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LncRNA CASC15 functions as an oncogene by sponging miR-130b-3p in bladder cancer

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Abstract. – OBJECTIVE: Recent studies have revealed that long noncoding RNAs (lncRNAs) are dysregulated in malignant tumors and participates in carcinogenesis. The purpose of our study was to uncover the mechanisms underlying lncRNA CASC15 in bladder cancer (BLCA).

PATIENTS AND METHODS: In this research, Real Time-quantitative Polymerase Chain Reaction (RT-qPCR) was performed to detect cancer susceptibility candidate 15 (CASC15) expression in BLCA samples and cells. Besides, the wound healing assay and transwell assay were performed in BLCA cells after CASC15 was knocked down. Furthermore, the bioinformatics analysis and dual-luciferase reporter assay were conducted to explore the target miRNA of CASC15, which was further verified through rescue experiments in BLCA cells.

RESULTS: CASC15 expression was upregulated in BLCA tissue samples. Moreover, CASC15 downregulated the miR-130b-3p expression and promoted cell migration and invasion in BLCA *in vitro*. The rescue experiments also revealed that the inhibitory effects by the silence of CASC15 could be reversed through the inhibition of miR-130b-3p.

CONCLUSIONS: Our study suggests a vital regulatory mechanism of CASC15 in BLCA and the CASC15/miR-130b-3p axis might serve as a new therapeutic interventional target for BLCA patients.

Key Words:
Long noncoding RNA, CASC15, BLCA, MiR-130b-3p.

Introduction

Bladder cancer (BLCA) is the most common genitourinary malignancy in the world and ranks the sixth most commonly diagnosed malignancy in men[1,2]. The incidence and mortality of BLCA are significantly increasing over the past decades. It is reported that there are approximately 380,000 new BLCA cases and 150,000 deaths every year worldwide[3]. The 5-year survival rate is [illegible] for BLCA patients who develops migration to sentinel lymph nodes, and 10% for those with migration to surrounding organs and distant organs (https://www.cancer.net/cancer-types/bladder-cancer/statistics). Therefore, it's very important to identify promising early detection markers and effective therapeutic methods to improve the outcomes of BLCA.

The long noncoding RNAs (lncRNAs) are a large class of non-protein-coding transcripts that are over 200 nt in length. LncRNAs have [illegible] key regulators of important biological processes and are widely involved in the development and progression of tumors. For example, lncRNA CAMTA1 contributed to cell proliferation and cell motility in breast cancer by targeting miR-20b[4]. The knockdown of DGCR8 suppressed cell proliferation, cell migration, and cell invasion in ovarian cancer[5]. Through the regulation of miR-34c expression and by targeting MUC2, lncRNA AF147447 repressed cell proliferation and cell invasion of gastric cancer patients infected with *Helicobacter pylori*[6]. Activated by H3K27, lncRNA CCAT1 promoted cell proliferation and cell migration in esophageal squamous cell carcinoma through the regulation of the expression of SPRY4 and HOXB13[7]. LncRNA CASC15 is a novel lncRNA discovered in numerous cancers. Moreover, miR-130b-3p acted as a tumor suppressor during tumorigenesis and was predicted as a potential target of the cancer susceptibility candidate 15 (CASC15). However, the role of CASC15/ miR-130b-3p in BLCA is still unclear.

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In this study, we firstly found out that CASC15 could regulate cell migration and invasion by sponging miR-130b-3p in BLCA cells, which might offer a new insight on the therapy of BLCA.

Patients and Methods

Clinical Samples

Human BLCA tissues and adjacent tissues were obtained from 56 BLCA patients at Shandong Provincial Hospital Affiliated to Shandong University from March 2016 to December 2018. This study was approved by the Hospital's Protection of Human Subjects Committee of Shandong Provincial Hospital Affiliated to Shandong University. Written informed consent was offered by every BLCA patient before the surgery.

Cell Culture

The BLCA cell lines (BIU, J82, SW780, UM-UC3) were obtained from American Type Culture Collection (ATCC; Manassas, VA, USA). The culture medium Dulbecco's Modifie[illegible]gle's Medium (DMEM; Hyclone, Thermo [illegible] Scientific, Waltham, MA, USA) and 10% [illegible] bovine serum (FBS; Gibco, Invitrogen, Carls[illegible] CA, USA) were used to incubate the cells.

Cell Transfection

For transfection, lentivi[illegible] e[illegible]ssing short-hairpin RNA (shRNA) [illegible]eting [illegible] was compounded and then [illegible] pLenti-EF1a-EGFP-F2A-Puro vec[illegible]r (B[illegible]c., San Diego, CA, USA). CAS[illegible] shRNA [illegible]15/shRNA) and negative [illegible]ol shRNA ([illegible]R-NA) were used for tr[illegible] in SW780 BLCA cells with Lipofecta[illegible] 20[illegible]rogen, Carlsbad, CA, USA).

RNA Extract[illegible] and Real Time-quan[illegible]tive [illegible]ymerase Chain Re[illegible] ([illegible]PCR)

TRIzol re[illegible]vitrog[illegible] Carlsbad, CA, USA) was utilize[illegible]e total RNA from tissues [illegible]lls. The [illegible] concentration was meas[illegible]sing an [illegible]traviolet spectrophoto[illegible] (Hi[illegible], Japan). The comple-m[illegible] D[illegible]ucleic Acid (cDNA) was syn[illegible]ccording to the instructions of the PrimeSc[illegible]T MasterMix kit (Invitrogen, Carlsbad, C[illegible]A). β-actin was used as the internal referen[illegible]e in the quantitative analysis of CASC15 expression. The [illegible] used as following: CASC15, fo[illegible]d 5'-[illegible]G-CATGGAAAACCCAG-3' [illegible] reverse 5'-[illegible]G-GACCTGAGCTGTAAG[illegible]'; β-actin, fo[illegible]rd 5'-GATGGAAATCGTCA[illegible]T-3' a[illegible] reverse 5'-TGGCACTT[illegible]GTTG[illegible]. The thermal cycle was [illegible]llows: 30 [illegible]5°C, 5 sec for 40 cycles [illegible]5°C, [illegible] sec at [illegible]C. The relative expressi[illegible]as ca[illegible]ated b[illegible] performing the $2^{-\Delta\Delta CT}$ meth[illegible]

Wound H[illegible]ing As[illegible]

Totally [illegible] 10^4 cells we[illegible]ded into a 6-well plate. T[illegible]ere scratched with a pipette tip and cu[illegible]ed [illegible]ium when growing to about confluen[illegible] of 90[illegible]d closure was viewed at [illegible]fic time poi[illegible] Each assay was independently repeated for three times.

[illegible]nswell Ass[illegible]

[illegible] migratio[illegible]say, the transfected cells were t[illegible] the top of migration chambers (24[illegible], 8-μm pore size; BD Biosciences, Franklin Lakes, NJ, USA) with 200 μL se[illegible]m-free DMEM. For the invasion assay, the cells [illegible]formed to the top of Matrigel-coated [illegible] chambers (24-well insert, 8-μm pore [illegible]e; BD Biosciences, Franklin Lakes, NJ, USA) [illegible]ith 200 μL serum-free DMEM. The bottom [illegible]hamber was added with DMEM and FBS as a [illegible]emoattractant. After being incubated for 48 h, [illegible]he non-invaded cells were removed from the inner part by a cotton swab. After being fixed in 4% formaldehyde, the cells on the lower membrane surface were stained with 0.1% crystal violet. The microscope was used to manually count invading cells in three randomly chosen fields and took the pictures.

Dual-Luciferase Reporter Assay

For the luciferase assay, the 3'-untranslated region (3'-UTR) of CASC15 was cloned into the pGL3 vector (Promega, Madison, WI, USA), which was identified as wild-type (WT) 3'-UTR. The quick-change site-directed mutagenesis kit (Stratagene, La Jolla, CA, USA) was used for the site-directed mutagenesis of the miR-101-3p binding site in CASC15 3'-UTR, which was named as mutant (MUT) 3'-UTR. The cells were transfected with WT-3'-UTR or MUT-3'-UTR and miR-ctrl or miR-101-3p for 48 h. Then, the luciferase assay was conducted according to the Dual-Luciferase Reporter Assay System (Promega, Madison, WI, USA).

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Statistical Analysis

The Statistical Product and Service Solutions (SPSS) 18.0 (SPSS Inc., Chicago, IL, USA) was utilized to conduct the statistical analysis. The two-tailed Student's *t*-test was performed to analyze the difference. $p<0.05$ was considered statistically significant.

Results

Expression Level of CASC15 in Tissues and Cells of BLCA

To determine the role of CASC15 in the tumorigenesis of BLCA, the RT-qPCR was performed to detect CASC15 expression in 56 patients' tissues and 4 BLCA cell lines. CASC15 was remarkably upregulated in the tumor tissue samples compared to that in the adjacent tissues (Figure 1A). Besides, the CASC15 expression level was the highest in SW780 cells among 4 BLCA cells (Figure 1B).

CASC15 Knockdown Repressed Cell [illegible] Growth Ability in BLCA Cells

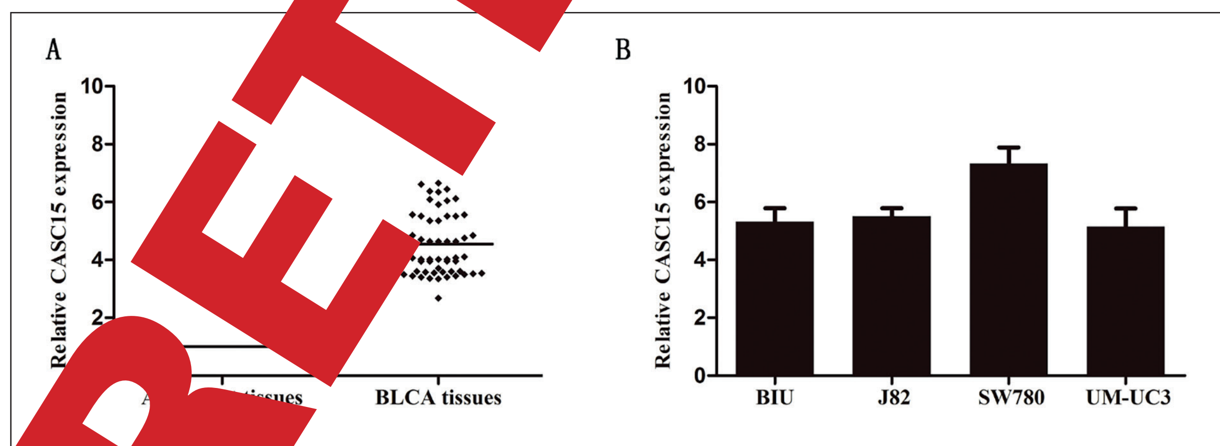
In our study, SW780 cells were chosen [illegible] knockdown of CASC15. The CASC15/shR[illegible] and NC/shRNA were synthesized and transduc[illegible] into SW780 cells. Then, the CASC[illegible]xpression was determined by RT-qPCR (Fi[illegible] Subsequently, the wound healing ass[illegible]esu[illegible]owed that the migrated area of BL[illegible] cell [illegible]ited by the knockdown of C[illegible]re 2B). Besides, the transwell assay re[illegible]d that the number of migrated and [illegible]aded [illegible]ells was reduced after CASC[illegible]was knocke[illegible]wn (Figures 2C and 2D).

CASC15 Inhibits [illegible]CA Tumor[illegible]is Via Sponging [illegible]-130[illegible]p

Then, we use[illegible]ANA[illegible]BASE Predicted v.2 (http://carolina[illegible]-innov[illegible]n.gr/diana_tools/web/in[illegible]ex.p[illegible]base[illegible]2Findex-predicted) to p[illegible]ct the p[illegible]get microRNAs of CASC[illegible]iR-130b-3p, a[illegible]g those predicted microR[illegible] reported to suppress tumorigenes[illegible] va[illegible]ors. The binding sequence of CASC15 by m[illegible]p was shown in Figure 3A[illegible]oreover, miR-[illegible]-3p was upregulated in [illegible]C15/shRNA group compared with that in the [illegible]/shRNA grou[illegible] (Figure 3B). Furthermore, the [illegible]lts of the lu[illegible]ase assay demonstrated that [illegible]uciferase [illegible]ity was significantly reduced [illegible]ansfection of CASC15-WT and mi[illegible] while no significant changes of the luciferase activity were observed in the cells [illegible]transfected CASC15-MUT and miR-130b-3p [illegible]).

The Association Between CASC15 and MiR-130b-3p in [illegible]LCA Migration and Invasion

To further identify the association between CASC15 and miR-130b-3p in the metastasis of BLCA, we conducted rescue experiments in



Figur[illegible] [illegible]sion levels of CASC15 were upregulated in BLCA tissues and cancer cell lines. **A,** CASC15 expression was significant[illegible]ted in the BLCA tissues compared with that in adjacent tissues. **B,** Expression levels of CASC15 relative to β-actin were [illegible]ined in the human BLCA cell lines by RT-qPCR. Data are presented as the mean ± standard error. *$p<0.05$, as compar[illegible]d with the adjacent tissues and control cells.

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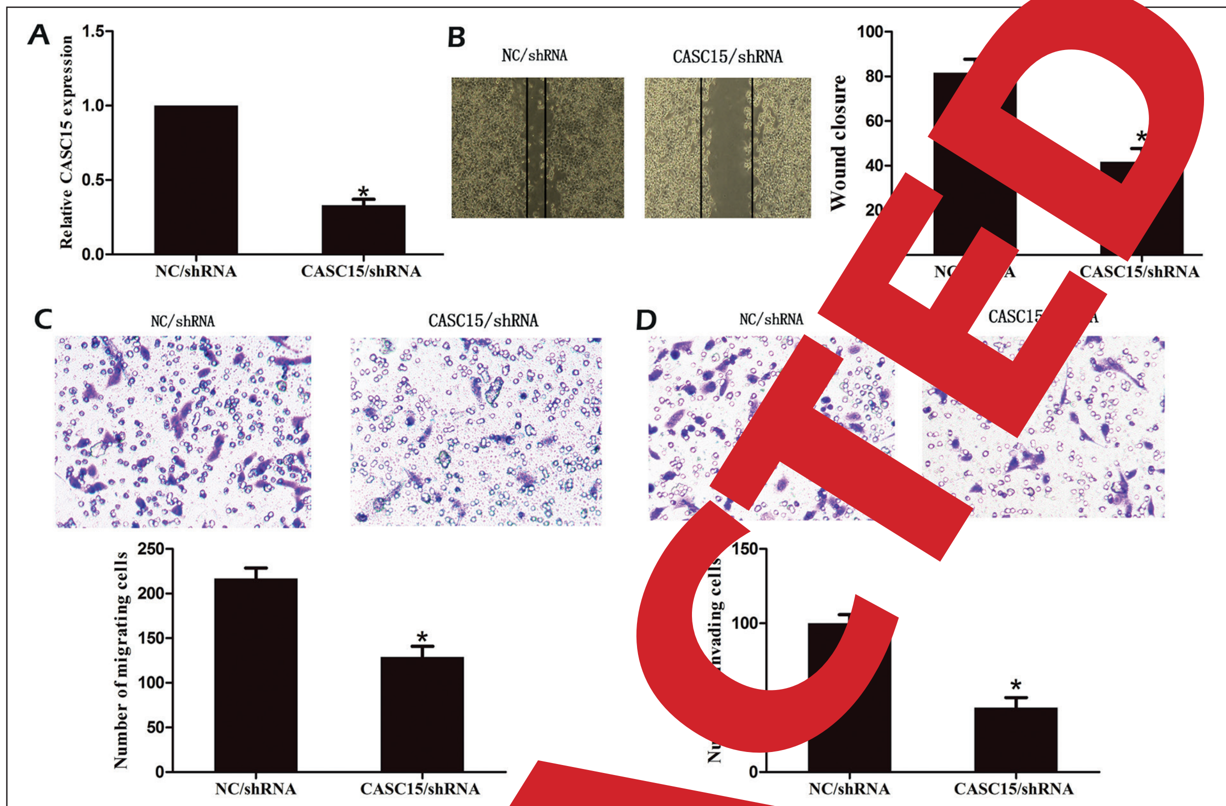
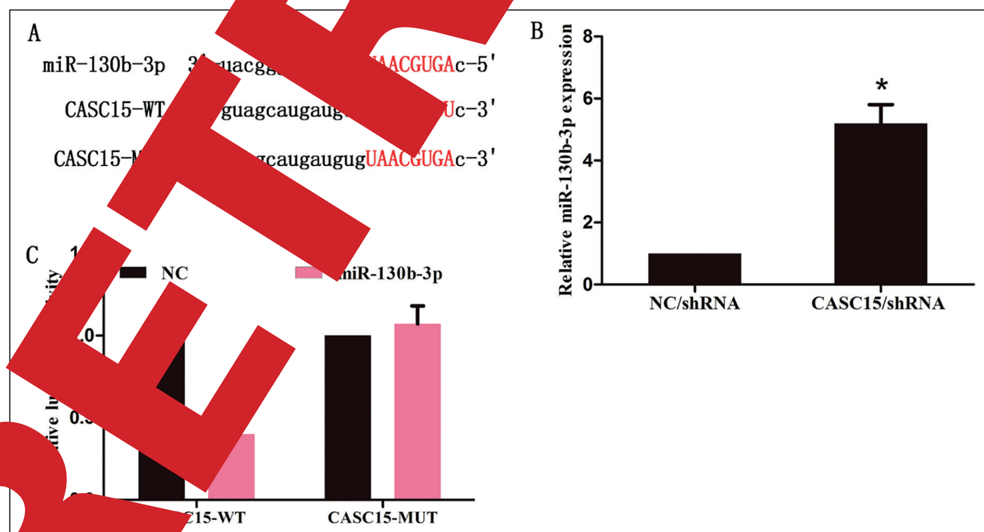


Figure 2. Knockdown of CASC15 inhibited BLCA c[illegible]ration a[illegible] **A,** CASC15 expression in BLCA cells transduced with NC/shRNA or CASC15/shRNA was detected by [illegible]R. β[illegible] was used as an internal control. **B,** Wound healing assay showed that the migrated area of the cells in CASC15/s[illegible] was significantly decreased compared with NC group in SW780 BLCA cells (magnification: 40×). **C,** The transwell [illegible]owed that the number of migrated BLCA cells was decreased in CASC15/shRNA group compared with NC[illegible] group ([illegible]ication: 40×). **D,** The transwell assay showed that number of invaded BLCA cells was decreased in CAS[illegible]A group c[illegible]d with NC/shRNA group (magnification: 40×). The results represent the average of three independe[illegible]ts (mean ± s[illegible]dard error). *p<0.05, as compared with the control cells.



Fi[illegible]action between miR-130b-3p and CASC15 in BLCA. **A,** The binding sites of miR-130b-3p on CASC15. **B,** The mi[illegible]expression was increased in CASC15/shRNA group compared with NC/shRNA group. **C,** Co-transfection of miR-130[illegible]ASC15-WT strongly decreased the luciferase activity, while the co-transfection of miR-130b-3p and CASC15-MUT [illegible] change the luciferase activity. The results represent the average of three independent experiments. Data are presented as the mean ± standard error. *p<0.05, as compared with the control cells.

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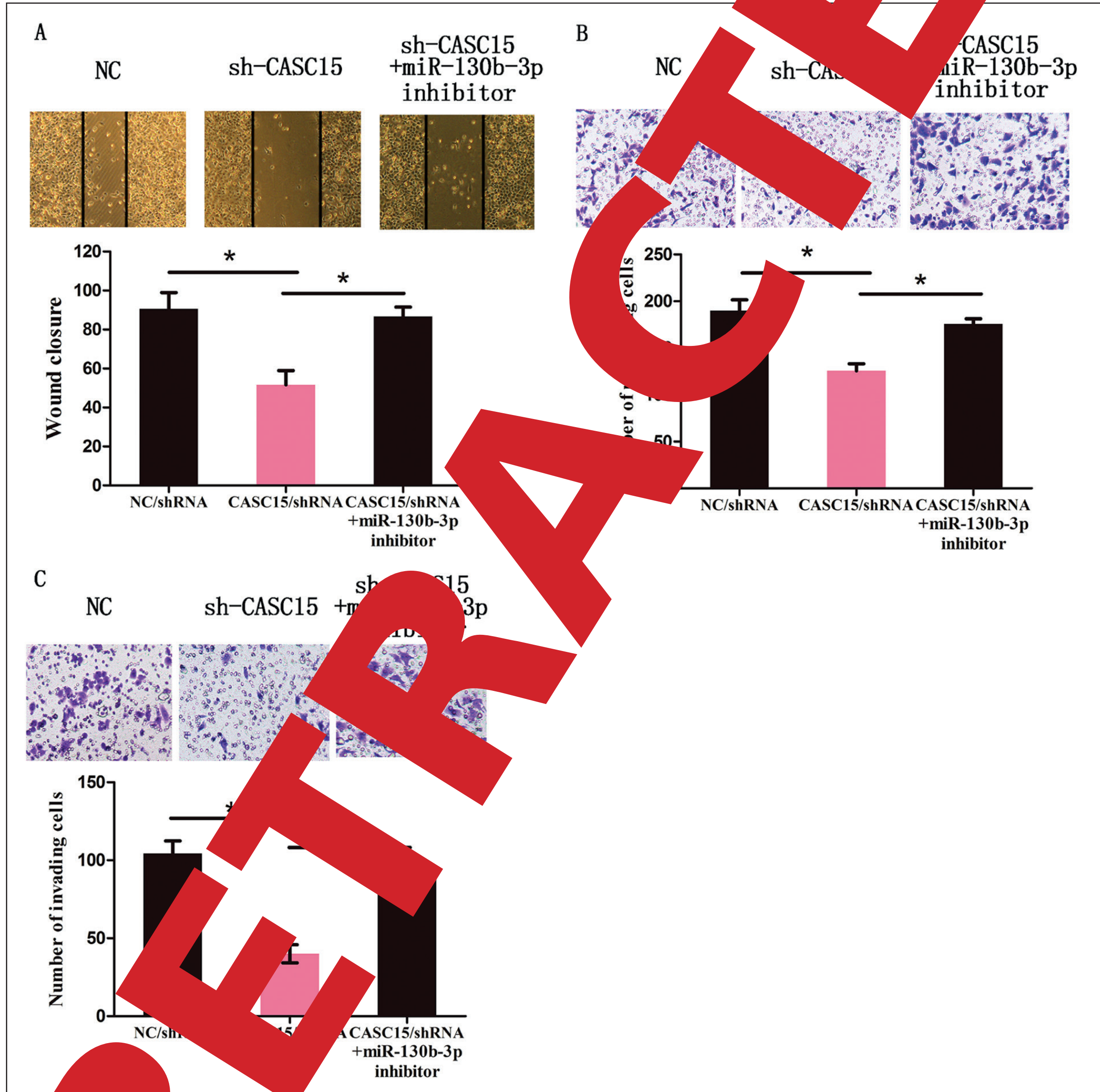
BLCA cells. The wound healing assay revealed that the knockdown of miR-130b-3p could reverse the inhibition of cell migration by the silence of CASC15 in the SW780 cells (Figure 4A). Besides, the results of the transwell assay showed that the knockdown of miR-130b-3p could reverse the inhibition of cell migratio[illegible]ce of CASC15 in SW780 cells (Fi[illegible]4B). M[illegible]ile, the results of the transwe[illegible]ssay also i[illegible]ed that the knockdown of m[illegible]0b-3p could [illegible]rse the inhibition of the cell i[illegible]y CAS[illegible] inhibition in SW780 ce[illegible]s (Figu[illegible]



Fi[illegible]4. Re[illegible]s showed that the knockdown of miR-130b-3p could reverse the effect of silenced CASC15 on B[illegible] migra[illegible]n. **A,** The wound healing assay showed that wound closure was significantly decreased after the sh[illegible]15 in BLCA cells, which could be reversed by the knockdown of miR-130b-3p (magnification: 40×). **B,** The transw[illegible]howed that the number of migrated cells was significantly decreased after the knockdown of CASC15 in BLCA cells, [illegible]ld be reversed by knockdown of miR-130b-3p (magnification: 40×). **C,** The transwell assay showed that the number of in[illegible] cells was significantly decreased after the knockdown of CASC15 in PC cells, which could be reversed by knockdown of m[illegible]R-130b-3p (magnification: 40×). *$p<0.05$, as compared with the control cells.

Discussion

Many studies have proved that lncRNAs participated in the regulation of BLCA development. For example, LncRNA CSC8 inhibited the cell proliferation in BLCA by regulating the glycolytic pathway, which is associated with an advanced stage of BLCA[8]. Through the interaction with miR-124, lncRNA XIST modulated the tumor growth cell migration in BLCA by targeting androgen receptor[9]. Besides, lncRNA linc00346 could enhance the malignant phenotypes of BLCA[10], while the knockdown of lncRNA FGFR3-AS1 inhibited cell proliferation and induced cell apoptosis and cell motility reduction in BLCA[11].

Recently, the cancer susceptibility candidate 15 (CASC15) has been indicated to play an important role in tumorigenesis in several cancers. For example, by regulating the expression of SOX4, CASC15 participated in RUNX1-rearranged acute leukemia[12]. CASC15 was also reported to promote cell proliferation in gastric cancer and might be a risk factor for gastric cancer progno-sis[13]. CASC15 enhanced cell metastasis and [illegible]tocarcinogenesis in hepatocellular carcino[illegible] was also correlated with poor prognosis f[illegible] patients[14]. Our study showed that the express[illegible] of CASC15 was upregulated in BLCA tissue[illegible]. Furthermore, after CASC15 was kn[illegible]d down, the cells' migration and invasi[illegible] were suppressed in BLCA. These d[illegible] indi[illegible]d that CASC15 functioned as an onc[illegible]e an[illegible]ed the metastasis of BLCA.

Then, a bioinformatics a[illegible]lysis [illegible] to predict the potential target [illegible]RNAs [illegible]15. MiR-130b-3p was chos[illegible]r our followin[illegible]dy for its vital role in re[illegible]he progression of numerous carcinom[illegible]or [illegible] miR-130b-3p was aberrantly expressed in pr[illegible]ncer and inhibited prosta[illegible]ancer metastas[illegible]ia targeting MMP2[15,16][illegible]o, mi[illegible]-130b could inhibit cell growth and p[illegible]ote c[illegible]poptosis via regulating CYLD in g[illegible]ca[illegible]. Be[illegible], miR-130b-3p was downreg[illegible]reast [illegible]er and inhibited cell invasion and [illegible]*vitro*[18].

The [illegible]tion b[illegible] miR-130b-3p and CAS[illegible]rther stud[illegible]. After CASC15 was kn[illegible]d do[illegible] miR-130b-3p showed a[illegible]. In addition, the luciferase [illegible]lts demonstrated that miR-130b-3p could b[illegible]targeted by CASC15. The above results reve[illegible]at CASC15 might realize its function in BL[illegible] via sponging miR-130b-3p. To further verify the interaction [illegible]SC15 and miR-130b-3p in BLCA[illegible] were then performed. We [illegible]nd that the [illegible]bition of BLCA cell migr[illegible] and invasio[illegible]gered by the silence of [illegible] were r[illegible]sed after miR-130b-3p wa[illegible] knock[illegible].

[illegible]nclu[illegible]ns

We showe[illegible] tha[illegible] C[illegible]15 could enhance BLC[illegible]ll migra[illegible]vasion through sponging [illegible]-130b-3p an[illegible]erved as a new biomark[illegible]rogression of BLCA.

C[illegible]t of Interest

T[illegible]uthors declare that they have no conflict of interests.

[illegible]owledge[illegible]ts

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