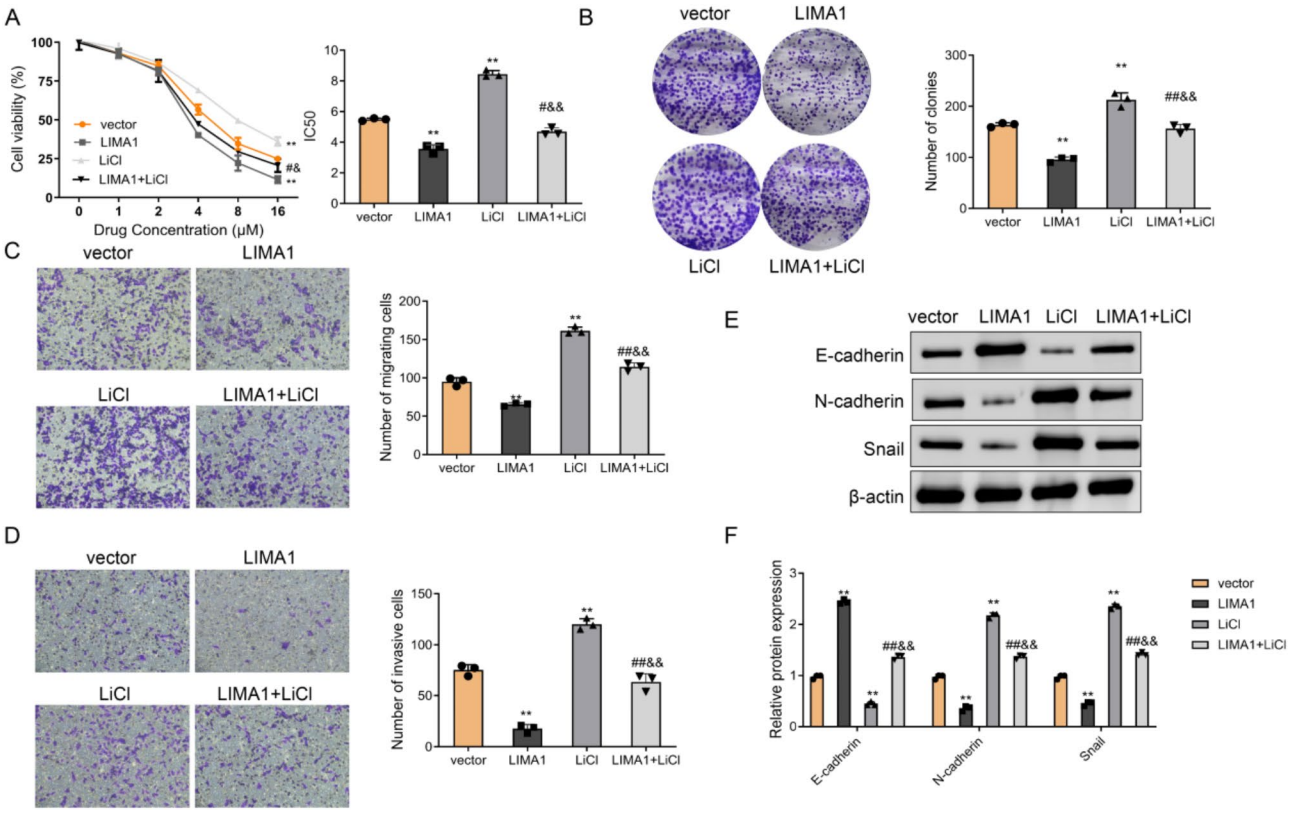

Fig. 5 Activation of Wnt/β-catenin pathway partially counteracts the effect of LIMA1 on BCa cells. (**A**) MTT assay was used to detect the cell survival rate of J82/CR cells in vector group, LIMA1 group, LiCl group, and LIMA1 + LiCl group under cisplatin treatment, and IC50 was calculated; (**B**) Colony formation assay was used to detect the proliferation ability of J82/CR cells in vector group, LIMA1 group, LiCl group, and LIMA1 + LiCl group; (**C**) Transwell assay was used to detect the migration ability of J82/CR cells in vector group, LIMA1 group, LiCl group, and LIMA1 + LiCl group; (**D**) Transwell assay was used to detect the invasion ability of J82/CR cells in vector group, LIMA1 group, LiCl group, and LIMA1 + LiCl group; (**E**) Western blot assay was used to detect protein levels of E-cadherin, N-cadherin, and Snail in J82/CR cells of vector group, LIMA1 group, LiCl group, and LIMA1 + LiCl group, with representative bands provided; (**F**) Results of statistical analysis of protein levels of E-cadherin, N-cadherin, and Snail in J82/CR cells of vector group, LIMA1 group, LiCl group, and LIMA1 + LiCl group. $N = 3$; $^{**}P < 0.01$ vs. vector group; $\#\#P < 0.01$ vs. LIMA1 group

The migration and invasion of BCa cells and the EMT process are important reasons for their tendency to infiltrate adjacent muscle layers and metastasize to distant organs [20]. Early-stage BCa is usually limited to the mucosal layer, but its abnormal migration and invasion help it infiltrate and spread to adjacent muscle layers and metastasize to organs far from the primary lesion through the blood or lymphatic system [25]. EMT process plays a significant role in the abnormal migration and invasion of BCa cells. EMT refers to the process where epithelial cells lose their apical-basal polarity and intercellular adhesion, thereby transforming into invasive mesenchymal cells [24]. During uncontrolled EMT in tumor cells, the zinc finger transcription factor Snail can bind to the E-box sequence, inhibiting the transcription process of the E-cadherin gene and thereby reducing the level of E-cadherin protein. Subsequently, as the level of E-cadherin protein, which maintains intercellular adhesion, decreases, N-Cadherin protein, which provides greater flexibility for epithelial cells, becomes dominant [26]. As a result, epithelial cell detachment and mobility is enhanced, ultimately tumor cells acquiring strong migration and invasion [26]. Our study found that knocking down LIMA1 enhanced the migration and invasion of BCa cells in vitro, concurrently downregulating E-cadherin protein levels and upregulating N-cadherin and Snail protein levels, whereas overexpression of LIMA1 produced the opposite effects. This study revealed for the first time that overexpression of LIMA1 can inhibit migration, invasion, and the EMT process in in vitro BCa cell models.

The underlying molecular mechanism of LIMA1 was further explored. We focused on the Wnt/β-catenin signaling pathway given its critical in embryonic development, internal environment homeostasis, and cancer progression. Upon receiving external activation signals, Wnt proteins, represented by Wnt3a, bind to the Frizzled receptor and LRP5/6 receptor on the cell membrane to form a trimeric complex. Then the activation signals transmit into cells to activate Dishevelled protein located

in the cytoplasm. Activated Dishevelled protein can effectively prevent the degradation of β-catenin, causing β-catenin to accumulate in the cytoplasm and translocate into the nucleus. β-catenin in the nucleus interacts with T-cell factor/lymphoid enhancing factor, ultimately promoting the expression of downstream target genes, such as C-myc [27]. Qi et al. found that in a hepatocellular carcinoma model, overexpression of LIMA1 could significantly promote the degradation of β-catenin, leading to reduced β-catenin protein levels and inactivation of the Wnt/β-catenin signaling pathway to consequently inhibit tumor progression [28]. Interestingly, our study results also revealed that overexpression of LIMA1 effectively downregulated the levels of key proteins in the Wnt/β-catenin signaling pathway, such as Wnt3a, β-catenin, and C-myc, effectively inhibiting the pathway. By artificially activating the Wnt/β-catenin signaling pathway in BCa cells with LiCl, the inhibitory effect of LIMA1 overexpression on the proliferation, migration, invasion, EMT, and cisplatin resistance of BCa cells was partially reversed. This suggests that LIMA1 exerts its anti-BCa effects through negatively regulating the Wnt/β-catenin signaling pathway. Some previous studies also demonstrated that applying traditional Chinese medicine Echinatin [29] or downregulating miR-148b-3p levels [30] could similarly inhibit BCa progression by suppressing the Wnt/β-catenin signaling pathway, aligning with the conclusions of this study.

However, this study has some limitations. First, the absence of animal experiments means the effects of LIMA1 on BCa progression could not be observed in vivo. Second, activating the Wnt/β-catenin signaling pathway only partially reversed the anti-tumor effect of LIMA1, suggesting other signaling pathways might also be involved in the regulation of BCa by LIMA1. Thus, future studies need to more comprehensively investigate the specific molecular mechanisms of LIMA1 in BCa.

Conclusion

The results of this study showed that low expression of LIMA1 was an important characteristic of BCa. This study was the first to confirm that LIMA1 inhibited the proliferation, migration, invasion, and cisplatin resistance of BCa cells by regulating the Wnt/β-catenin pathway, promising to be a new target for clinical treatment of BCa.

Abbreviations

BCa	Bladder cancer
EPLIN	Epithelial protein lost in neoplasms
LIMA1	LIM domain and actin binding 1
siRNA	Small interfering RNA
SREBP3	Sterol regulatory element-binding protein 3

Supplementary Information

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Supplementary Material 1

Supplementary Material 2

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Author contributions

Study concept and design: HRW; Analysis and interpretation of data: ZL, SCZ; Drafting of the manuscript: HRW; Critical revision of the manuscript for important intellectual content: ZL, SCZ; Statistical analysis: all authors; Study supervision: all authors; all authors have read and approved the manuscript.

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Data availability

The data used to support the findings of this study are available from the corresponding author upon request.

Declarations

Ethics approval and consent to participate

This study was approved by the Ethics Committee of Changzhou Wujin People's Hospital (Ethics number: 2024-SR-016). All patients provided written informed consent prior to surgery.

Consent for publication

All participants in this study have been informed a consent for publication. We ensure that all personal and identifiable information has been appropriately handled to protect the privacy of the participants.

Competing interests

The authors declare no competing interests.

Informed consent

N/A.

Registry and the registration no. of the study/trial

N/A.

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