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NLK Promotes the Proliferation, Migration, Invasion, and EMT of Bladder Cancer Cells by Regulating Paxillin

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Abstract

Objective: This research was intended to exploring the effect and mechanism of Nemo like kinase (NLK) and paxillin on proliferation, migration, invasion, and epithelial mesenchymal transition (EMT) of bladder cancer cells, and providing theoretical basis for basic research, clinical prevention and treatment of bladder cancer. **Methods:** The expression of NLK in bladder cancer tissue was analyzed by bioinformatics analysis. The proliferation, migration and invasion of bladder cancer cells were detected by EdU staining assay, clone formation assay, and transwell assay. RNA pull down assay was used to detect the interaction between NLK and paxillin in bladder cancer cells. Western blot assay was performed to measure the expressions of NLK, p-paxillin, and EMT-related proteins in bladder cancer cells. **Results:** NLK and p-paxillin were highly expressed in bladder cancer. NLK interacted with paxillin. NLK silencing inhibited the proliferation, migration, invasion, EMT, and p-paxillin expression of bladder cancer cells. Paxillin overexpression reversed the effect of NLK silencing on proliferation, migration, invasion, EMT, and p-paxillin expression in bladder cancer cells. **Conclusion:** NLK promotes the proliferation, migration, invasion and EMT of bladder cancer cells by regulating paxillin, providing theoretical basis for basic research, clinical prevention and treatment of bladder cancer.

1 Introduction

Bladder cancer, also known as urological cancer or bladder cancer, is the tenth most common cancer in the world, with its incidence rate rising steadily worldwide [1]. Although the survival rate of bladder cancer has been improved with the introduction of early diagnosis, robotic surgery and immunotherapy, bladder cancer is still an important factor with rising cases in the global cancer burden, especially in developed countries [2]. Therefore, a better understanding of the pathological mechanism and potential risk factors of bladder cancer is essential for its prevention and treatment.

Nemo like kinase (NLK) is an evolutionarily conserved mitogen activated serine/threonine protein kinase [3]. The Nemo gene mutation, which reduces the survival rate of fruit flies and leads to abnormal head and eye development, was first reported in 1994. The mammalian homolog of Nemo was cloned in 1998 and officially named NLK [4]. A large amount of evidence suggested that abnormal expression of NLK is closely related to the occurrence and progression of human cancers. NLK has been found to be highly expressed in cancers such as lung cancer, gallbladder cancer, laryngeal cancer, and colorectal cancer [5-8]. The promoting effect of NLK on these cancers is intimately associated with its regulation of cell proliferation, migration, invasion, and chemoresistance [9-11]. However, the role of NLK in bladder cancer is poorly studied. Studies have shown that downregulation of NLK can inhibit the phosphorylation of paxillin [12]. The phosphorylation of paxillin has been proved to promote the migration and invasion of bladder cancer cells [13]. Therefore, we wonder whether NLK can play a regulatory role in bladder cancer by promoting the phosphorylation of paxillin.

Thus, this study first investigated the effects of NLK on bladder cancer cells and further explored the regulation of paxillin by NLK in bladder cancer, aiming to provide a theoretical basis for the basic research and clinical prevention and treatment of bladder cancer.

2 Materials and methods

2.1 Bioinformatics analysis

The expression level of NLK in bladder cancer tissues was analyzed using the UALCAN website (http://ualcan.path.uab.edu/index.html) based on the TCGA database.

2.2 Cell culture

Human ureteral epithelial cells SV-HUC-1 (CRL-9520; ATCC, USA) as well as human bladder cancer cell lines RT4 (HTB-2; ATCC, USA) and T24 (HTB-4; ATCC, USA) were purchased. The cells were cultured in RPMI-1640 complete medium (PM150110B; Procell, China) in a cell culture incubator (37 ℃ , 5% CO_2, saturated humidity). When the cell density reached 80%-90%, the cells were digested with 0.25% EDTA trypsin (PB180224; Procell, China) and passaged.

2.3 Cell transfection

ShNLK, paxillin overexpression plasmids, and negative control plasmids (shNC and NC) were purchased from You-Bio (China) and VectorBuilder (China) for transfection experiments to silence NLK and overexpress paxillin. RT4 and T24 cells were cultured in 6-well plates, and when the cell density reached 80%, the complete medium was replaced with serum-free medium. The cells were transfected with shNC, NC, shNLK, or paxillin overexpression plasmids and transfection reagent (L7800; Solarbio, China). After 48 h, the cells were collected for subsequent experiments.

2.4 RNA pull down assay

An RNA pull down kit (20164) was purchased from Thermo Fisher Scientific (USA). RT4 and T24 cells were collected, and lysed with protein lysis buffer to

obtain cell proteins. Biotin-labeled NLK full-length (FL) probes and control probes (NC) were used to incubate the extracted cell proteins to form RNA-protein complexes. The complexes were washed with elution buffer, and the protein samples were collected. The expression level of paxillin in the samples was detected by Western blot using a paxillin monoclonal antibody (ab32084, Abcam, USA) to analyze the interaction between NLK and paxillin in bladder cancer cells.

2.5 qRT-PCR assay

After transfection, RT4 and T24 cells were collected, and 1 mL Trizol (15596026; Solarbio, China) was added to the cell samples, incubated at room temperature for 5 min and mixed well. Then, 200 μL of chloroform (A23432; Wokai, China) was added, incubated at room temperature for 3 min, and centrifuged at 15000 rpm for 15 min at 4 ℃. The supernatant was collected, and twice the sample volume of isopropanol (E15794; Wokai, China) was added and centrifuged at 15000 rpm for 10 min at 4 ℃. The RNA precipitate was collected and washed with 75% ethanol (E130059; Aladdin, China). The RNA concentration was measured. Next, the RNA samples were reverse-transcribed into cDNA using a reverse transcription kit (D7168S; Beyotime, China). The cDNA was mixed with SYBR QPCR Master Mix (D7260; Beyotime, China) and primers for NLK and paxillin, and the amplification reaction was performed in a real-time PCR instrument (QuantStudio 6; Applied Biosystems, USA). The expression levels of NLK and paxillin were assessed by the $2^{-\triangle\triangle CT}$ method.

2.6 EdU staining assay

An EdU cell proliferation detection kit (C0088L) was purchased from Beyotime (China). After transfection of RT4 and T24 cells, the original culture medium was discarded and 1 mL 20 μM EdU working solution was added to the culture wells for 2-h incubation. Subsequently, cells were cultured with 1 mL fixative solution for 15 min and 1 mL permeabilizing solution for 10 min at room temperature, and washed with washing solution for 5 min. The cells were then incubated with 0.5 mL Click reaction solution in the dark for 30 min and washed three times with washing solution for 5 min. The nuclei were stained with 1 mL Hoechst33342 staining solution (C1022; Beyotime, China) in the dark for 10 min, and rinsed thrice with washing solution for 5 min each. The cells were imaged using a fluorescence microscope (DM2500; Leica, Germany) to detect EdU-positive cells, and the cells were counted using ImageJ software to assess the effects of shNLK and paxillin on cell proliferation.

2.7 Transwell assay

The Transwell chamber (140629; Thermo Fisher, USA) was pre-placed into a 24 well plate (for invasion detection, 100 μL matrix gel (354230; Corning, USA) was placed into the Transwell chamber in advance). The transfected RT4 and T24 cells were collected, and cultured in serum-free medium to prepare a single-cell suspension. 100 μL cell suspension containing 1×10^6 RT4 or T24 cells was transferred into the Transwell chamber. Subsequently, 700 μL complete culture medium was added to the lower chamber of a 24-well plate for 24-h incubation. The culture medium in the chamber was aspirated, and the cells inside and outside the chamber were fixed with 4% paraformaldehyde (R20497; Wokai, USA) for 15 min, stained with crystal violet (D10739; Wokai, USA) for 15 min, and washed with PBS. Cells that had not migrated through the membrane into the outer layer of the chamber were wiped off with a cotton swab. The cells on the outer layer of the chamber were imaged using an optical microscope (DMi8 S; Leica, Germany), and were counted using ImageJ software to assess the effects of shNLK and paxillin on cell migration and invasion.

2.8 Colony formation assay

Transfected RT4 and T24 cells were collected, and

made into single-cell suspensions with complete medium. 2 mL cell suspension containing 1,000 RT4 or T24 cells was added to each well of a 6-well plate and cultured in a cell culture incubator for 2 weeks. During the culture, the complete medium was changed every 3 days. After 2 weeks, the original culture medium was aspirated, and the cell colonies in each well were fixed with 1 mL 4% paraformaldehyde for 15 min and stained with 1 mL crystal violet for 15 min. After washing with PBS, the cell colonies in each well were photographed using a camera, and the total number of colonies was counted using ImageJ software to evaluate the effects of shNLK and paxillin on cell proliferation.

2.9 Western blot assay

Transfected RT4 and T24 cells were collected, washed three times with PBS, and lysed with RIPA lysis buffer (R21236; Vokai, USA). The lysates were centrifuged at 14000 rpm for 20 min at 4 ℃, and the supernatant was collected as the cell protein sample. The protein concentration was measured using a BCA kit (PC0020; Solarbio, China) according to the instructions. An appropriate amount of protein loading buffer (P1040; Solarbio, China) was added to the protein samples, followed by thermal denaturation in a 100 ℃ metal bath for 5 min. Subsequently, 50 μg protein sample was loaded onto a polyacrylamide gel (P1200; Solarbio, China) for electrophoresis and transferred to a membrane. The membrane was blocked with 5% skim milk for 1 h, eluted thrice with TBST (T1085; Solarbio, China) for 10 min each, and incubated overnight at 4 ℃ with monoclonal antibodies against NLK (ab97642; Abcam, USA), p-paxillin (ab32084; Abcam, USA), and epithelial-mesenchymal transition (EMT)-related proteins (E-cadherin (ab231303; Abcam, USA), Vimentin (ab92547; Abcam, USA), N-cadherin (ab76011; Abcam, USA), Snail (ab216347; Abcam, USA), Slug (ab51772; Abcam, USA), Twist (ab50887; Abcam, USA)). The next day, the membrane was eluted three times with TBST for 10 min each and incubated with the corresponding secondary antibodies at room temperature for 2 h. After elution three times with TBST, ECL developing solution (PE0010; Solarbio, China) was added to the membrane, and the protein bands were imaged using an Image Lab 3.0 imaging system (Bio-Rad; USA). Finally, the data were analyzed using ImageJ software, with GAPDH (ab8245; Abcam, USA) as an internal reference, to evaluate the effects of shNLK and paxillin on the expressions of NLK, p-paxillin, and EMT-related proteins in the cells.

2.10 Statistical methods

All experimental results were analyzed using GraphPad Prism 8.0 software. Comparisons among multiple groups were performed using one-way ANOVA. Quantitative data were expressed as mean ± standard deviation. A p-value of less than 0.05 was considered statistically significant.

3 Results

3.1 NLK and p-paxillin were highly expressed in bladder cancer, and shNLK suppressed the proliferation of bladder cancer cells

Using UALCAN, the analysis data revealed that NLK was highly expressed in bladder cancer tissues (Figure 1A). Subsequently, Western blot assays were performed to detect the expressions of NLK and p-paxillin in bladder cancer cells. As shown in Figure 1B-C, compared with human ureteral epithelial cells SV-HUC-1, bladder cancer cells RT4 and T24 displayed upregulation of NLK and p-paxillin ($p < 0.01$). After transfection with shNLK, the expression of NLK in RT4 and T24 cells was significantly reduced ($p < 0.001$; Figure 1D), confirming successful transfection. According to Figure 1E-F, relative to shNC, shNLK decreased EdU-positive cells in RT4 and T24 cells ($p < 0.01$), indicating that shNLK inhibited the proliferation of bladder cancer cells.

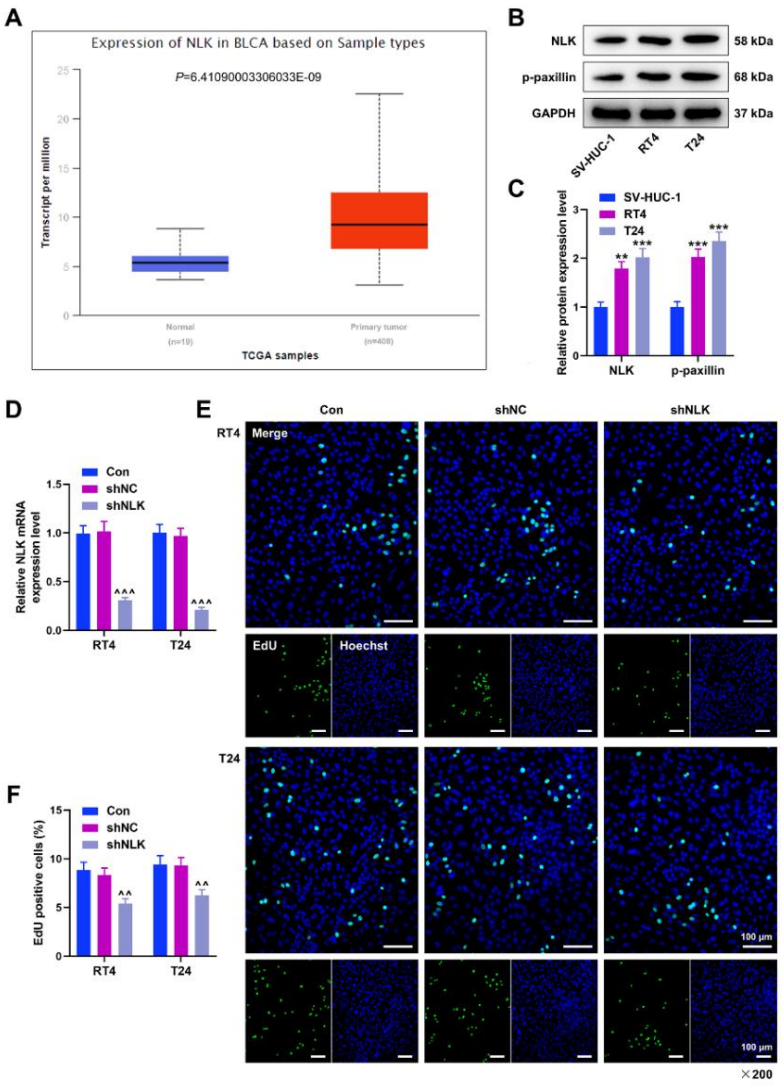

Figure 1 NLK and p-paxillin were highly expressed in bladder cancer, and shNLK suppressed bladder cancer cell proliferation. (A) Analysis of NLK expression levels in bladder cancer tissues using the UALCAN website. (B-C) Western blot assays to detect the expression levels of NLK and p-paxillin in bladder cancer cells. (D) QRT-PCR assays to assess the transfection efficiency of shNLK. (E-F) EdU staining assays to examine the proliferation of bladder cancer cells after shNLK transfection (200×). Compared with the SV-HUC-1 group, ** $p < 0.01$, *** $p < 0.001$; compared with the shNC group, ^^ $p < 0.01$, ^^^ $p < 0.001$.

3.2 ShNLK inhibited migration, invasion, and proliferation of bladder cancer cells

Transwell assays were used to detect changes in cell migration and invasion after transfection. As shown in Figure 2A-D, compared with the shNC, shNLK reduced the proportion of migration and invasion of RT4 and T24 cells ($p < 0.001$). Subsequently, colony formation assays were conducted to detect changes in cell proliferation after transfection. In light of Figure 2E-F, shNLK reduced the proportion of colony formation in RT4 and T24 cells ($p < 0.001$), further indicating that shNLK inhibited the proliferation of bladder cancer cells.

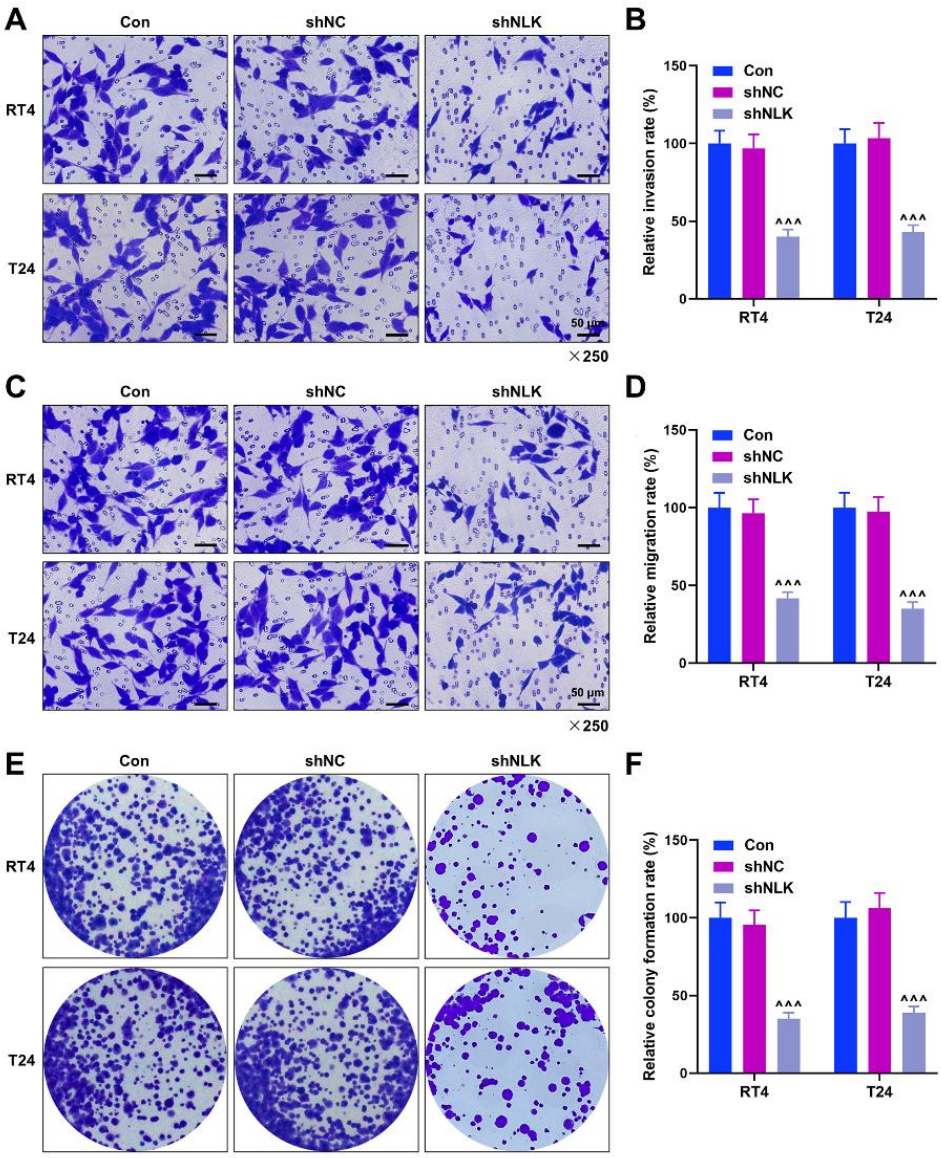

Figure 2 ShNLK dampened migration, invasion, and proliferation of bladder cancer cells. (A-B) Transwell assays to detect the invasion of bladder cancer cells after shNLK transfection (250×). (C-D) Transwell assays to test the migration of bladder cancer cells after shNLK transfection (250×). (E-F) Colony formation assays to examine the proliferation of bladder cancer cells after shNLK transfection. Compared with the shNC group, ^^^ $p < 0.001$.

3.3 ShNLK repressed EMT in bladder cancer cells and NLK interacted with paxillin

Western blot assays were carried out to detect the expressions of EMT-related proteins in bladder cancer cells. In contrast with shNC, shNLK increased the expression of E-cadherin in RT4 and T24 cells ($p <$ 0.001; Figure 3A-D) and decreased the expressions of Vimentin, N-cadherin, Snail, Slug, and Twist ($p < 0.01$; Figure 3A-D), indicating that shNLK inhibited EMT in bladder cancer cells. As described in Figure 3E, the RNA pull down assay data revealed that full-length NLK can while NC cannot downregulate p-paxillin protein, denoting that NLK interacted with paxillin.

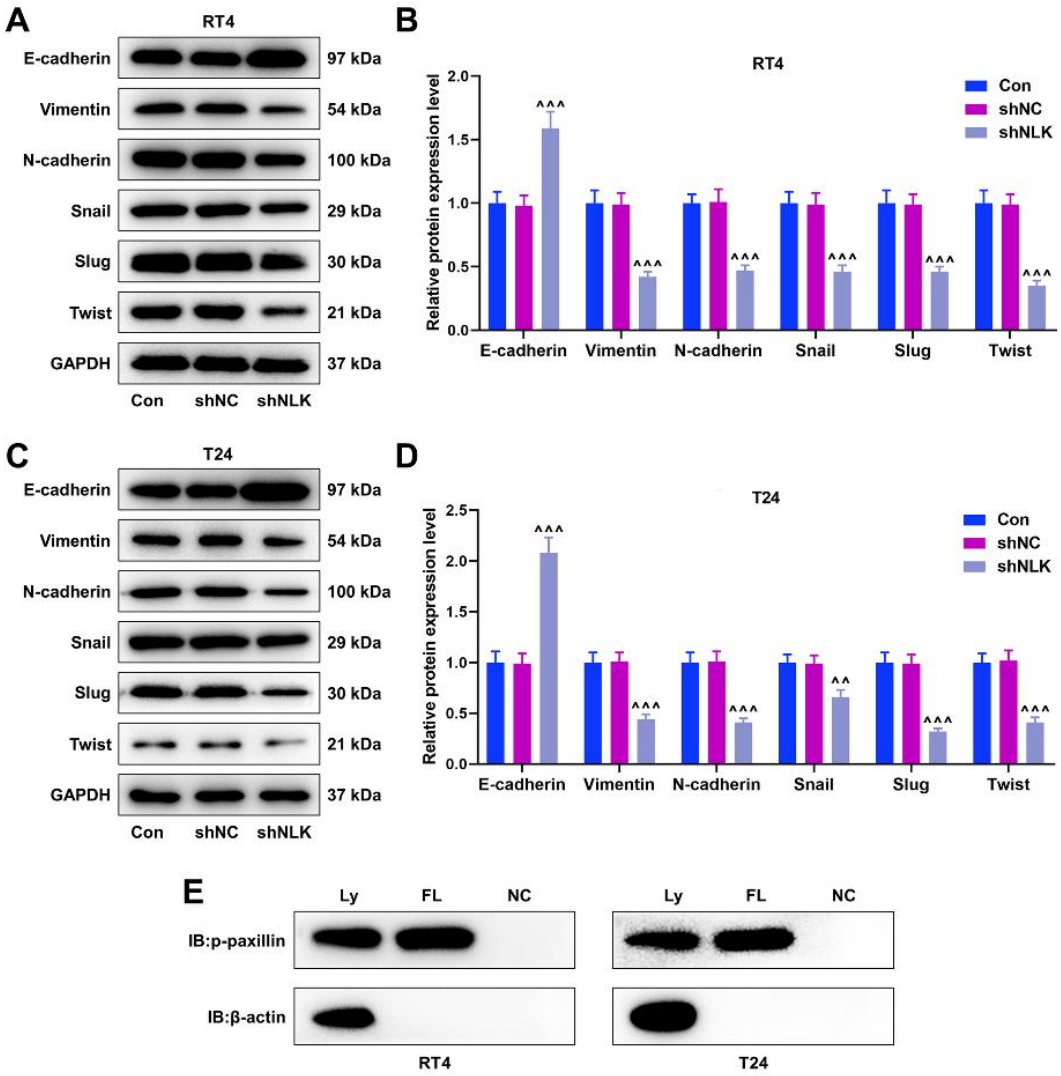

Figure 3 ShNLK repressed EMT in bladder cancer cells and NLK interacted with paxillin. (A-D) Western blot assays to measure the expressions of EMT-related proteins in bladder cancer cells after shNLK transfection. (E) RNA pull-down assays to determine the interaction between NLK and paxillin in bladder cancer cells. Compared with the shNC group, $^{\wedge\wedge}p < 0.01$, $^{\wedge\wedge\wedge}p < 0.001$.

3.4 ShNLK inhibited bladder cancer cell proliferation by downregulating p-paxillin expression

As shown in Figure 4A-B, relative to shNC, shNLK inhibited the expression of p-paxillin in RT4 and T24 cells ($p < 0.01$). Subsequently, cells were transfected with paxillin overexpression plasmid, which significantly increased paxillin expression in RT4 and T24 cells ($p < 0.001$; Figure 4C), confirming successful transfection. Co-transfection of shNLK and paxillin overexpression plasmid was then performed, and EdU staining assays were used to determine cell proliferation. As displayed in Figure 4D-E, paxillin overexpression elevated the proportion of EdU-positive cells in the shNLK+NC group ($p < 0.01$), hinting that paxillin overexpression reversed the inhibitory effect of shNLK on bladder cancer cell proliferation. This further proved that shNLK curbed bladder cancer cell proliferation by waning p-paxillin expression.

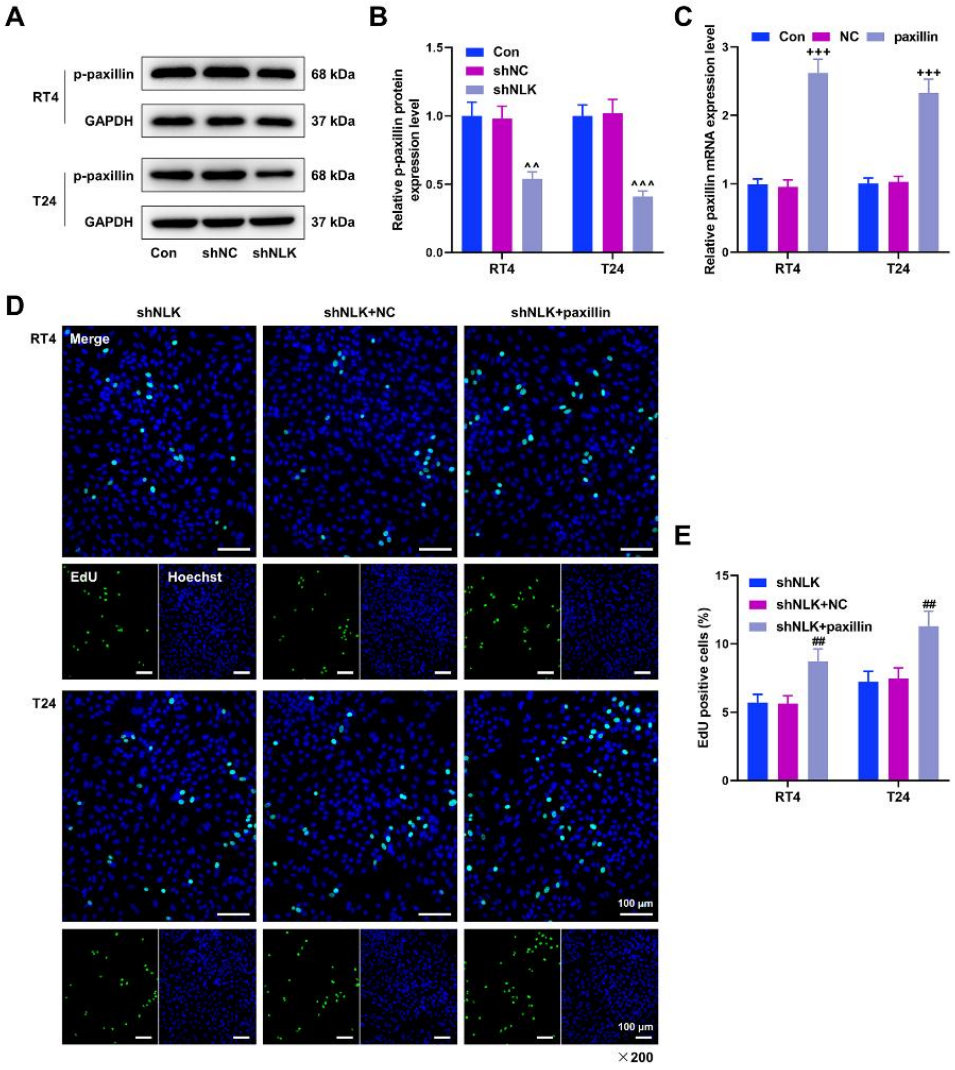

Figure 4 ShNLK hindered bladder cancer cell proliferation by downregulating p-paxillin expression. (A-B) Western blot assays to detect p-paxillin expression in bladder cancer cells after shNLK transfection. (C) QRT-PCR assays to assess the transfection efficiency of paxillin overexpression plasmid. (D-E) EdU staining assays to examine the proliferation of bladder cancer cells after co-transfection of shNLK and paxillin overexpression plasmid (200×). Compared with the shNC group, $^{\wedge\wedge}p < 0.01$, $^{\wedge\wedge\wedge}p < 0.001$; compared with the NC group, $^{+++}p < 0.001$; compared with the shNLK+NC group, $^{\#\#}p < 0.01$.

3.5 ShNLK inhibited migration, invasion, and proliferation of bladder cancer cells by diminishing p-paxillin expression

Transwell assays were used to detect changes in cell migration and invasion after transfection. In line with Figure 5A-D, compared with shNLK+NC, paxillin overexpression increased the proportion of migration and invasion in RT4 and T24 cells ($p < 0.001$). The colony formation assays were carried out to determine changes in cell proliferation after transfection. As shown in Figure 5E-F, compared with shNLK+NC, paxillin overexpression elevated the proportion of colony formation in RT4 and T24 cells ($p < 0.001$). These results indicated that shNLK inhibited migration, invasion, and proliferation of bladder cancer cells by regulating paxillin.

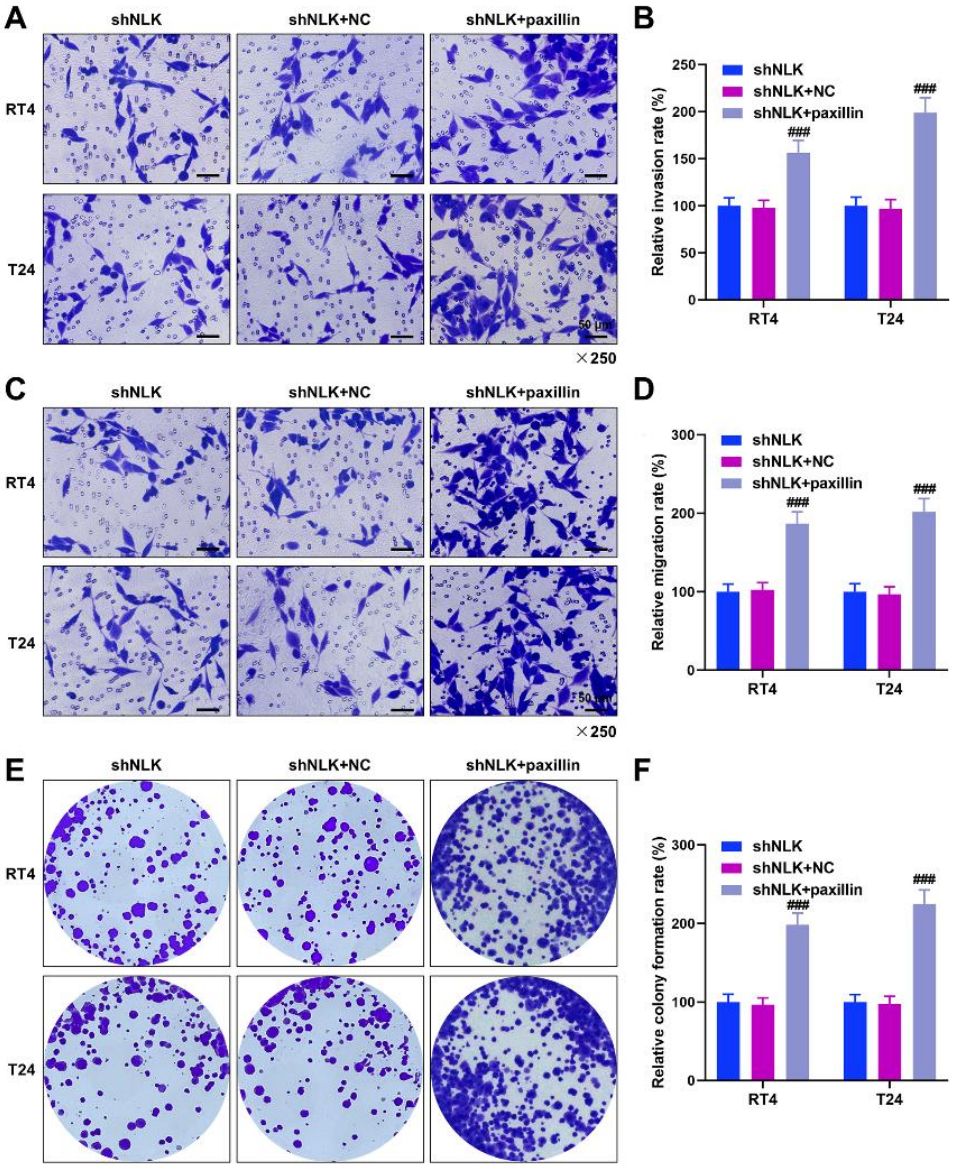

Figure 5 ShNLK suppressed migration, invasion, and proliferation of bladder cancer cells by downregulating p-paxillin expression. (A-B) Transwell assays to detect the invasion of bladder cancer cells after co-transfection of shNLK and paxillin overexpression plasmid (250×). (C-D) Transwell assays to measure the migration of bladder cancer cells after co-transfection of shNLK and paxillin overexpression plasmid (250×). (E-F) Colony formation assays to gauge the proliferation of bladder cancer cells after co-transfection of shNLK and paxillin overexpression plasmid. Compared with the shNLK+NC group, $^{###}p < 0.001$.

3.6 ShNLK dampened EMT in bladder cancer cells by repressing p-paxillin expression

As shown in Figure 6A-D, compared with shNLK+NC, paxillin overexpression decreased E-cadherin expression in RT4 and T24 cells ($p < 0.01$) and increased the expressions of Vimentin, N-cadherin, Snail, Slug, and Twist ($p < 0.001$). These results indicated that shNLK inhibited EMT in bladder cancer cells by regulating paxillin.

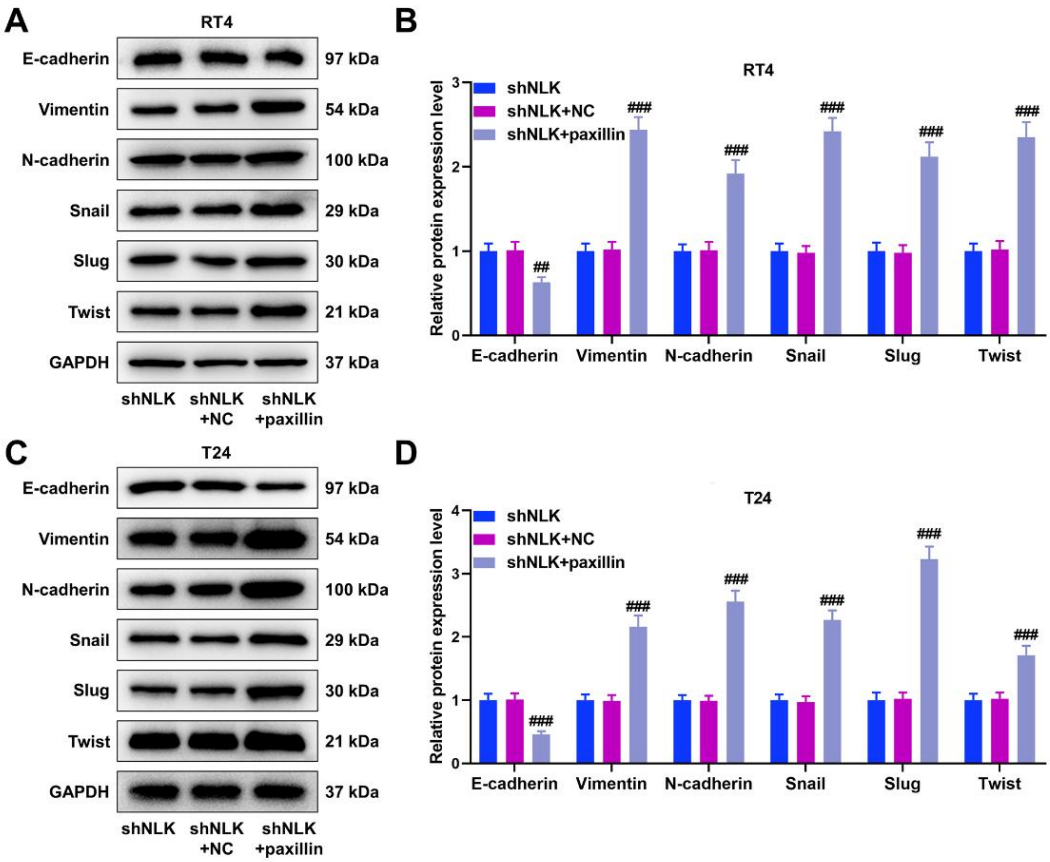

Figure 6 ShNLK suppressed EMT in bladder cancer cells by downregulating p-paxillin. (A-D) Western blot assays to quantify the expressions of EMT-related proteins in bladder cancer cells after co-transfection of shNLK and paxillin overexpression plasmid. Compared with the shNLK+NC, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$.

4 Discussion

The incidence rate of bladder cancer is still rising with the continuous improvement of diagnosis and treatment technology, better understanding the pathological mechanism of bladder cancer and finding biomarkers of bladder cancer are of great importance for research, prevention and treatment.

NLK is an evolutionarily conserved mitogen activated serine/threonine protein kinase that has been confirmed to have oncogenic effects in various cancers. Its abnormally high expression has been found in cancers such as lung cancer, gallbladder cancer, laryngeal cancer, and colorectal cancer [5-8]. This is the first study finding that NLK is highly expressed in bladder cancer tissues and cells, suggesting that NLK also has a regulatory role in the development of bladder cancer. The regulatory role of NLK in cancer is closely related to its regulation of various biological functions of cancer cells. For example, NLK can promote the progression of colorectal cancer by facilitating the cell cycle [9]. NLK can maintain the proliferation and stemness of non-small cell lung cancer cells to promote their development [14,15]. NLK silencing can dampen the proliferation and invasion of laryngeal cancer cells, thereby exerting an inhibitory effect on laryngeal cancer [7]. Through transfection and biological function experiments, this study found for the first time that silencing of NLK can inhibit the proliferation, migration, and invasion of bladder cancer cells, hinting that abnormally overexpressed NLK in bladder cancer can promote the development of bladder cancer.

EMT is a process in which epithelial cells acquire mesenchymal characteristics. After undergoing EMT,

tumor cells lose their original polarity and tight junctions, and their abilities to infiltrate, migrate, and invade are enhanced, allowing them to detach from the primary site and form metastases [16]. When cancer cells undergo EMT, they gradually lose epithelial cell characteristics and the expressions of related proteins (such as E-cadherin) and increase mesenchymal characteristics and the expressions of related proteins (such as Vimentin, N-cadherin, Snail, Slug, and Twist). Suppressing EMT in bladder cancer cells can prevent the metastasis and development of bladder cancer [17,18]. Studies have confirmed that NLK can promote the EMT process in lung cancer cells by upregulating N-cadherin and downregulating E-cadherin [11]. This study is the first to find that silencing of NLK elevates E-cadherin expression in bladder cancer cells and diminishes the expressions of Vimentin, N-cadherin, Snail, Slug, and Twist, indicating that silencing of NLK inhibits the EMT process in bladder cancer cells. This further confirms that the abnormally overexpressed NLK in bladder cancer promotes EMT, and its mechanism needs further in-depth research.

As the main substrate of Src tyrosine kinase, paxillin plays a crucial role in regulating the assembly and organization of focal adhesions [19]. The high expression of paxillin has been verified to be positively correlated with tumor recurrence and metastasis, and inhibiting the expression of paxillin can suppress the EMT and metastasis processes of colorectal cancer [20]. Meanwhile, paxillin has been proven to promote the migration, invasion, proliferation, and angiogenesis of bladder cancer cells [21,22]. Additionally, studies have found that downregulation of NLK can inhibit the phosphorylation of paxillin. Consistent with the above reports, this study also found that p-paxillin is highly expressed in bladder cancer cells, and NLK interacts with paxillin and regulates its phosphorylation. Furthermore, this study provides the first evidence that overexpression of paxillin reverses the effects of NLK silencing on the proliferation, migration, invasion, EMT, and p-paxillin expressions of bladder cancer cells, further proving that NLK promotes EMT in bladder cancer by regulating the phosphorylation of paxillin.

In summary, this study found that NLK and p-paxillin levels are upregulated in bladder cancer. NLK interacts with paxillin. NLK silencing inhibits the proliferation, migration, invasion, and EMT of bladder cancer cells and suppresses p-paxillin expression, while overexpression of paxillin reverses these effects of NLK silencing. This study is the first to prove that NLK silencing inhibits the proliferation, migration, invasion, and EMT of bladder cancer cells by suppressing p-paxillin expression, providing a theoretical basis for the basic research and clinical prevention and treatment of bladder cancer.

Acknowledgements

Not applicable.

Conflicts of Interest

All authors declare that the research was conducted in the absence of any commercial or fnancial relationships that could be construed as a potential confict of interest.

Author Contributions

J.L. wrote the initial paper, Q.Y. designed and drew the figures, L.C. revised the paper.

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Availability of Data and Materials

The original contributions presented in the study are included in the article, further inquiries can be directed to the corresponding authors.

Supplementary Materials

Not applicable.

References

[1] Saginala K, Barsouk A, Aluru JS, et al. Epidemiology of Bladder Cancer. *Medical Sciences* 2020; 8(1): 15.

[2] Bray F, Ferlay J, Soerjomataram I, et al. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: A Cancer Journal for Clinicians* 2018; 68(6): 394-424.

[3] Brott BK, Pinsky BA, Erikson RL. Nlk is a murine protein kinase related to Erk/MAP kinases and localized in the nucleus. *Proceedings of the National Academy of Sciences of the United States of America* 1998; 95(3): 963-968.

[4] Choi KW, Benzer S. Rotation of photoreceptor clusters in the developing Drosophila eye requires the nemo gene. *Cell* 1994; 78(1): 125-136.

[5] Lv M, Li Y, Tian X, et al. Lentivirus-mediated knockdown of NLK inhibits small-cell lung cancer growth and metastasis. *Drug Design, Development and Therapy* 2016; 10: 3737-3746.

[6] Li M, Zhang S, Wang Z, et al. Prognostic significance of nemo-like kinase (NLK) expression in patients with gallbladder cancer. *Tumor Biology* 2013; 34(6): 3995-4000.

[7] Shen N, Duan XH, Wang XL, et al. Effect of NLK on the proliferation and invasion of laryngeal carcinoma cells by regulating CDCP1. *European Review for Medical and Pharmacological Sciences* 2019; 23(14): 6226-6233.

[8] Zhang W, He J, Du Y, et al. Upregulation of nemo-like kinase is an independent prognostic factor in colorectal cancer. *World Journal of Gastroenterology* 2015; 21(29): 8836-8847.

[9] Li SZ, Zeng F, Li J, et al. Nemo-like kinase (NLK) primes colorectal cancer progression by releasing the E2F1 complex from HDAC1. *Cancer Letters* 2018; 431: 43-53.

[10] Zou D, Wang D, Li R, et al. MiR-197 induces Taxol resistance in human ovarian cancer cells by regulating NLK. *Tumor Biology* 2015; 36(9): 6725-6732.

[11] Lei L, Wang Y, Zheng YW, et al. Overexpression of Nemo-like Kinase Promotes the Proliferation and Invasion of Lung Cancer Cells and Indicates Poor Prognosis. *Current Cancer Drug Targets* 2019; 19(8): 674-680.

[12] Ishitani T, Ishitani S, Matsumoto K, et al. Nemo-like kinase is involved in NGF-induced neurite outgrowth via phosphorylating MAP1B and paxillin. *Journal of Neurochemistry* 2009; 111(5): 1104-1118.

[13] Chiu HY, Sun KH, Chen SY, et al. Autocrine CCL2 promotes cell migration and invasion via PKC activation and tyrosine phosphorylation of paxillin in bladder cancer cells. *Cytokine* 2012; 59(2): 423-432.

[14] Wei YB, Yan PY, Yang XF. The mechanism of tumor microenvironment in the occurrence and development of bladder cancer. *Chemistry of Life* 2022; 42(9): 1665-1671.

[15] Suwei D, Liang Z, Zhimin L, et al. NLK functions to maintain proliferation and stemness of NSCLC and is a target of metformin. *Journal of Hematology & Oncology* 2015; 8: 120.

[16] Lamouille S, Xu J, Derynck R. Molecular mechanisms of epithelial-mesenchymal transition. *Nature Reviews Molecular Cell Biology* 2014; 15(3): 178-196.

[17] Zhang Y, Weinberg RA. Epithelial-to-mesenchymal transition in cancer: complexity and opportunities. *Frontiers of Medicine* 2018; 12(4): 361-373.

[18] Chi M, Liu J, Mei C, et al. TEAD4 functions as a prognostic biomarker and triggers EMT via PI3K/AKT pathway in bladder cancer. *Journal of Experimental & Clinical Cancer Research* 2022; 41(1): 175.

[19] Ma X, Hammes SR. Paxillin actions in the nucleus. *Steroids* 2018; 133: 87-92.

[20] Wen L, Zhang X, Zhang J, et al. Paxillin knockdown suppresses metastasis and epithelial-mesenchymal transition in colorectal cancer via the ERK signalling pathway. *Oncology Reports* 2020; 44(3): 1105-1115.

[21] Hou T, Zhou L, Wang L, et al. Leupaxin Promotes Bladder Cancer Proliferation, Metastasis, and Angiogenesis Through the PI3K/AKT Pathway. *Cellular Physiology and Biochemistry* 2018; 47(6): 2250-2260.

[22] Monami G, Gonzalez EM, Hellman M, et al. Proepithelin promotes migration and invasion of 5637 bladder cancer cells through the activation of ERK1/2 and the formation of a paxillin/FAK/ERK complex. *Cancer Research* 2006; 66(14): 7103-7110.