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LncRNA DLEU1 accelerates the malignant progression of clear cell renal cell carcinoma *via* regulating miRNA-194-5p

G.-Z. HE[1], S.-Y. YU[2], Q.-P. ZHOU[1], M.-L. CHEN[1], Y.-W. ZHANG[3], Y. Z[illegible]G[4], Z.-B. ZHANG[5], Z.-Y. HAN[6], J. YU[6]

[1]Department of Ultrasound, University of Chinese Academy of Sciences Sh[illegible]en Ho[illegible]al, Department of Interventional Ultrasound, Chinese PLA General Hospital, [illegible]her[illegible]ina
[2]Department of Interventional Ultrasound, Tianyou Hospital Affiliated [illegible]Yu[illegible]versity Science and Technology, Wuhan, China
[3]Department of General Surgery, University of Chinese Academy [illegible]nces Shenz[illegible]spital, Shenzhen, China
[4]Entre of Medical Heredity and Molecular Diagnosis, University [illegible] Chine[illegible]ademy of Sciences-Shenzhen Hospital, Shenzhen, China
[5]Department of Urology, University of Chinese Academy o[illegible]s-Shenzhen H[illegible]al, Shenzhen, China
[6]Department of Interventional Ultrasound, Chinese Pl[illegible]eneral Hospital, Beijing, China

Abstract. – OBJECTIVE: The aim of this study was to illustrate the role of long non-[illegible] RNA (lncRNA) DLEU1 in regulating the [illegible]g nant progression of clear cell renal cell [illegible]i-noma (ccRCC) by targeting microRNA-19[illegible] (miRNA-194-5p).

PATIENTS AND METHODS: DL[illegible] expressio[illegible] level in ccRCC tissues and para[illegible]s tissues was determined by quantita[illegible] Rea[illegible]e-Poly-merase Chain Reaction (q[illegible]CR). T[illegible] correla-tion between DLEU1 exp[illegible]n and [illegible] indexes of ccRCC pat[illegible]nts [illegible]sed. [illegible] the silence of DLUE[illegible]e pro[illegible]e and migra-tory abilities of AC[illegible]nd 786-O [illegible]vere evalu-ated. Furthermo[illegible]-Luciferase [illegible]er gene assay and res[illegible] exp[illegible]nts were co[illegible]ducted to identify the ro[illegible] of DLE[illegible]RNA-194-5p in regu-lating the [illegible]CC progress[illegible] *in vitro*.

RESU[illegible]: DLEU1 expre[illegible] was marked-ly up[illegible]ulated [illegible] ccRCC tis[illegible]ues when com-pare[illegible]th par[illegible]ncerous tissues. The rates of lym[illegible]s m[illegible]tasis a[illegible]d distant metastasis in ccRC[illegible]s with [illegible]gh level of DLEU1 were [illegible]nifica[illegible]igher[illegible]ereas the prognosis was [illegible]cant[illegible]ransfection of si-DLEU1 re-ma[illegible]ly atte[illegible]d proliferative and migratory abi[illegible]s of ACHN and 786-O cells. MiRNA-194-5p w[illegible]as the target gene of DLEU1. In [illegible]tion, t[illegible]knockdown of miRNA-194-5p could [illegible]verse the regulatory effect of DLEU1 on the [illegible]iferative and metastatic abilities of ccRCC.

[illegible]CLUSIONS: DLEU1 is closely related to lym[illegible]atic metastasis, distant metastasis, and poor prognosis of ccRCC. It aggravates the progression of ccRCC by targeting miRNA-194-5p.

Key w[illegible]
LncRNA [illegible]U1, MiRNA-194-5p, Clear cell renal cell [illegible]cinoma (CcRCC), Metastasis.

Introduction

Renal cell carcinoma (RCC) is the third most common malignancy in the urinary system, which originates from the renal tubular epithelium[1-3]. Due to the widespread application of ultrasound and CT examinations, the early diagnostic rate of RCC has been remarkably improved. However, the mortality of RCC is still on the rise, especially among people over 60 years[4,5]. Pathological subtypes of RCC are diverse, mainly including clear cell renal cell carcinoma, papillary carcinoma, and chromophobe cell carcinoma[6,7]. Clear cell renal cell carcinoma (ccRCC) is the most common histopathological subtype, which accounts for about 75% of all renal solid tumors[8,9]. Current studies have found that the occurrence, development, and metastasis of ccRCC involve a variety of molecular mechanisms. VHL, PBRM1, MET, and PTEN are important molecules affecting the progression of ccRCC. Therapeutic strategies for ccRCC include active monitoring, radio-frequency ablation, renal radical resection, and partial resection either with lymphadenectomy or not[10]. About one-third of RCC patients are accompanied by distant metastases. Meanwhile, these

Corresponding Author: Jie Yu, MD; e-mail: jiemi301@163.com

patients cannot achieve benefits from conventional systemic chemotherapy and/or radiotherapy[11]. Individualized therapy based on VEGF antibody and mTOR inhibitor is preferred for ccRCC patients with distant metastasis. However, the therapeutic efficacy is far from satisfactory for those in advanced stage[12].

Increasing evidence has proved that lncRNAs regulate multifunction of genetic information processing in living cells[13]. Long non-coding RNA (lncRNA) has been confirmed involved in the regulation of cellular activities and molecular pathways. Dysregulation of certain lncRNAs may be related to the pathogenesis of malignant tumors[14]. Previous studies[15-18] have indicated that lncRNAs are involved in the incidence and progression of ccRCC, which may be utilized as therapeutic targets. For example, lncRNA DLEU1 is a functional lncRNA participating in the regulation of multiple pathological processes[19-21].

LncRNA acts as competitive endogenous RNA (ceRNA) by competitively binding to microRNA (miRNA) regulatory genes with the same miRNA response element (MRE), thus influencing cellular functions[22]. The aim of this research was to investigate the miRNA sponge function of DLEU1 in regulating ccRCC progression and the p underlying mechanism.

Patients and Met

Patients and CcRCC Sa es

CcRCC tissues and -cance were collected from 4 pat g cal surgery. None o enrol CC patients received preopera anti-tumor ies. Clinical indexes we ed from pat for further analyses nform nsent was obtained from patie and their fa s before the study. This in gation was app by the Ethics Comm e of U versity of Chinese Academy of Sci s Shen Hospital.

Cell C

nal t elial cell line (HK-2) and cc cell l aki-1, Caki-2, 769P, ACHN, and 6-O) were purchased from the American T ollection (ATCC; Manassas, VA, A). All cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM; Gibco, Rockville, USA) containing 10% fetal bovine serum (FB Gibco, Rockville, MD, USA) and maintained in a 37°C, 5% CO_2 incubator. Cell passage was conducted at 80-90% of confluence.

Cell Transfection

Transfection plasmids were provided by GenePharma (Shanghai, China). Cells were seeded into 6-well plates. At 70% of confluence, cell tion was performed according to the ructio of Lipofectamine 2000 (Invitro Carlsbad, CA, USA). 48 h later, transfected were harvested for verification of transfection cy and subsequent experiments.

Cell Counting Kit-8 (CCK-8) ay

Cells were first s into well pl s at a density of 2×10^3 c pe day 1 , and 4, absorbance v at 450 each ple was recorded usi CCK-8 kit Laboratories, Kuma n), respecti . Finally, the viability c ve was ed.

Tr Migration y

lls were first adjusted to a dose of 2.0×10^5/ 200 µL of suspension was added to the r side of th nswell chamber (Millipore, B ca, MA, A) inserted in 24-well plates. Me L of medium containing 10% FBS wa to the bottom side. After 48 h of incubation, cells migrated to the bottom side were ith methanol for 15 min and dyed with ys olet for 20 min. The number of migratory cells was counted under a microscope (magnification 10×). 5 fields were randomly selected for each sample.

Wound Healing Assay

Cells were seeded in 24-well plates with 5.0×10^5 cells/well. An artificial wound was created in the confluent cell monolayer using a 200 µL pipette tip. Wound closure images were taken using an inverted microscope at 0 and 24 h, respectively. Finally, the percentage of wound closure was calculated.

Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR)

Total RNA was extracted from cells using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) and purified by DNase I treatment. Subsequently, extracted RNA was reverse transcribed into complementary deoxyribose nucleic acid (cDNA) using Primescript RT Reagent (TaKaRa, Otsu, Shiga, Japan). Then, the obtained cDNA was subjected to qRT-PCR using SYBR®Premix Ex Taq™ (TaKaRa, Otsu, Shiga, Japan). Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and U6 were used as internal references for mRNA and miRNA, respec-

tively. Each sample was performed in triplicate. The relative expression level of genes was calculated by the $2^{-\Delta\Delta Ct}$ method. Primer 5.0 was used for designing qRT-PCR primers. Primer sequences used in this study were as follows: DLEU1, F: 5'-CCAGTAACGTTCCATCATTATC-3', R: 5'-GCTCGCATGAGACAACGTAAGTGA-3'; miRNA-194-5p, F: 5'-GCCTGCTACAACCATCGTCGACTG-3', R: 5'-AGGTTGCTTGATGGCCGTCG-3'; U6: F: 5'-GCTTCGGCAGCACATATACTAAAAT-3', R: 5'-CGCTTCAGAATTTGCGTGTCAT-3'; GAPDH: F: 5'-CGCTCTCTGCTCCTCCTGTTC-3', R: 5'-ATCCGTTGACTCCGACCTTCAC-3'.

Dual-Luciferase Reporter Gene Assay

ACHN and 786-O cells were co-transfected with pmirGLO-DLEU1-WT/pmirGLO-DLEU1-MUT/pmirGLO and NC/miRNA-194-5p mimic using Lipofectamine 2000. 24 h later, co-transfected cells were harvested. Luciferase activity was determined using a Dual-Luciferase reporter assay system (Promega, Madison, WI, USA).

Statistical Analysis

Statistical Product and Service Solutions (SPSS) 22.0 (IBM, Armonk, NY, USA) was used for all statistical analyses. Experimental [illegible] expressed as mean ± standard dev[illegible]. Inte[illegible] group differences were analyzed by [illegible]st. The Kaplan-Meier curve was introduced [illegible]ival analysis. Chi-square test was perfo[illegible]ate the correlation between DLEU[illegible]el with [illegible]gical indexes of ccRCC pa[illegible]s. Spearman c[illegible]tion test was conducte[illegible] assess[illegible] relation[illegible] between expressions [illegible]LEU[illegible]d miR-194-5p. $p<0.05$ was consid[illegible]d s[illegible]y signi[illegible]t.

Results

Up-regulation of D[illegible]1 in [illegible] Tissues and [illegible] Lines

[illegible]RT-PCR results indicated that DLEU1 was [illegible]ly expressed [illegible] ccRCC tissues relative to pa[illegible]ncerous tiss[illegible] (Figures 1A, 1B). Similarly,

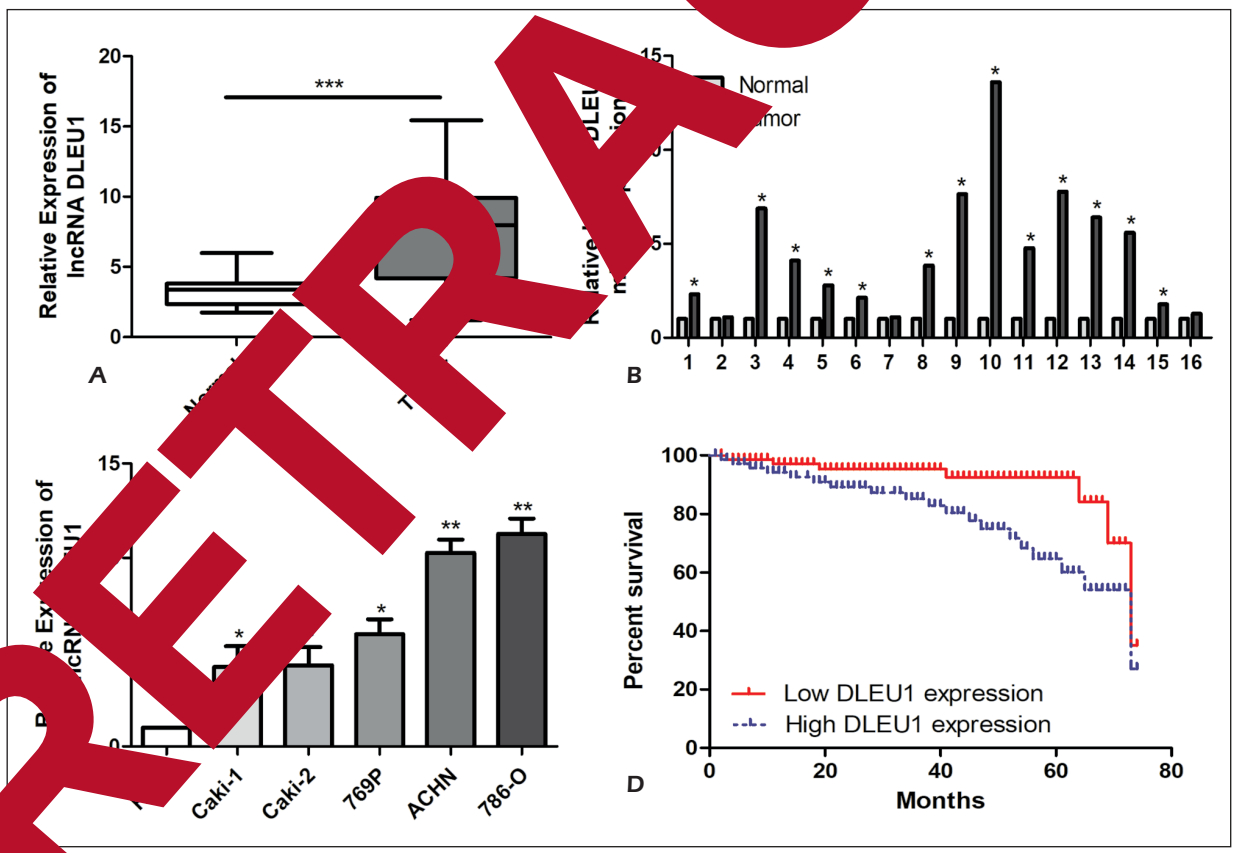


Figure 1. Up-regulation of DLEU1 in ccRCC tissues and cell lines. **A**, Relative level of DLEU1 in ccRCC tissues and para-cancerous tissues. **B**, Relative level of DLEU1 in 16 paired ccRCC tissues and para-cancerous tissues. **C**, Relative level of DLEU1 in renal tubular epithelial cell line (HK-2) and ccRCC cell lines (Caki-1, Caki-2, 769P, ACHN, and 786-O). **D**, Kaplan-Meier curve was introduced to evaluate the survival of ccRCC patients in low DLEU1 expression group and high DLEU1 expression group.

Table I. Association of lncRNA DLEU1 expression with clinicopathologic characteristics of renal cell cancer.

Parameters features	No. of cases (n=51)	LncRNA DLEU1 expression		*p*-value*
		Low (%)	High (%)	
Age (years)				0.800
<60	15	9	6	
≥60	25	16	9	
Gender				0.462
Male	19	13	6	
Female	21	12	9	
T stage				0.1
T1-T2	25	18	7	
T3-T4	15	7	8	
Lymph node metastasis				.004
No	27	21	6	
Yes	13	4		
Distance metastasis				0.0
No	30	22		
Yes	10	3	7	

DLEU1 expression was significantly up-regulated in ccRCC cells when compared with renal tubular epithelial cells (Figure 1C). Based on the median expression level of DLEU1, 40 ccRCC pa[illegible]ts were divided into high DLEU1 expressio[illegible] and low DLEU1 expression group. Kaplan-[illegible]er curve revealed that survival rate was mark[illegible] worse in ccRCC patients in high DLEU1 expr[illegible]sion group (Figure 1D).

Clinical data were collect[illegible]RCC pa-tients for analysis. As sho[illegible] Table[illegible] DLEU1 level was positively co[illegible]ed wit[illegible] metastasis and distant me[illegible]s [illegible]CRC [illegible]tients, rather than a[illegible]ender, [illegible]mor staging. These results ind[illegible]d that DL[illegible]ould be a hallmark for pr[illegible]he malignan[illegible]gression of ccRCC.

Knock[illegible]n of DLEU1 S[illegible]essed Proli[illegible]tive a[illegible]d Migratory Ab[illegible]s of C[illegible]C

[illegible]f si-DL[illegible]U1 markedly down-regulated [illegible] level [illegible]ACHN and 786-O cells [illegible]re 2[illegible]K [illegible]ssay showed that the proli[illegible]ve abi[illegible]CHN and 768-O cells transfect[illegible] with si-DL[illegible]EU1 was significantly inhibited ([illegible] [illegible]ranswell assay revealed that the [illegible]gratory [illegible]ility of ccRCC cells transfected with [illegible]DLEU1 was remarkably suppressed relative to [illegible]f control cells (Figure 2C). In addition, a dec[illegible]sed percentage of wound closure indicated inhibited migratory ability of ccRCC cells after silence of DLEU1 (Figure 2D).

[illegible]EU1 Bound[illegible] MiRNA-194-5p

[illegible]vious bio[illegible]rmatics method has predicted t[illegible]ationship between DLUE1 and miRNA[illegible]p. In this study, the Dual-Luciferase [illegible]porter gene assay was conducted to verify this [illegible]Luciferase activity in ACHN and 786-O [illegible]ls [illegible]transfected with pmirGLO-DLEU1-WT and miRNA-194-5p mimic decreased significantly, confirming the binding relationship between DLEU1 and miRNA-194-5p (Figure 3A) Transfection of si-DLEU1 markedly up-regulated miRNA-194-5p level in ccRCC cells (Figure 3B). Besides, miRNA-194-5p level was significantly down-regulated in both ccRCC tissues and cell lines (Figures 3C, 3D). A negative correlation was identified between the expression levels of DLEU1 and miRNA-194-5p (Figure 3E).

DLEU1/MiRNA-194-5p Regulatory Axis in CcRCC

To uncover the involvement of miRNA-194-5p in the malignant progression of ccRCC influenced by DLEU1, rescue experiments were conducted. Transfection efficacy of miRNA-194-5p inhibitor in ACHN and 786-O cells was verified by qRT-PCR. Down-regulated expression of DLEU1 in ccRCC cells transfected with si-DLEU1 was markedly up-regulated after co-transfection of miRNA-194-5p inhibitor (Figure 4A). Transfection of miRNA-194-5p inhibitor significantly enhanced the proliferative and migratory abilities of ccRCC cells. Meanwhile, attenuated viabilities of ACHN and 786-O cells transfected with

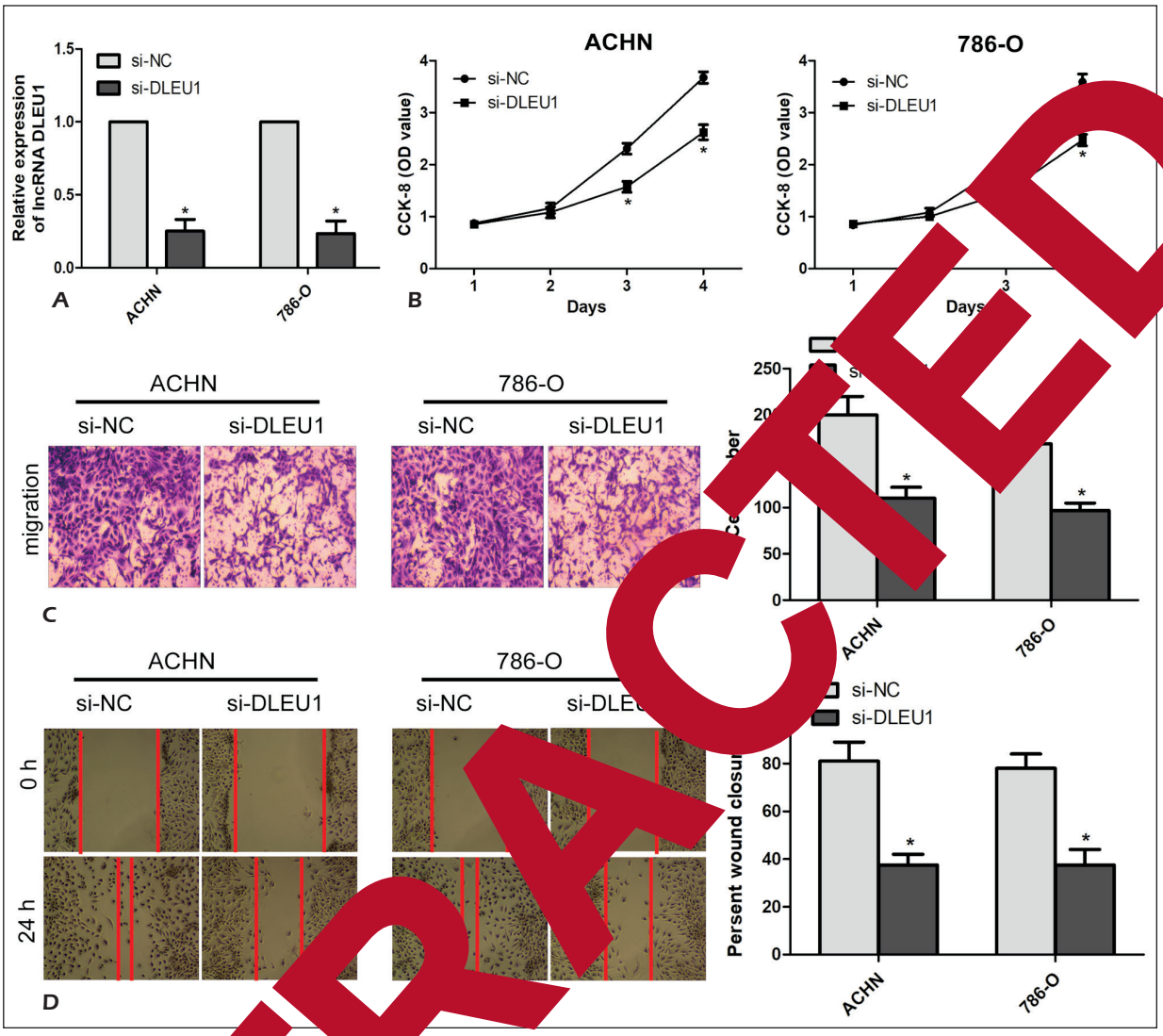


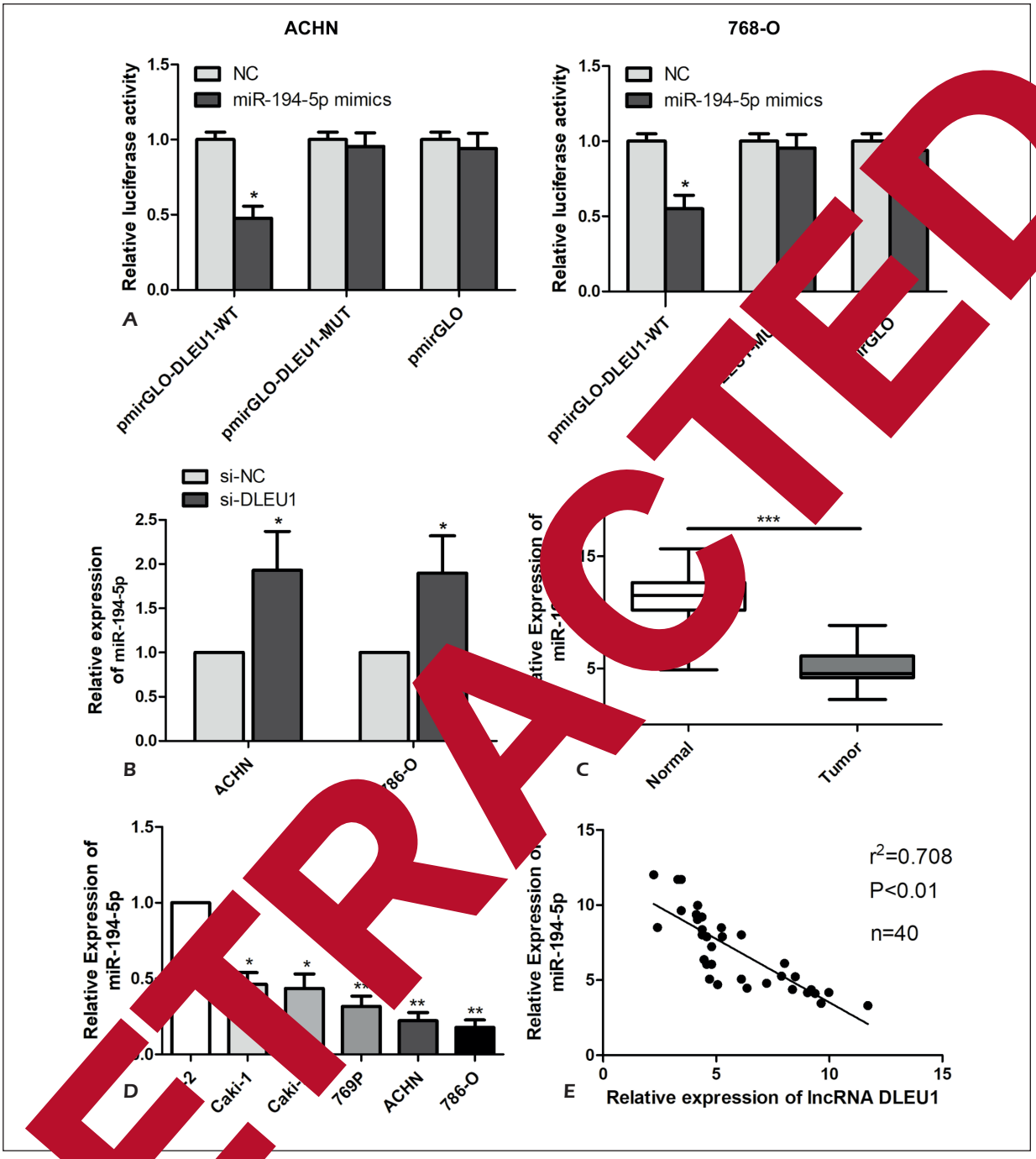
Figure 2. Knockdow[n] LEU1 supp[resses] proliferative and migratory abilities of ccRCC cells. **A**, Transfection efficacy of si-DLEU1 in ACHN [and] 6-O cells. **B**, [Viability] of ACHN and 786-O cells transfected with si-NC or si-DLEU1 at day 1, 2, 3 and 4, respectiv[ely]; [migrat]ory ability of [AC]HN and 786-O cells transfected with si-NC or si-DLEU1 (magnification 10×). **D**, Wound closu[re] ACH[N] 786-O cells transfected with si-NC or si-DLEU1 (magnification 10×).

si-DL[EU1] could[d] be partially reversed by knockdow[n] miR[NA]-194-5p (Figure 4B). Transwell and [wound] healing ass[ays] proved that silence of miRNA-[194-]5p coul[d] reverse the regulatory effect of DL[EU1] on [the] migratory ability of ccRCC *in vitro* (Figure 4C, 4D).

Discussion

RCC is a common malignant tumor of the urinary system. The incidence of RCC in urinary malignancies is second only to bladder cancer and prostate cancer[1-3]. In recent years, the mortality of RCC is on the rise[4-6]. LncRNAs have been found extensively involved in the progression of RCC, which are capable of determining its malignant level[13]. The specificity of cancer-related lncRNAs indicates its diagnostic and prognostic potentials in urological malignancies[23].

Human Genome Sequencing uncovers less than 3% of protein-coding genes in the whole genome. More than 80% of genes are transcribed into RNAs without protein-coding functions[13,14]. LncRNAs are a type of ncRNAs with over than 200 nt in length[13,15]. Compared with proteins, lncRNAs are highly tissue-specific[16]. Through regulations of cis-acting, trans-acting and miRNA interaction, lncRNAs mediate the synthesis



re 3. U1 bo miR-194-5p. **A**, Luciferase activity in ACHN and 786-O cells co-transfected with pmirGLO-WT/p EU1-MUT/pmirGLO and NC/miR-194-5p mimic. **B**, Relative level of miR-194-5p in ACHN and 786 lls trans with si-NC or si-DLEU1. **C**, Relative level of miR-194-5p in ccRCC tissues and para-cancerous tissues. **D**, R ve level of R-194-5p in renal tubu-lar epithelial cell line (HK-2) and ccRCC cell lines (Caki-1, Caki-2, 769P, ACHN, an egative correlation was observed between expression levels of DLEU1 and miR-194-5p.

proteins[17,18]. Scholars[16-18] have demonstrated the f lncRNAs at the chromosomal, transcription and post-transcriptional levels. Furthermore, they are widely involved in the occurrence and progression of malignancies.

DLEU1 is up-regulated in various cancer tissues, such as liver cancer and gastric cancer. Its expression level is closely related to pathological features and clinical prognosis of tumor patients. This suggests its carcinogenic role in malignant

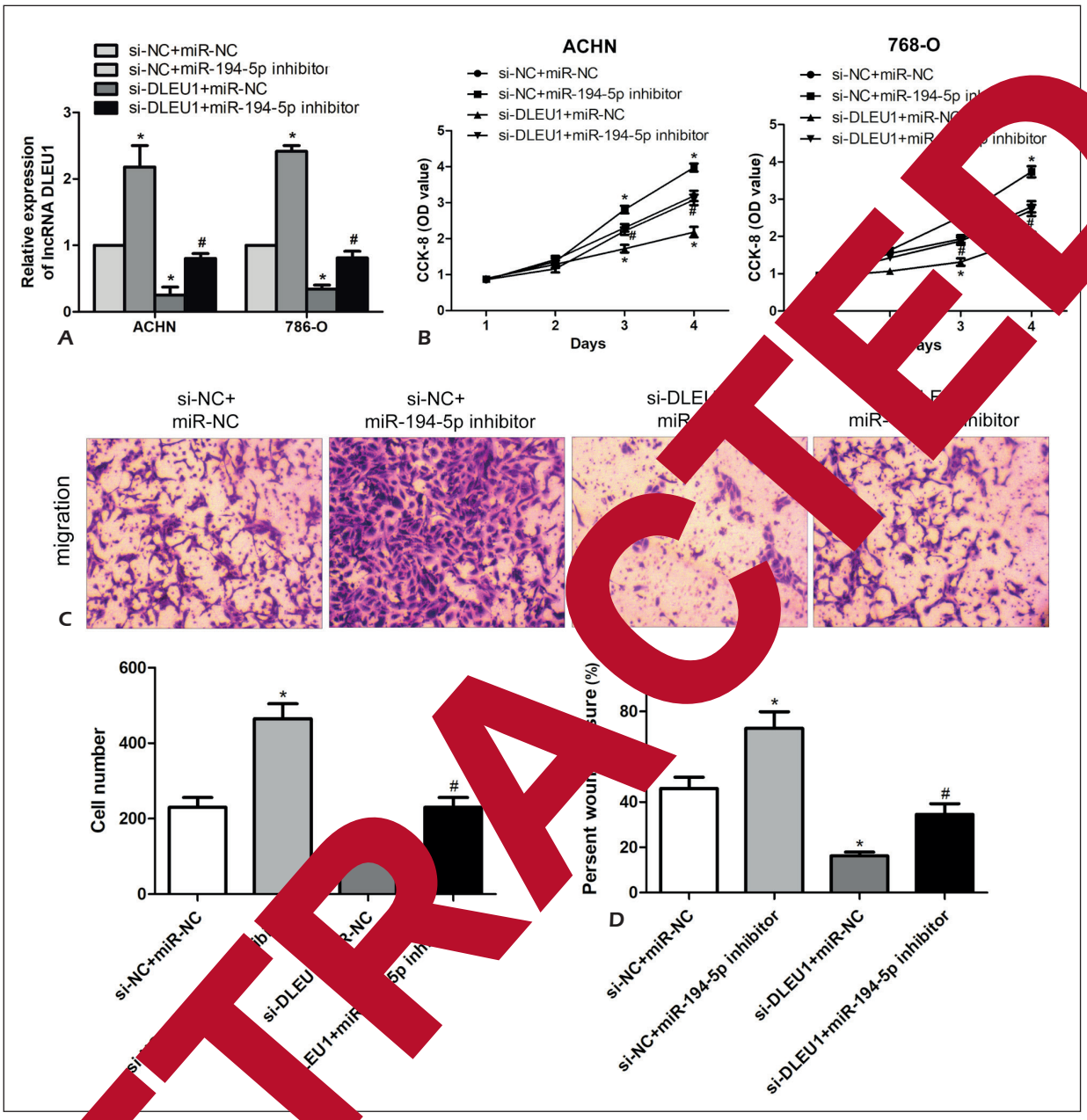


Figure LEU1/ iR-194-5p regu ory axis in ccRCC. ACHN and 786-O cells were transfected with si-NC+miR-NC, si-NC+ 94-5p i tor, si-DLEU1+miR-NC or si-DLEU1+miR-194-5p inhibi-tor. **A**, Relative level of DLEU1; **B**, Viability at d 3, a especti ly; **C**, Migratory cell number (magnification 10×); **D**, Wound closure.

s, in g CC[19-21]. In this paper, 40 pa ccRCC s and para-cancerous tissues we rst colle ed. QRT-PCR results showed D ighly expressed in ccRCC tissues ccRCC ell lines. Moreover, DLEU1 level s positively correlated with lymphatic metastad distant metastasis, suggesting its oncogenic r in ccRCC. *In vitro* studies demonstrated that silence of DLEU1 could attenuate the proliferative and migratory abilities of ccRCC cells.

Recent investigations[22] have highlighted the well-concerned function of lncRNA as a miRNA sponge[2]. Previous bioinformatics method has predicted the binding relationship between DLUE1 and miRNA-194-5p. In this study, the Dual-Luciferase reporter gene assay verified this finding. Our results revealed significantly down-regulated miRNA-194-5p in ccRCC. Silence of miRNA-194-5p enhanced the viability and migratory abilities of ACHN and 786-O cells. Notably, silence of miR-

NA-194-5p could reverse the regulatory effect of DLEU1 on cell proliferation and migration. As a result, a regulatory loop DLEU1/miRNA-194-5p was identified to aggravate the malignant progression of ccRCC.

Conclusions

DLEU1 is closely related to lymphatic metastasis, distant metastasis and poor prognosis of ccRCC. Furthermore, it aggravates the progression of ccRCC by targeting miRNA-194-5p.

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Conflict of Interests

The Authors declare that they have no conflict of interests.

References

1) Dudani S, Savard MF, Heng DYC. An upda... predictive biomarkers in metastatic renal cell... cinoma. Eur Urol Focus 2019 Apr 19. pii: S24... 4569(19)30111-7. doi: 10.101...19.04.00... [Epub ahead of print]
2) Grange C, Brossa A, Busso... Extra...lar vesicles and carried miRNAs... progre...a of renal cell carcinoma. Int J Mo... 2019;...
3) Ouzaid I, Capitanio U... CG, Leibo...h BC, Ljungberg B,... Poppel ... salah K Young Academic Urolo... idney Cancer... g Group of the European... n of Urolog...ical metastasecto... re... l carcinoma: a systematic review. Eur Urol Onc... 2: 141-149.
4) Di Piet..., Luu HN, Spi... Sexton W, Dickinson S, P... A, Park JY. Biom... and new therapeutic targets in renal cell carcinoma. Eur Rev Pharm... Sci 2018; 22: 5874-5891.
5) ... L, Ro... J, Petro... J, Reniero L, Cicora F. Renal cell carcinoma: a report of the current literature. Curr U... ep 2019; 20: 4.
6) Williams... K, Cheng L. Renal cell carcinoma staging: falls, challenges, and up-dates. Histopathology 2019; 74: 18-30.
7) ... B, Eble JN, Egevad L, Samaratunga H. ... renal cell carcinoma. Histopathology 2019; 74: 4-17.
8) Santoni M, Cimadamore A, Cheng L, Lopez-Beltran ... Battelli N, Massari F, Scarpelli M, Ga-losi AB, ...acarda S, Montironi R. Circulating tumor cells in renal cell carcinoma: recent findings and future challenges. Front Oncol 2019; 9: 228.
9) Tian ZH, Yuan C, Yang K, Gao XL. Systematic identification of key genes and pathways in ... renal cell carcinoma on bioinformati... Ann Transl Med 2019; 7: 89.
10) Kotecha RR, Motzer RJ, Voss MH. ...ards individualized therapy for metastatic ... ll car-cinoma. Nat Rev Clin Oncol 2019 Apr ... 10.1038/s41571-019-0209-1. [Epub ...ead of ...
11) Taneja K, Williamson SR... dates in ... staging and histologic... ing of renal cell... noma. Surg Pathol ... 2018; 1... 7-812.
12) Kammerer-Jacquet S... fuze A... ut J, Mathieu R, Laguerre B, Ver...oest... , Belau...ureau MA, Bensalah... ioux... N. T...ing the PD-1/PD-L1... way in r... ...noma. Int J Mol Sci... 20. pii: E16...
13) Gugnoni... cchi A. Long...ncoding RNA and ...elial ...chymal transition in cancer. Int J Mol Sci 201... 1924.
14) ...uo DX, Mo Y... rging roles of lncRNAs ... post-transcriptio... regulation in cancer. Genes Dis 2019; 6: 6-15.
15) Liu X, Hao Y, ...W, Yang X, Luo X, Zhao J, Li J, Hu X, Li L. Long ...coding RNA emer-gence during ...enal cell car...ma tumorigenesis. Cell Physiol ...hem 20...7: 735-746.
16) ...E, Xue B, Shan Y, Weng G, Shen B. Long ...oding RNAs in urologic malig-nancies: functional roles and clinical translation. J Cancer ...016; 7: 1842-1855.
17) ...M, Hutterer GC, Kiesslich T, Pummer K, Berindan-Neagoe I, Perakis S, Schwarzenbacher D, Stotz M, Gerger A, Pichler M. Current insights into long non-coding RNAs in renal cell car-cinoma. Int J Mol Sci 2016; 17: 573.
18) Jiang X, Lei R, Ning Q. Circulating long noncoding RNAs as novel biomarkers of human diseases. Biomark Med 2016; 10: 757-769.
19) Li Y, Shi B, Dong F, Zhu X, Liu B, Liu Y. Long Non-coding RNA DLEU1 promotes cell pro-liferation, invasion, and confers cisplatin resistance in bladder cancer by regulating the miR-99b/HS3ST3B1 axis. Front Genet 2019; 10: 280.
20) Gao S, Cai Y, Zhang H, Hu F, Hou L, Xu Q. Long noncoding RNA DLEU1 aggravates pancreatic ductal adenocarcinoma carcinogenesis via the miR-381/CXCR4 axis. J Cell Physiol 2019; 234: 6746-6757.
21) Liu T, Han Z, Li H, Zhu Y, Sun Z, Zhu A. LncRNA DLEU1 contributes to colorectal cancer progression via activation of KPNA3. Mol Cancer 2018; 17: 118.
22) Zhang C, Ge C. A simple competing endogenous RNA network identifies novel mRNA, miRNA, and lncRNA markers in human cholangiocarcinoma. Biomed Res Int 2019; 2019: 3526407.
23) Zhu D, Singh S, Chen X, Zheng Z, Huang J, Lin T, Li H. The landscape of chimeric RNAs in bladder urothelial carcinoma. Int J Biochem Cell Biol 2019; 110: 50-58.