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Original Article

Circ-ITCH inhibits bladder cancer progression through miR-184/FOXO3 axis

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Abstract: Objective: This study aimed to explore the role of circ-ITCH in the progression of bladder cancer (BCa). Methods: Kaplan-Meier analysis was performed to evaluate the prognostic significance of miR-184 in bladder cancer. Clustering analysis compared miR-184 expression levels across various BCa cell lines. Cell Counting Kit-8 (CCK-8) and transwell assays were used to assess cell proliferation and migration. Dual-luciferase reporter assays were employed to examine the regulatory relationship among circ-ITCH, miR-184, and FOXO3. Western blot analysis was conducted to investigate the post-transcriptional regulation of the circ-ITCH/miR-184/FOXO3 axis. Results: The study demonstrated a correlation between elevated miR-184 expression and poor prognosis in bladder cancer. Compared to SV-HUC, a normal bladder tissue cell line, most BCa cell lines exhibited increased miR-184 expression. Additionally, miR-184 was found to promote BCa cell progression. Importantly, circ-ITCH was identified as a natural sponge for miR-184 in BCa. Overexpression of circ-ITCH in BCa significantly reduced miR-184 expression, thereby inhibiting cell proliferation and migration. Moreover, FOXO3, a target of miR-184, is regulated by circ-ITCH. The suppression of FOXO3 by miR-184 was counteracted by circ-ITCH, which diminished the tumor-promoting effects of miR-184. Conclusions: This study underscores the pivotal role of the circ-ITCH/miR-184/FOXO3 axis in regulating BCa cell proliferation and migration. It introduces a potential therapeutic target for bladder cancer, suggesting that strategies like circ-ITCH overexpression and miR-184 inhibition could offer promising treatment options.

Keywords: miR-184, circ-ITCH, bladder cancer, proliferation, migration

Introduction

Bladder cancer (BCa), the most common malignancy of the urinary system, and it is the 10th most frequently diagnosed cancer worldwide. It is more prevalent in men, where it ranks as the sixth most common cancer and the ninth leading cause of cancer-related deaths [1]. BCa is classified into two types: muscle-invasive bladder cancer (MIBC) and non-muscle-invasive bladder cancer (NMIBC), with NMIBC accounting for around 80% of cases [2]. Despite advances in treatment, particularly through molecular profiling and checkpoint blockade immunotherapy [2, 3], the 5-year survival rate for advanced BCa remains low due to high rates of postoperative recurrence and distant metastasis [4]. Currently, there are no approved targeted therapies for BCa, emphasizing the need for further understanding of its development and for identifying key molecular biomarkers for treatment and prognosis.

MicroRNAs (miRNAs) are small non-coding RNAs, approximately 22 nucleotides long, that regulate the expression of protein-coding and noncoding RNAs [5]. Extensive research has shown that dysregulated miRNAs are deeply involved in cancer development, making them attractive targets for new therapeutic approaches [6-8]. For instance, miR-184 is downregulated in colon cancer, where it inhibits proliferation and invasion by suppressing C-MYC and BCL-22 [9], or directly targeting IGF-1R [10]. In non-small cell lung cancer (NSCLC), miR-184 acts as a tumor suppressor by inhibiting cell proliferation and invasion through targeting CDC25A and c-Myc [11]. However, in gastric cancer (GC), overexpression of miR-184 promotes proliferation, epithelial-mesenchymal transition (EMT), and inhibits apoptosis [12]. Despite these findings, the role of miR-184 in BCa progression remains unclear.

Circular RNAs (circRNAs), a unique class of RNAs with a covalently-closed loop structure,

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are gaining attention for their regulatory roles in various diseases [13]. Recent studies show that many circRNAs are involved in cancer progression through diverse mechanisms, either promoting or inhibiting tumor growth [14, 15]. In BCa, circRNAs play critical roles by acting as miRNA sponges, influencing cell proliferation, apoptosis, cell cycle regulation, migration, invasion, angiogenesis, and resistance to cisplatin chemotherapy, positioning them as potential therapeutic targets [16, 17].

FOXO3, also known as FOXO3a, is a member of the forkhead transcription factor family and plays a crucial role in tumor suppression. Evidence suggests that FOXO3 is linked to reduced cell proliferation, growth, and survival in various cancers [18]. Overexpression of FOXO3 has been shown to inhibit tumor growth in breast cancer, hepatocellular carcinoma, glioblastoma, and gastric cancer [19-22]. The regulation of FOXO3 is complex, involving post-transcriptional suppression by miRNAs [23]. The 3'-untranslated region (3'-UTR) of FOXO3 mRNA contains target sequences for miRNAs, including miR-155, miR-132, and miR-212 [24, 25]. However, the role of FOXO3 in BCa remains insufficiently explored.

In this study, we found that miR-184 expression was significantly upregulated in BCa and negatively correlated with overall survival. Overexpression of miR-184 in BCa cell lines promoted cell proliferation and migration, whereas miR-184 inhibitors suppressed these effects. Notably, we identified that circ-ITCH functions as a competing endogenous RNA (ceRNA) for miR-184, increasing FOXO3 expression by sequestering miR-184, thus inhibiting tumor progression in BCa. Our findings reveal a novel mechanism involving the circ-ITCH/miR-184/FOXO3 axis in BCa progression.

Materials and methods

Cell lines and cell culture

The BCa cell lines (EJ, T24, 253J, RT4) and normal bladder tissue cell line SV-HUC were provided by the Cell Bank of the Chinese Academy of Sciences (Shanghai, China). All cells were cultured in Dulbecco's modified Eagle's medium (DMEM) (Gibco, Life Technologies, Carlsbad, CA, USA) containing 10% fetal bovine serum (FBS) (Gibco). Cells were cultured at 37°C in a humidified incubator containing 5% CO_2.

Cell transfection and plasmids construction

miR-184 mimics, inhibitors and their related negative control oligonucleotides were designed and synthesized by Ribobio (Guangzhou, China). To establish stable overexpression of circ-ITCH, cDNA of Circ-ITCH was cloned into pcDNA3.1(+) CircRNA Mini Vector. FOXO3 siRNAi: AAUGUGACAUGGAGUCCAUUA.

RNA extraction, reverse transcription PCR and quantitative real-time PCR

Total RNA was extracted using TRIzol reagent (Invitrogen, Carlsbad, CA) following the manufacturer's instructions. For Circ-ITCH, first-strand cDNA was synthesized using Reverse Transcriptase kit (Tiangen, China). The abundance of miR-184 was measured using quantitative stem-loop reverse transcription polymerase chain reaction (stem-loop RT-PCR). Mature miR-184 and U6 snRNA were transcribed with the ImProm-II™ Reverse Transcription System (Promega) using the Stem-loop RT primer miR-184-RT and random nonadeoxyribonucleotide primers. Quantitative Real-Time PCR (qPCR) was performed using the SYBR Green method (Applied Biosystems, USA) on the 7900 Real-Time PCR System with the SDS 2.4 software sequence detection system (Applied Biosystems, USA). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) or U6 small nuclear RNA (U6 snRNA) was used as a control for mRNA or miRNA, respectively. The expression levels were calculated using the 2-ΔΔCt method. Primer sequences were synthesized by Sangon Biotech (Shanghai, China) as follows: circ-ITCH-F: 5'-GCAGAGGCCAACACTGGAA-3'; circ-ITCH-R: 5'-TCCTTGAAGCTGACTACGCTGAG-3'; Stem-loop RT primer sequence for miR-184: 5'-GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACACCCTT-3'; miR-184-F: 5'-CGCGTGGACGGAGAACTGAT-3'; miR-184-R: 5'-ACCCUUAUCAGUUCUCCGUCCA-3'; U6-F: 5'-CGCTTCGGCAGCACATATAC-3'; U6-R: 5'-CAGGGGCCATGCTAATCTT-3'; GADPH-F: 5'-GCTGTAGCCAAATCGTTGT-3'; GAPDH-R: 5'-CCAGGTGGTCTCCTCTGA-3'.

Western blot

Cells were lysed in RIPA buffer (Beyotime, China) supplemented with 1% protease/phos-

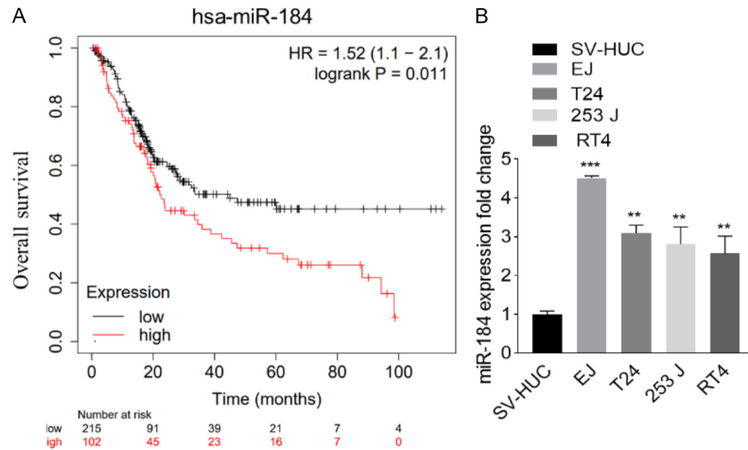


Figure 1. MiR-184 is associated with poor outcomes in bladder cancer (BCa) and is upregulated in BCa cells. A. Kaplan-Meier survival analysis of the correlation of miR-184 expression and overall survival in BCa patients from KM-plotter online database. B. Relative expression level of miR-184 in a normal bladder tissue cell line SV-HUC and bladder cancer cell lines EJ, T24, 253J, RT4 by qRT-PCR. Statistical significance was indicated by **P<0.01, ***P<0.001.

phatase inhibitors (Thermo, USA). The lysates were resolved into 10% SDS-PAGE gels and separated proteins were then transferred onto PVDF membranes (Millipore, USA). After blocking with 5% skim milk powder dissolved in tris-buffered saline for 1 h, the membranes were incubated with specific primary antibodies overnight at 4°C. Primary antibodies used were anti-β-Actin (81115-1-RR, Proteintech, USA) and anti-FOXO3 (10849-1-AP, Proteintech, USA). The next day, membranes were incubated for 1 h with the suitable secondary antibodies, and immunoreactions were visualized and imaged by a using chemiluminescent detection reagents (Azure Biosystems, USA).

Cell migration ability

Cell migration and invasion were assessed using Corning transwell insert chambers (8 μm pore size; Corning) and BD BioCoat Matrigel Invasion Chambers (BD Biosciences, Bedford, MA), respectively. The chemoattractant was 500 ul or 600 ul medium containing 10% FBS which were added into the lower well of each chamber. About 4 × 10^4 prepared cells were added into the chamber and incubated for 18-20 hours at 37°C.

Cell proliferation assay

The cells were seeded in 96-well plates (3 × 10^3 cells per well) and incubated for 4 days at 37°C. The changes of cell proliferation were monitored every day using CCK-8 reagent (Dojindo, Kumamoto, Japan) and the absorbance values were measured at 450 nm via a Hybrid Reader (BioTek Laboratory Instrument).

Luciferase reporter assay

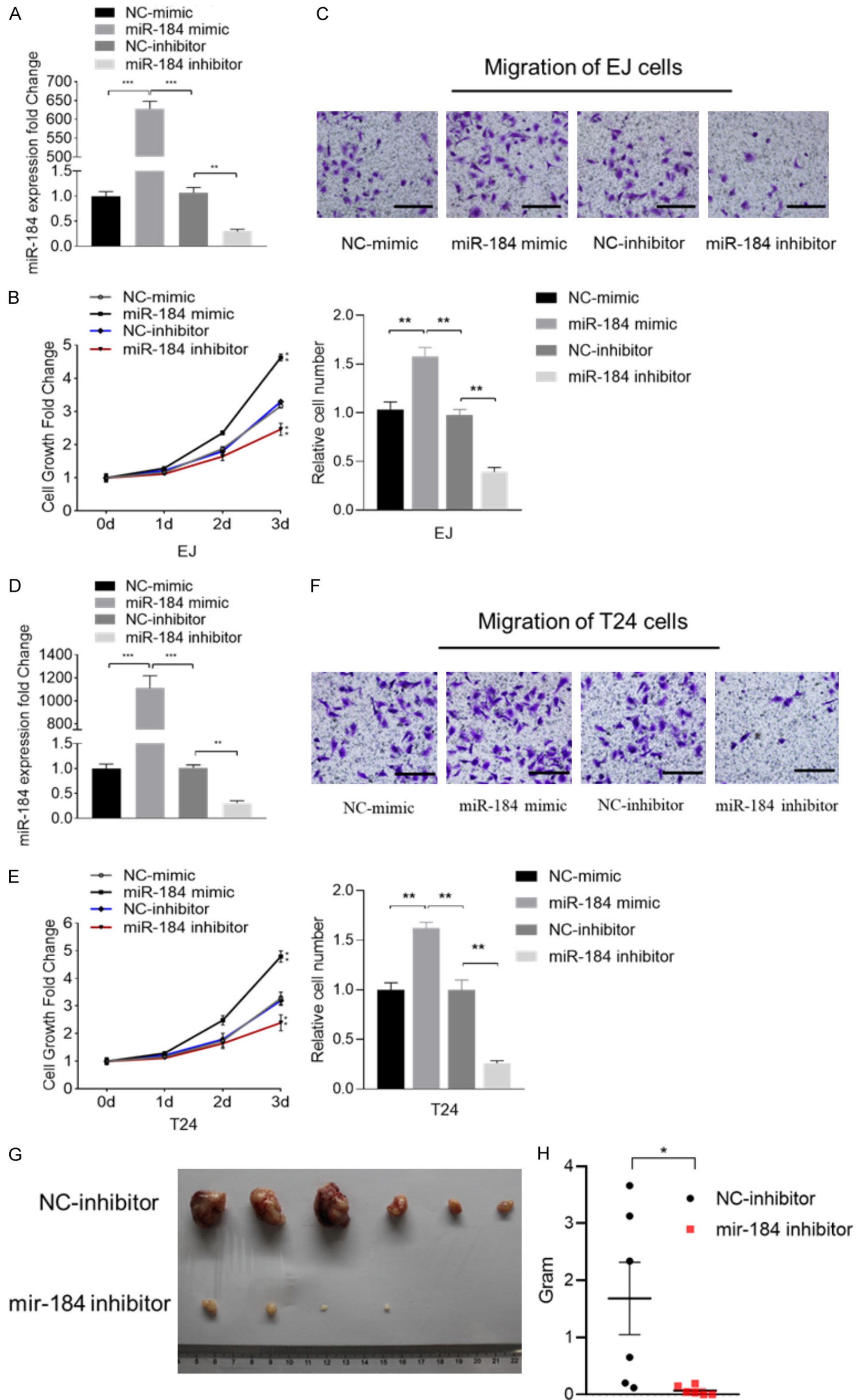
EJ cells were seeded in 24-well plates at the density of 1.0 × 10^5 cells per well. After 24 h, each well was transiently co-transfected with 100 ng of the indicated wild-type or mutant 3'-UTR psiCHECK-2 plasmid and 60 pmol NC or miR-184 mimics using 1.44 μl Lipofectamine reagent (Invitrogen). Cell lysates were collected 24 h after transfection, and Renilla and firefly luciferase activities were measured with a Dual-Luciferase Reporter System (Promega). The Renilla luciferase activities normalized to firefy luciferase activities was the value of relative luciferase activity.

Subcutaneous xenograft models

A total of 5 × 10^6 infected T24 cells were subcutaneously injected into the right back of 6-week-old male BALB/c-nude mice (Shanghai Slack Laboratory animal Co., Ltd). On day 30, the mice were killed under anesthesia and tumors were isolated and weighed.

Statistical analysis

Kaplan-Meier analysis (https://kmplot.com/analysis/) was performed to access the correlation of miR-184 expression and overall survival in BCa patients. The best cut-off method was performed as previously described [26]. All statistical analyses were performed using GraphPad Prism 8 (GraphPad Software, La Jolla, CA, USA). Two-sided Student's t tests were used to assess differences between groups, and results are shown as the mean ± SD of three independent experiments (n=3). Statistical significance was indicated by *P<0.05, **P<0.01, ***P<0.001.



A
NC-mimic
miR-184 mimic
NC-inhibitor
miR-184 inhibitor
miR-184 expression fold Change
C
Migration of EJ cells
NC-mimic
miR-184 mimic
NC-inhibitor
miR-184 inhibitor
B
Cell Growth Fold Change
0d
1d
2d
3d
EJ
Relative cell number
D
F
Migration of T24 cells
E
T24
G
NC-inhibitor
mir-184 inhibitor
H
Gram

Figure 2. miR-184 promotes bladder cancer cell progression. A. Relative expression level of miR-184 in EJ cells transfected with miR-184 mimic or inhibitor. B. Cell proliferation ability was carried out by CCK8 assay in EJ cells. C. Cell migration ability was carried out by transwell assay. Bar =200 μm. D. Relative expression level of miR-184 in T24 cells transfected with miR-184 mimic or inhibitor. E. Cell proliferation ability was carried out by CCK8 assay in T24 cells. F. Cell migration ability was carried out by transwell assay in T24 cells. Bar =200 μm. G. Photographs of tumors excised 30 days after inoculation of T24 cells into nude mice (NC-inhibitor vs mir-184 inhibitor). H. The tumor weight from each nude mouse at the end of the excised days. Statistical significance was indicated by *P<0.05, **P<0.01, ***P<0.001.

Table 1. List of potential circRNA targets of hsa-miR-184 predicted by circinteractome and starbase online databases

Circinteractome	Starbase
circFCGBP	circAGRN
circHTT	circCPSF3L
circPIEZO1	circGNB1
circBCORL1	circRER1
circARHGAP35	circH6PD
circITCH	circATP13A2
circACOT11	circUBR4
circTNR	circPHACTR4
circTPCN2	hsa_circ_000570
circGEMIN4	circSRSF4
circZZEF1	circHDAC1
circNEURL4	circEIF2C3
circSBNO2	circSLC6A9
circAP3D1	circPPAP2B
circMAST3	circLEPROT
circSNRNP200	circMAN1A2
circWHSC1	circPSMB4
circFGFR1	circS100A16
circNOL6	circFLAD1
circGRIN2D	circNES
circAGO2	circC1orf112
circSPAG5	circCACNA1E
circEPHA2	circSMG7
circPTPRF	circINTS7
circDPYD	circCENPF
circHMCN1	TCONS_l2_00001808
circIDE	circTP53BP2
circFBXW4	circFBXO28
circSTT3A	circWDR26
circNCAPD3	circTRIM11
circANO6	circPPPDE1
	circHADHB
	circCAD
	circMSH6
	circSH3BP4
	circKIF1A
	circD2HGDH
	circBHLHE40
	circUBP1
	circCPOX
	circHEG1
	circPLXNA1
	circTBL1XR1
	circANKRD17
	hsa_circ_001160
	circENC1
	circREEP5
	circSMAD5
	circHMGXB3
	circLARP1
	circNPM1
	circDBN1
	circRUFY1
	circTRIM41
	circKIAA0240
	hsa_circ_000438
	circREV3L
	circMICALL2
	TCONS_l2_00025633
	circFBXL18
	circFSCN1
	circGARS
	circMRPS17
	circKCTD7
	circPOM121
	circGTF2I
	circBAZ1B
	circPOM121C
	circLAMB1
	circSND1
	circINSIG1
	circDLC1
	circYWHAZ
	circZFPM2
	circSQLE
	circMYC
	circEIF2C2
	TCONS_l2_00029343
	circTLE1
	circSEC61B
	circSUSD1
	circZER1
	circLRRC8A
	circASS1

circSURF4
circCOL5A1
circNOTCH1
circKLF6
circANK3
circJMJD1C
circTRIM8
circCTBP2
circRIC8A
circTPP1
circNAV2
circNAT10
circCREB3L1
circMTCH2
circINCENP
circWDR74
circCOX8A
circSF1
circPLEKHB1
circFOXM1
circFGFR1OP2
circADCY6
circMCRS1
circDIP2B
circPTGES3
circGNS
circOSBPL8
circATXN2
circALDH2
circGCN1L1
circMLEC
circCAMKK2
circPITPNM2
circLATS2
circIRS2
circHNRNPC
circKTN1
circKIAA0247
circZDHHC22
circCYFIP1
circMAP1A
circDIS3L
circCSK
circIDH2
circTELO2
circTSC2
circSRRM2
circTNRC6A
circNFATC2IP
circGOT2
circST3GAL2
circGABARAPL2
circCENPN
circZCCHC14
circSLC7A5
circFAM57A
circELAC2
circSUZ12
circLASP1
circKRT19
circC17orf58
circCANT1
circBAIAP2
circARHGDIA
circCEP192
circEPG5
circPTBP1
circC19orf25
circSH3GL1
circHDGFRP2
circFARSA
circCALR
circAKAP8
circELL
circSNRPA
circPVR
circPVRL2
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circLIG1
circHRC
circPRR12
circPPP6R1
circTBC1D20
circCDC25B
circTM9SF4
circITCH
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circSRC
circPPP1R16B
circBMP7
circSCAF4
circRRP1B
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circTNRC6B
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circKDM6A
circSTAG2
circSLC6A8
circSLC25A6

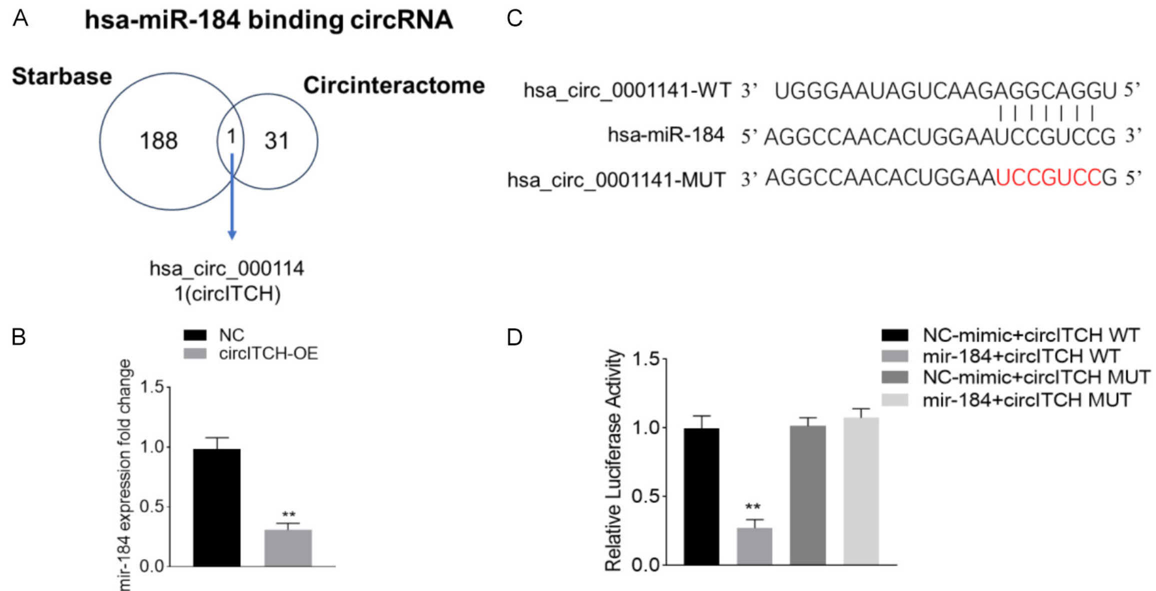

Figure 3. Circ-ITCH functions as a sponge of miR-184 in bladder cancer. A. Venn diagram showing the overlap of the target circRNAs of hsa-miR-184 predicted by circinteractome and starbase online databases. B. Relative expression level of mir-184 transfected with circ-ITCH in bladder cancer cell lines EJ. C. A schematic model shows potential-binding sites for miR-184 and circ-ITCH, and a schematic of miR-184 and wild-type/mutant circ-ITCH binding site sequences with luciferase reporter vectors. D. Luciferase activity of the circ-ITCH luciferase reporter vector (WT or MUT) in EJ cells co-transfected with the miR-184 mimic or NC. Statistical significance was indicated by **P<0.01.

Results

MiR-184 is associated with poor outcomes in bladder cancer (BCa) and is upregulated in BCa cells

To better understand the role of miR-184 in BCa, we analyzed its correlation with BCa using an online database. Kaplan-Meier analysis showed that patients with high miR-184 expression had worse overall survival (**Figure 1A**). We further examined miR-184 expression in BCa cell lines using qRT-PCR. Results revealed that miR-184 levels were significantly higher in BCa cell lines (EJ, T24, 253 J, and RT4) compared to the normal bladder tissue cell line SV-HUC (**Figure 1B**). Together, these findings indicate that miR-184 is associated with poor prognosis and is upregulated in BCa cells.

MiR-184 promotes bladder cancer cell progression

To explore the function of miR-184 in BCa, we used a miR-184 mimic and inhibitor to assess its effects on BCa cells. RT-qPCR confirmed the effectiveness of the miR-184 mimic and inhibitor in EJ cells (**Figure 2A**). The miR-184 mimic enhanced migration and proliferation of EJ cells, while the miR-184 inhibitor had the opposite effect (**Figure 2B**, **2C**). The same validation was performed in T24 cells, where the miR-184 mimic promoted migration and proliferation, and the inhibitor caused the reverse effects (**Figure 2D-F**). Subcutaneous tumor transplantation formation experiment in nude mice showed that miR-184 inhibitor significantly decreased tumor growth capability (**Figure 2G**, **2H**). These findings suggest that miR-184 promotes BCa progression.

Circ-ITCH functions as a sponge of miR-184 in bladder cancer

Circular RNAs (circRNAs) have been reported to function as 184-184es BCa rimen for miRNAs, regulating the expression of downstream genes. We conducted a cross-analysis using CircInteractome and starBase databases (**Table 1**), identifying circ-ITCH as a potential miR-184 binding partner (**Figure 3A**). StarBase predicted the binding sites between circ-ITCH and miR-184. Circ-ITCH was found to decrease miR-184 expression (**Figure 3B**), suggesting that it targets miR-184. To validate the sponge effect of circ-ITCH on miR-184, we constructed wild-

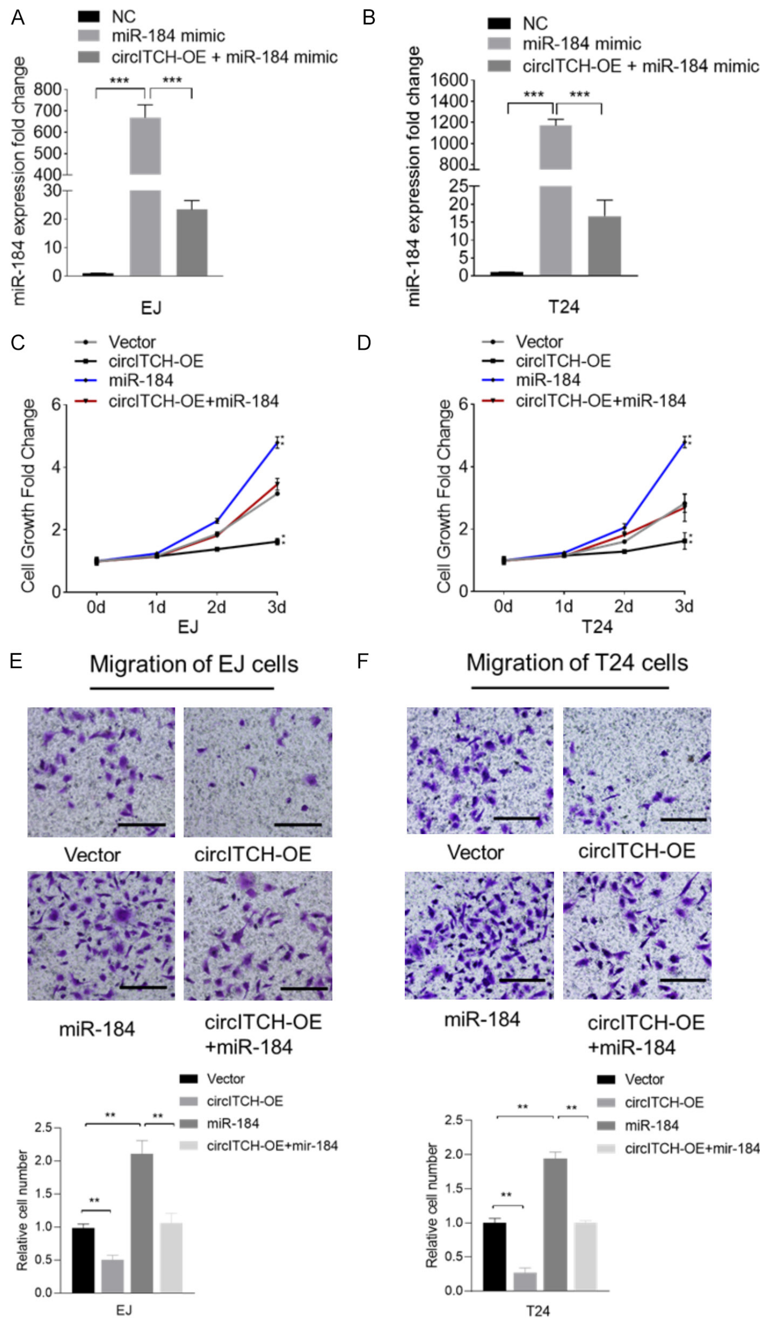

Figure 4. Circ-ITCH reverses the pro-tumor effects of miR-184 on bladder cancer progression. A. Relative expression level of miR-184 transfected with miR-184 mimic or/and circ-ITCH in EJ cells. B. Relative expression level of miR-184 transfected with miR-184 mimic or/and circ-ITCH in T24 cells. C. Cell proliferation ability of EJ cells transfected with miR-184 mimic or/and circ-ITCH by CCK8 assay. D. Cell proliferation ability of T24 cells transfected with miR-184 mimic or/and circ-ITCH by CCK8 assay. E. Cell migration ability of EJ cells transfected with miR-184 mimic or/and circ-ITCH by transwell assay. Bar =200 μm. F. Cell migration ability of T24 cells transfected with miR-184 mimic or/and circ-ITCH by transwell assay. Bar =200 μm. Statistical significance was indicated by *P<0.05, **P<0.01, ***P<0.001.

type circ-ITCH (circ-ITCH WT) and a mutant form (circ-ITCH MUT) in a dual-luciferase vector psiCHECK™siCHECKT in (**Figure 3C**). Luciferase assays revealed that miR-184 significantly suppressed the activity of circ-ITCH WT but had no effect on circ-ITCH MUT (**Figure 3D**). These results indicate that circ-ITCH directly regulates miR-184 expression in BCa cells.

Circ-ITCH reverses the pro-tumor effects of miR-184 on bladder cancer progression

To investigate whether circ-ITCH inhibits miR-184-mediated BCa proliferation and migration, rescue experiments were conducted by co-infecting cells with miR-184 mimics and circ-ITCH overexpression plasmids. Overexpression of circ-ITCH significantly reduced miR-184 levels after miR-184 mimic treatment in both EJ and T24 cells (**Figure 4A**, **4B**). Circ-ITCH overexpression also reversed the miR-184-induced increase in proliferation and migration in both cell lines, whereas the control vector did not affect miR-184-mediated changes (**Figure 4C-F**). These findings indicate that circ-ITCH mitigates the tumorigenic and metastatic effects of miR-184 in BCa cells.

Circ-ITCH regulated FOXO3 through competing for miR-184 in BCa cells

Using the TargetScan database, we identified several potential miR-184 targets, with FOXO3 standing out due to its tumor-suppressive role in cancer [23] (**Figure 5A**). FOXO3 mRNA expression was reduc-

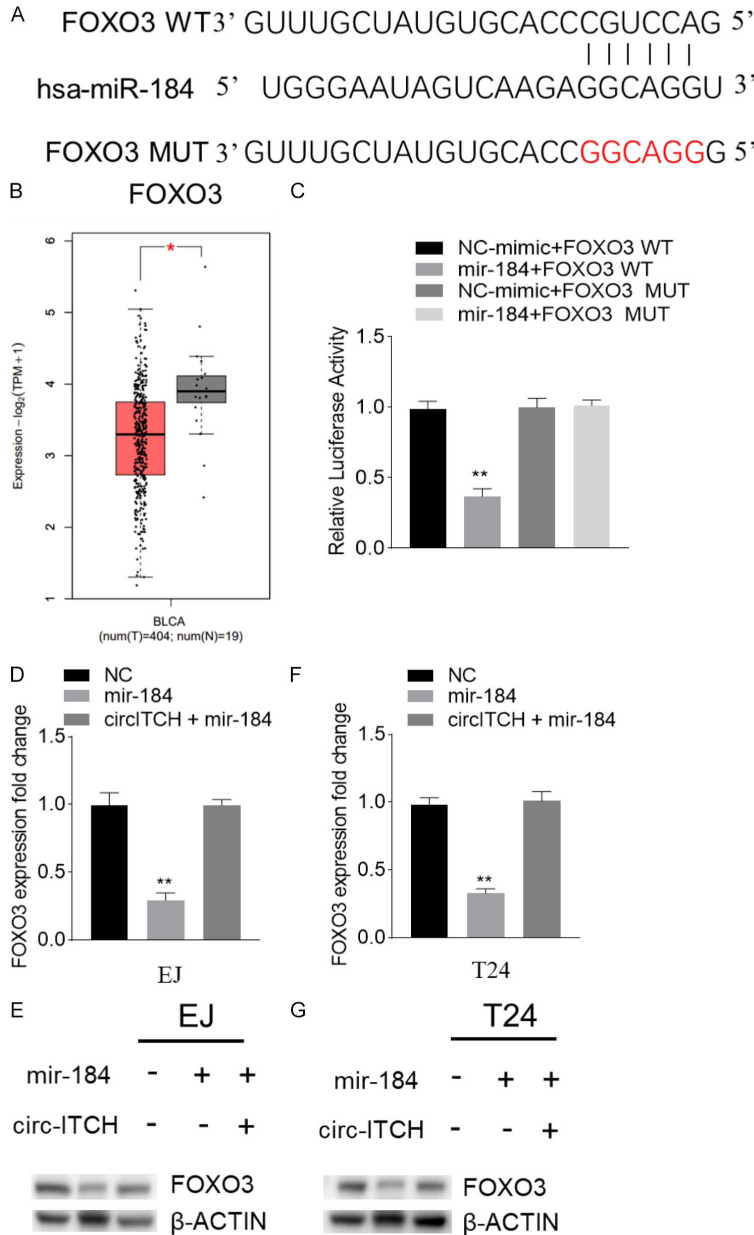

Figure 5. Circ-ITCH regulated FOXO3 through competing for miR-184 in BCa cells. A. A schematic model shows potential-binding sites for miR-184 and FOXO3, and a schematic of miR-184 and wild-type/mutant FOXO3 binding site sequences with luciferase reporter vectors. B. FOXO3 expression of tumor and normal bladder tissue from TCGA database. C. Luciferase activity of the FOXO3 luciferase reporter vector (WT or MUT) in EJ cells cotransfected with the miR-184 mimic or NC. D. Relative mRNA expression level of FOXO3 transfected with mir-184 mimic or/and circ-ITCH in EJ cells. E. The protein level of FOXO3 transfected with mir-184 mimic or/and circ-ITCH in EJ cells. F. Relative mRNA expression level of FOXO3 transfected with mir-184 mimic or/and circ-ITCH in T24 cells. G. The protein level of FOXO3 transfected with mir-184 mimic or/and circ-ITCH in T24 cells. Statistical significance was indicated by *P<0.05, **P<0.01.

ed in BCa tissues, as shown by TCGA data (**Figure 5B**). Dual-luciferase assays confirmed that miR-184 significantly suppressed the activity of FOXO3 WT, but had no effect on FOXO3 MUT (**Figure 5C**). Additionally, miR-184 reduced FOXO3 mRNA and protein levels, but circ-ITCH overexpression reversed this effect (**Figure 5D-G**). These results demonstrate that the circ-ITCH/miR-184 axis regulates FOXO3 in BCa cells.

Circ-ITCH overexpression suppresses BCa cells progression by modulating FOXO3

To further explore the role of circ-ITCH and FOXO3 in BCa progression, we co-infected cells with circ-ITCH overexpression plasmids and FOXO3 siRNA. Circ-ITCH overexpression suppressed BCa cell proliferation and migration, but this effect was reversed by FOXO3 silencing (**Figure 6A-F**). Overall, these results suggest that circ-ITCH suppresses BCa progression by regulating FOXO3.

Discussion

The expression pattern of microRNAs (miRNAs) is associated with cancer types, stages, and other clinical variables, making miRNAs profiling a valuable tool for cancer diagnosis and prognosis [27]. MiR-184 has been shown to play varying roles across different tumors. For example, serum levels of miR-184 are significantly lower in non-small cell lung cancer (NSCLC) patients compared to those with pneumonia and healthy individuals. These levels are closely correlated with smoking history, tumor-node-metastasis (TNM) stage, and the degree of pathological differentiation. The area under the curve (AUC) of serum miR-184 for predicting 3-year survival in NSCLC

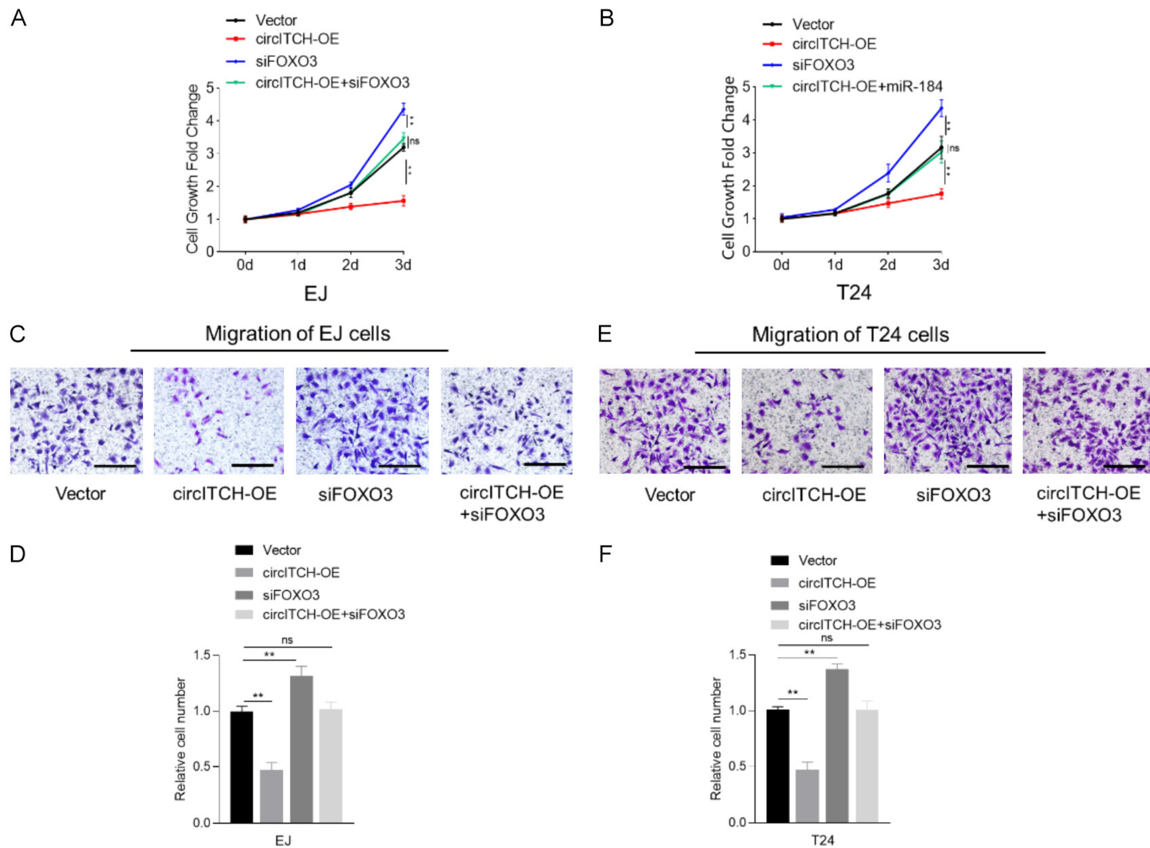

Figure 6. Circ-ITCH overexpression suppresses BCa cell progression by modulating FOXO3. A. Cell proliferation ability of EJ cells transfected with circ-ITCH or/and siFOXO3 in EJ cells by cck8 assay. B. Cell proliferation ability of T24 cells transfected with circ-ITCH or/and siFOXO3 in T24 cells by cck8 assay. C, D. Cell migration ability of EJ cells transfected with circ-ITCH or/and siFOXO3 in EJ cells by transwell assay. Bar =200 μm. E, F. Cell migration ability of T24 cells transfected with circ-ITCH or/and siFOXO3 in T24 cells by transwell assay. Bar =200 μm. Statistical significance was indicated by **P<0.01. ns, not significant.

patients was 0.869, suggesting its potential as a diagnostic and predictive molecular marker [28]. Additionally, low miR-184 levels in NSCLC predict worse prognosis, with miR-184 inhibiting cell proliferation and invasion by targeting CDC25A and c-Myc [11]. In addition, miR-184 is down-regulated in oral cancer [29], colorectal cancer [10] and breast cancer [30]. Conversely, overexpression of miR-184 has been found to promote proliferation and inhibit apoptosis in gastric cancer (GC) [12], osteosarcoma [31], head and neck squamous cell carcinoma [32], and hepatocellular carcinoma [33]. Prior to this study, the role of miR-184 in bladder cancer (BCa) had not been explored.

In our research, we observed that higher miR-184 expression is correlated with shorter overall survival in BCa patients, indicating that miR-184 is significantly associated with poor prognosis. MiRNAs are involved in various aspects of tumor biology, including proliferation, apoptosis, invasion/metastasis, and angiogenesis, thus playing either oncogenic or tumor-suppressive roles. Compared to SV-HUC, a normal bladder tissue cell line, miR-184 was highly expressed in most BCa cell lines. Moreover, overexpression of miR-184 promoted BCa cell proliferation and migration, while miR-184 inhibitors had the opposite effect, suggesting that miR-184 acts as an oncogene in BCa. MiR-184 influences tumorigenic signaling pathways, like how AKT/mTORC1 and Wnt/nt/R-184 influences tumorigenic signaling pathways; such as Myc and apoptotic proteins like Bcl-2 [34]. We found that miR-184 significantly reduced the mRNA and protein levels of FOXO3 (also known as FOXO3a or FKHRL1), a gene associated with longevity that generally functions as a tumor suppressor in BCa and other cancers [35, 36]. FOXO3 mRNA expression in BCa tissues was

significantly lower than in normal tissues (P< 0.05), and its negative expression was identified as a risk factor for poor prognosis in BCa [18].

Circular RNAs (circRNAs), a newly recognized class of non-coding RNA molecules, have regulatory capabilities characterized by their stability, high abundance, and evolutionary conservation [37, 38]. With advancements in high-throughput sequencing and bioinformatics, thousands of circRNAs have been identified, many of which are expressed in tissue- and disease-specific manners, acting as miRNA sponges, protein scaffolds, transcriptional regulators, and more [39]. CircRNAs have been shown to play various biological roles in multiple cancers, including BCa. In our study, we identified circ-ITCH as an endogenous miR-184 sponge. Overexpression of ci-rc-ITCH in BCa significantly suppressed miR-184 expression, thereby inhibiting BCa progression by restoring FOXO3 function. Our findings further underscore the tumor-suppressive role of circ-ITCH in BCa [16, 40]. We provide evidence that the circ-ITCH/miR-184/FOXO3 axis is involved in BCa progression, highlighting two promising molecular targets for BCa therapeutic intervention, although further research is required to fully elucidate the detailed mechanisms. In conclusion, our findings suggested that Circ-ITCH can inhibit bladder cancer progression through the miR-184/FOXO3 axis.

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Disclosure of conflict of interest

None.

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