European Review for Medical and Pharmacological Sciences 2019; 23: 6602-6611

LncRNA RUSC1-AS1 promotes the proliferation of breast cancer cells by epigenetic silence of KLF2 and CDKN1A

C.-C. HU[1], Y.-W. LIANG[1], J.-L. HU[2], L.-F. LIU[1], J.-W. LIANG[1], R. WANG[3]

[1]Department of Oncology, the Affiliate Hospital of Chengde Medical Univer[illegible] Chengde, China
[2]Department of Clinical Pharmacy, the Affiliate Hospital of Chengde Medi[illegible]niver[illegible] Chengde, China
[3]Department of Radiology, the Affiliate Hospital of Chengde Medical [illegible]ersi[illegible]ngde[illegible]a

Chanchan Hu and Yunwei Liang contributed equally to this work.

Abstract. – OBJECTIVE: To clarify the potential function of long non-coding RNA (lncRNA) RUSC1-AS1 in regulating the progression of breast cancer (BCa) and the underlying mechanism.

PATIENTS AND METHODS: RUSC1-AS1 level in BCa tissues and adjacent normal tissues was first determined by quantitative Real Time [illegible]merase Chain Reaction (qRT-PCR). The [illegible]tion between RUSC1-AS1 expression with [illegible]of size, clinical stage and overall survival o[illegible]a patients was analyzed. Influences of RU[illegible] AS1 knockdown on viability, clonality, cell cy[illegible] and apoptosis of BCa cell li[illegible]CF-7 an[illegible] BT549 were evaluated. Targe[illegible]RUSC1-AS1 were predicted by bioi[illegible]matic[illegible]nd their interaction was further c[illegible]rmed b[illegible]NA immunoprecipitation (RIP),[illegible]natin[illegible] cipitation (ChIP) and [illegible]cu[illegible]ents.

RESULTS: A hi[illegible] abun[illegible] of RUSC1-AS1 was identifi[illegible]n BCa tiss[illegible]elative to controls. The [illegible]ion level of [illegible]C1-AS1 was positive[illegible]on[illegible]d to tumor size and clinical grade, but neg[illegible]y correlated to the overall su[illegible]al of BCa pa[illegible]. The silence of RUSC1-[illegible] markedly inhibi[illegible]iability, clonality, ce[illegible]cle pr[illegible]ression, and induced apoptosis [illegible]CF-7 a[illegible]BT549 cells. Finally, CDKN1A and[illegible]2 w[illegible]found[illegible]be the target genes of RU[illegible], whic[illegible]ere tumor-suppressor [illegible]es i[illegible]d in[illegible]SC1-AS1-mediated BCa [illegible]ssio[illegible].

[illegible]CLUSI[illegible]RUSC1-AS1 is highly expre[illegible]d in BCa, which promotes the progressi[illegible]through mediating CDKN1A and [illegible]. RU[illegible]AS1 may serve as a potential hallm[illegible]rk for BCa.

K[illegible]rds
B[illegible]st cancer, RUSC1-AS1, Epigenetics, KLF2, CDKN1A, Proliferation.

Introduction

Breast cance[illegible]Ca) is one of the most com[illegible] malignant [illegible]ors in women and usually o[illegible]ted from[illegible]east epithelial tissues. It is repo[illegible]incidence of BCa accounts for 7-10% o[illegible]alignancies[1]. Each year, there are [illegible].35 million newly onsets of BCa globally[2]. Pre[illegible]dies have found a variety of oncogenes [illegible]d t[illegible]or-suppressor genes involving in BCa, which help to improve the diagnostic rate and therapeutic efficacy of BCa. However, there are still many difficulties in the effective treatment of BCa[3]. Therefore, it is necessary to elucidate the molecular mechanism underlying the tumorigenesis of BCa, thus providing novel therapeutic approaches.

Long non-coding RNA (lncRNA) is produced by RNA polymerase II transcription with 200 nt in length, which distributes in the nucleus or cytoplasm. Due to the lack of ORF (open reading frame), lncRNAs could not encode proteins and only regulate gene expressions at different levels in the form of RNAs. LncRNA exerts multiple functions, which are involved in the regulation of tumor cell behaviors. A growing number of studies have indicated the close relationship between lncRNAs and tumorigenesis. Differentially expressed lncRNAs in tumor tissues and normal tissues have been identified. For example, lncRNA MALAT1 is highly specific in lung cancer and considered to be a specific marker for NSCLC and early-stage metastatic adenocarcinoma of lung[4]. MALAT1 is upregulated in lung cancer patients, and is able to accelerate growth and migration of tumor cells[5,6]. H19 is one of the earliest identified

Corresponding Author: Rui Wang, MD; e-mail: chanchanhu@126.com

imprinted genes. Upregulation of H19 promotes the proliferative ability of hepatoma cells, and its downregulation achieves the opposite trend[7].

LncRNA RUSC1-AS1 is located on human chromatin 1q22. So far, researches on RUSC1-AS1 are rarely reported. This study aims to explore the role of RUSC1-AS1 in BCa and its underlying mechanism.

Patients and Methods

Sample Collection

BCa tissues and adjacent normal tissues (n=48) were collected from BCa patients undergoing surgery for the first time in the Affiliated Hospital of Chengde Medical University from March 2015 to March 2017. None of the enrolled patients were treated with pre-operative anti-tumor therapy. They were not affected by other diseases. Samples were immediately preserved in liquid nitrogen within 15 min ex vivo. The investigation was approved by the Medical Ethics Committee and patients were informed consent. Clinical data of enrolled patients were illustrated in Table I[illegible]

Cell Culture and Transfection

BCa cell lines (MDA-MB-231, M[illegible] BT549) and breast epithelial cell line (MCF-1[illegible] were provided by Cell Bank, C[illegible] Academ[illegible] of Science (Shanghai, China). [illegible] cultured in Roswell Park Memorial I[illegible]ute-16[illegible] (RPMI-1640; HyClone, South Lo[illegible]UT, US[illegible]ontaining 10% fetal bovine seru[illegible]BS; [illegible]ville, MD, USA) and 1% penicillin/streptomycin, and preserved in a 37°C, 5% CO_2 incubator.

One day prior to transfection, cells w[illegible]ed into a 6-well plate with 1×10^5 ce[illegible] Until 60-70% of confluence, cells [illegible] subjected to transfection using Lipofectam[illegible]2000 (Invitrogen, Carlsbad, CA, USA). Trans[illegible] vectors were provided by Genscript [illegible]anjing, [illegible]).

RNA Extraction and [illegible]antit[illegible]ive Real Time-Polyme[illegible] Chai[illegible]eaction (qRT-PCR)

Total RNA w[illegible]xtra[illegible]ing TR[illegible] (Invitrogen, Carlsb[illegible]CA, USA)[illegible]nti[illegible] and purified by U[illegible]trophotome[illegible]A samples with 1.8-2[illegible] density (OD 260/280 were considered [illegible]o be qu[illegible]. 1 μg of total RNA was revers[illegible] transcribed [illegible] complementary deox[illegible]nucleic acid ([illegible]A). QRT-PCR was p[illegible]rmed under the conditions of 95°C for 30 s, [illegible]C for 5 s, an[illegible]0°C for 31 s, for a total of 40 [illegible]s. Glyceral[illegible]de 3-phosphate dehydrogena[illegible] (GAPDH) [illegible] used as an internal reference. Rela[illegible] genes were calculated using the 2–ΔΔCt [illegible]. Primer sequence were as follows: RUSC1-AS1: F: AGTGGATGAGGACTGGCT, [illegible]AGGTCTAGATTTCCA; Bcl-2: F: GCG-CC[illegible]GTTTGATTTCTC, R: TTTGGGGCAGGCATGTTGAC; Bax: F: ACCATCTTTGTGGCGGGAG, R: GGAAAAACACAGTCCAAGGCA; KLF2: F: CTGCACATGAAACGGCACAT, R: CAGTCACAGTTTGGGAGGGG; PTEN: F: TGGATTCGACTTAGACTTGACCT, R: GGTGGGTTATGGTCTTCAAAAGG; CD-

Table I. Correlation [illegible] RUSC1-AS1 [illegible]n and pathological features in breast cancer patients (n = 48).

Clinicopath[illegible] features	[illegible]umber of Cases	RUSC1-AS1 expression		*p*-value
		Low (n=24)	High (n=24)	
Age [illegible]				0.3861
[illegible]	25	11	14	
[illegible]	23	13	10	
Gen[illegible]				0.7726
Male	23	12	11	
[illegible]male	25	12	13	
T[illegible] size				0.0038*
[illegible]m	26	18	8	
[illegible]	22	6	16	
[illegible]M stage				0.0205*
I-II	22	15	7	
III-IV	26	9	17	
[illegible] node metastasis				0.0014*
A[illegible]sent	27	19	8	
Present	21	5	16	

KN1A: F: AAGTCAGTTCCTTGTGGAGCC, R: GGTTCTGACGGACATCCCCA; CDKN2B: F: GGACTAGTGGAGAAGGTGCG, R: GGGCGCTGCCCATCATCATG;LSD1:F:AGCGTCATGGTCTTATCAA, R: GAAATGTGGCAACTCGTC; EZH2: F: TGCACATCCTGACTTCTGTG, R: AAGGGCATTCACCAACTCC; GAPDH: F: CGCTCTCTGCTCCTCCTGTTC, R: ATCCGTTGACTCCGACCTTCAC.

Cell Counting Kit-8 (CCK-8)

Transfected cells were seeded in a 96-well plate with 2×10^4 cells per well. At the appointed time points, 10 μL of CCK-8 solution (Dojindo, Kumamoto, Japan) was applied per well. After incubation for 2 hours, the recorded absorbance at 450 nm using a microplate reader was used for plotting the growth curves.

Cell Cycle Determination

Cells were digested and fixed in pre-cold 75% ethanol at -20°C overnight. At the other day, cells were incubated with 150 μL of RNase A for 30 minutes, dyed with 100 μL of propidium iodide (PI) for another 30 minutes in the dark and f… subjected to flow cytometer determinatio… SCalibur; BD Biosciences, Detroit, MI, US…

Cell Apoptosis Determination

Transfected cells for 48 hours… suspende… in 500 μL of binding buffer a… d with 5… μL of Annexin V and 5 μL …I for … minutes in the dark. Percentage of …ptotic c… ana-lyzed by a BD FACSC…lib… Bioscience, Detroit, … USA) … 1 hour.

Colony Forma… Assay

Transfecte… …s … hours were seeded in the 6-well p…ate with 1×1… …s per well. The medium wa… …aced every 2… …d cells were incubated … 1-2 weeks until ed… contact of colonies … ixation … % paraformaldehyde and dye wi… % vio… rystal f… 20 min, colonies were captu… …unting … a microscope.

…ern B…

… RIPA … …oimmunoprecipitation assay) pro… …sate (Beyotime, Shanghai, China) was … the total protein in each group of …s. The BCA (bicinchoninic acid) method was …rmed to quantitate the protein concentration. T… …tein samples were electrophoresed on polyacry…mide gels and then transferred to polyvinylidene difluoride (PVDF) membranes (Merck Millipore, Billerica, MA, USA). After blocking with 5% skimmed milk, the membranes were incubated with primary antibody (Cell Signaling Te… Danvers, MA, USA) at 4°C overnigh… …m… brane was incubated with the seco… …y antibody after rinsing with the Tris-Buff… Saline and Tween (TBST) solution. Chemilu… …nce was used to expose the protein ba… on the … rane. Bands were exposed by e… …ced chemi… cence (ECL) and anal… by I…age Sof… (NIH, Bethesda, MD, …A).

RNA Immuno…ipi… …(RIP)

Cells were … …ected and … …ad … …rding to the procedu… …Millipore (Bi… …MA, USA) Magna R… …M… Binding Pr…ein Immunoprecipitati… Kit. … …ll lysate was incubated with a… …Ago2 or ant… …antibody at 4°C for 6 h… …tein-RNA com… …x was captured and … …ted with 0.5 mg/mL proteinase K contain… 0.1% sodiu… …decyl sulphate (SDS) to ex… RNA. The … …gnetic beads were repeatedly w… …d with RI… …ashing buffer to remove nonspe… …n as much as possible. Finally, the extra… …RNA was subjected to qRT-PCR.

…atin Immunoprecipitation (ChIP)

… were cross-linked with 1% formaldehyde for 10 min at room temperature. Subsequently, the cross-linked cells were lysed using lysis buffer and sonicated for 30 min. Finally, the sonicated lysate was immuno-precipitated with corresponding antibodies and anti-IgG.

Statistical Analysis

Statistical Product and Service Solutions (SPSS) 17.0 (SPSS Inc., Chicago, IL, USA) was used for all statistical analysis. Data were represented as mean ± SD (standard deviation). The t-test was used for analyzing intergroup differences. The Kaplan-Meier method was introduced for survival analysis. $p<0.05$ indicated a significant difference.

Results

Upregulated RUSC1-AS1 in BCa

To uncover the dysregulated lncRNAs in BCa, we downloaded the lncRNA expression profiles of BCa from GEO datasets. RUSC1-AS1 was found to be significantly up-regulated in BCa (Figure 1A and 1B). We detected RUSC1-AS1 ex-

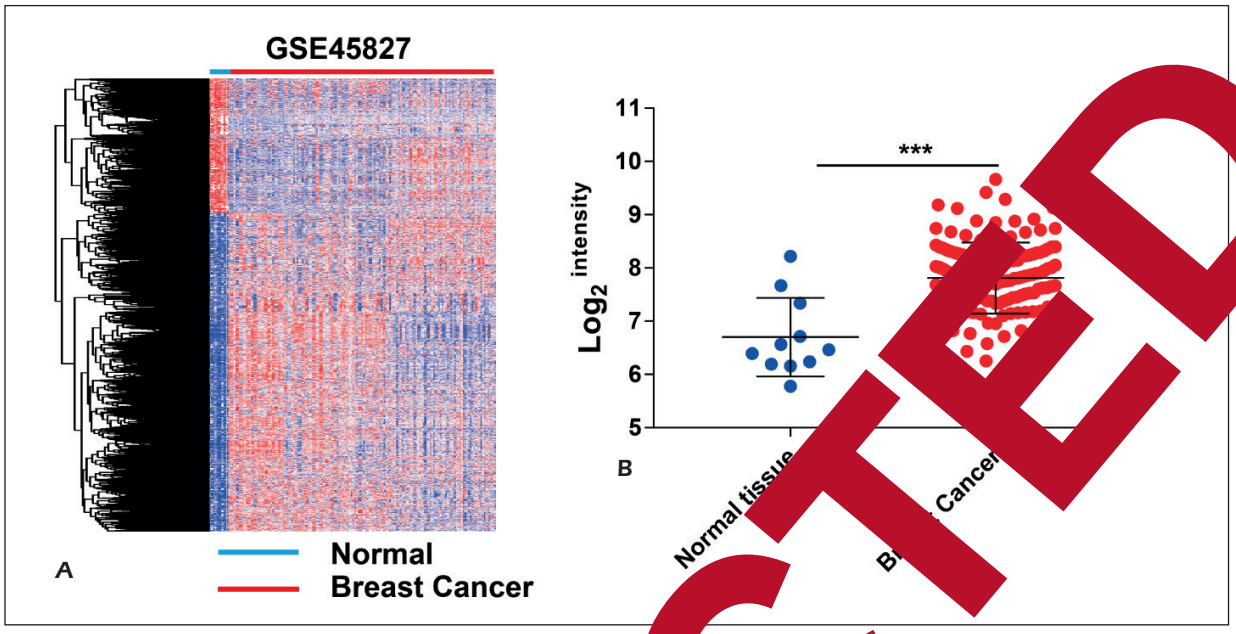


Figure 1. RUSC1-AS1 is upregulated in GSE45827. ***A,*** Heatmap of dysregulated lncRNAs in GSE45829. ***B,*** RUSCI-AS1 is up-regulated.

pression in 48 pairs of BCa tissues and adjacent normal tissues. QRT-PCR data revealed a high level of RUSC1-AS1 in BCa tissues relative to controls (Figure 2A). Furthermore, correlations were found between RUSC1-AS1 expression with tumor size, clinical stage and lymph node metastasis of BCa patients (Table 1). Particularly, RUSC1-AS1 expression was markedly higher in BCa tissues larger than 2 cm in tumor size relative to those with smaller tumor size. Meanwhile, BCa tissues with stage III-IV had a higher level of RUSC1-AS1 compared with those with stage I-II (Figure 2C). The Kaplan-Meier method was introduced to the survival analysis. BCa patients with high-level RUSC1-AS1 had the worse overall survival than those with a low level (Figure 2D).

Silencing of RUSC1-AS1 Inhibited Proliferation Ability of BCa Cells

Identified that in BCa tissues, RUSC1-AS1 expression was higher in BCa cell lines (MDA-MB-231, MCF-7 and BT549) than that in breast epithelial cell line (MCF-10A) (Figure 3A). It is suggested RUSC1-AS1 may serve as an oncogene to be related to the occurrence and progression of BCa. Among the selected BCa cell lines, MCF-7 and BT549 cells expressed a relatively high level of RUSC1-AS1, which were chosen for subsequent experiments. Transfection of si-RUSC1-AS1 1# or si-RUSC1-AS1 2# could markedly downregulate RUSC1-AS1 level in MCF-7 and BT549 cells (Figure 3B). As CCK-8 assay indicated, RUSC1-AS1 knockdown reduced the viability of BCa cells in a time-dependent manner (Figure 3C). Colony formation and transwell assays further demonstrated the inhibited proliferative, migrated, and invasive ability of BCa cells due to RUSC1-AS1 knockdown (Figure 3D). Flow cytometry showed that the silence of RUSC1-AS1 arrested BCa cells in G0/G1 phase (Figure 3E). However, apoptosis was pronounced in BCa cells transfected with si-RUSC1-AS1 (Figure 3F). The above data illustrated that silence of RUSC1-AS1 inhibited proliferation, migration, and invasion, but induced apoptosis of BCa cells.

RUSC1-AS1 Silenced KLF2 and CDKN1A by Binding to LSD1 and EZH2

LncRNAs regulate tumor cell behaviors by binding to the RNA-binding proteins. To explore the mechanism of RUSC1-AS1 in proliferation, we examined the mRNA levels of proliferation-related genes in MCF-7 and BT549 cells with RUSC1-AS1 knockdown. The silence of RUSC1-AS1 upregulated levels of Bax, KLF2, PTEN, and CDKN1A, while levels of Bcl-2 and CDKN2B did not change (Figure 4A). Through bioinformatics methods (http://pridb.gdcb.iastate.edu/RPISeq/), it was predicted that RUSC1-AS1 could be tar-

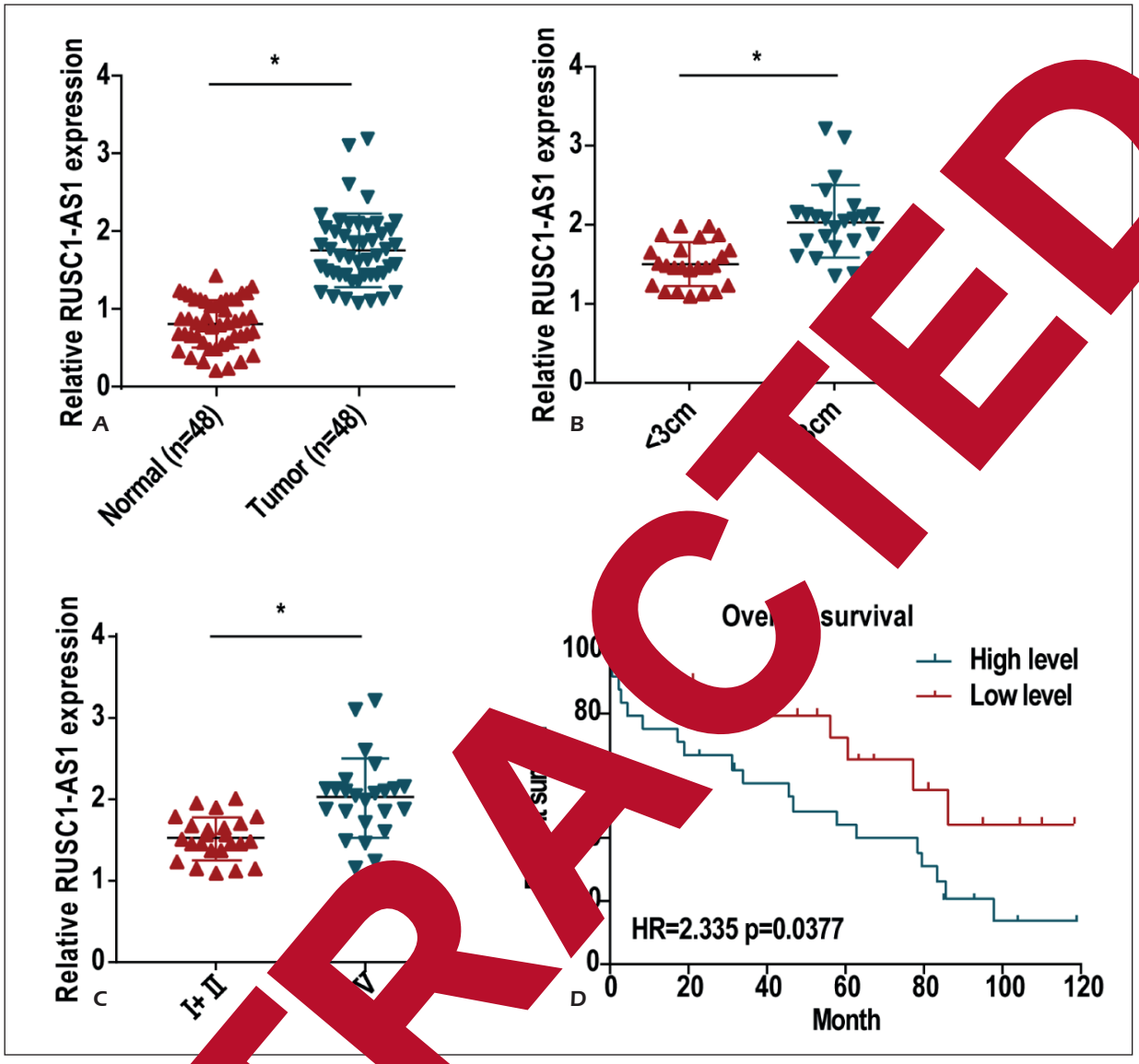
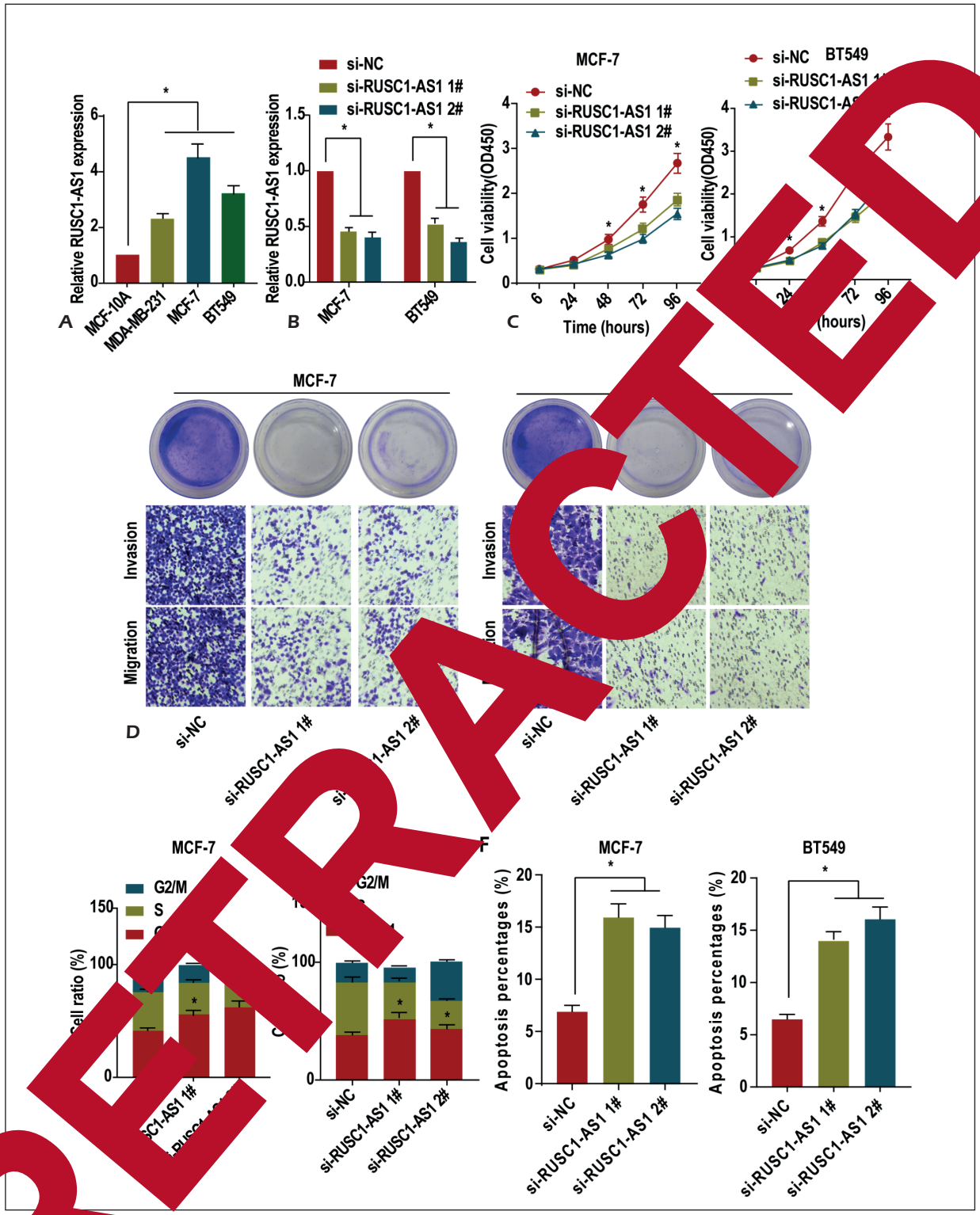


Figure 2. Upregu[lation] of RUSC1-AS1 in BCa[tissues]. [A] qRT-PCR data revealed a higher level of RUSC1-AS1 in BCa tissues relative to controls. ***B,*** [RUSC1]-AS1 expression was higher in BCa tissues larger than 3 cm in diameter relative to those with smaller than 3 cm. ***C,*** BCa tissues with stage III-IV had a higher level of RUSC1-AS1 compared with those of stage I-II. ***D,*** BCa patients with high level RUSC1-AS1 had the worse overall survival than those with low level.

get[ting] EZH2 and LSD1 (Figure 4B). RIP assay in[dicated] that RUSC1-AS1 directly bound to EZH2 and LSD1 in MCF-7 cells (Figure 4C). To explore [the] functions of LSD1 and EZH2, we examined expression changes of proliferation-related genes in MCF-7 cells after knockdown of LSD1 or EZH2. Transfection efficacies of si-LSD1 (1#, 2#) and si-EZH2 (1#, 2#) were verified in MCF-7 cells (Figure 4D, E). The mRNA levels of Bax, PTEN, and CDKNA1A were upregulated, while Bcl-2 was downregulated by silencing LSD1. However, the relative level of CDKN2B did not change by LSD1 knockdown (Figure 4F). In comparison, knockdown of EZH2 upregulated levels of Bax, KLF2, CDKN1A, and CDKN2B, whereas levels of Bcl-2 and PTEN did not change (Figure 4G). Based on the above data, only KLF2 and CDKN1A were upregulated by transfection of si-RUSC1-AS1, si-LSD1 or si-EZH2 (Figure 4H). Studies[8-10] have shown that CDKN1A and KLF2 serve as tumor-suppressor genes. EZH2 is a negative regulator of histone 3 lysine 27 (H3K27me3) trimethylation, and LSD1 negatively regulates histone 3 lysine 4 (H3K4me2) demethylation[11,12].



F of RUSC1-AS1 inhibited the proliferative ability of BCa cells. ***A,*** RUSC1-AS1 expression was higher in cell lines (MDA-MB-231, MCF-7, and BT549) than that in breast epithelial cell line (MCF-10A). ***B,*** Transfection of si-C1-AS1 1# or si-RUSC1-AS1 2# could markedly downregulate RUSC1-AS1 level in MCF-7 and BT549 cells. ***C,*** CCK-8 howed that the viability of BCa cells was reduced by RUSC1-AS1 knockdown in a time-dependent manner. ***D,*** Colony fo and transwell assays showed that RUSC1-AS1 knockdown inhibited proliferative, invasive, and migrated ability of BCa ells (magnification × 20). ***E,*** Flow cytometry showed that RUSC1-AS1 knockdown arrested BCa cells in G0/G1 phase. ***F,*** Flow cytometry showed that RUSC1-AS1 knockdown induced apoptosis of BCa cells.

Thus, RUSC1-AS1 may inhibit the expressions of CDKN1A and KLF2 by recruiting them to their promoter regions, resulting in H3K27 trimethylation or H3K4 demethylation. In this paper, the ChIP assay showed that the enrichment of EZH2, LSD1, and H3K27me3 to the CDKN1A and KLF2 promoter regions were reduced after knockdown of RUSC1-AS1. Conversely, the enrichment of H3K4me2 to the CDKN1A and KLF2 promoter regions was elevated (Figure 4I, 4J). These data indicated that RUSC1-AS1 exerted its biological function by inhibiting expressions of CDKN1A and KLF2 via binding to EZH2 and LSD1.

RUSC1-AS1 Negatively Regulated Expressions of KLF2 and CDKN1A

KLF2 and CDKN1A were lowly expressed in BCa tissues relative to controls (Figure 5A, 5B). Transfection of si-KLF2 or si-CDKN1A markedly downregulated both mRNA and protein levels in MCF-7 cells (Figure 5C-5F). Viability of MCF-7 cells was elevated by the silence of KLF2 or CDKN1A (Figure 5G). Rescue experiments indicated that the inhibited viability and clonality of MCF-7 cells due to RUSC1-AS1 knockdown were partially reversed by KLF2/CDKN1A knockdown (Figure 5H, 5I).

Discussion

LncRNAs could be ser as on enes or tumor-suppressor genes a, sho lose relationship to the pro of ample, HOTAIR is gula oth primary and metastatic BC ich prom oliferation and inhibits apo f cancer cell TAIR is positively cor ed metastasis a mortality of BCa[14]. Tao et al[15] d that estrogen can inhibit m A-148a level h binding to G protein pled estrogen rec r (GPER), thus upre ing H IR to promote the metastasis Ca. L NA RO is a molecular marker of BC red wi jacent tissues, ROR is ly ex d in tissues. ROR is affected ogen sterone, which is related to the ical sym ns and metastasis of tumors[16]. work, RUSC1-AS1 was highly ex- tissues and cell lines. Moreover, SC1-AS1 level was positively correlated with r size and clinical stage, but negatively cor- with survival of BCa patients. Knockdown of RUSC1-AS1 inhibited the proliferative rate of BCa cells, arrested cell cycle progression in the G0/G1 phase, and induced apoptosis of BCa cells. We believed that RUSC1-AS1 may exert an oncogenic role in BCa. Furthermore, biologic tions and functional verifications su RUSC1-AS1 directly bound to EZ and LSD1 in MCF-7 cells.

Many lncRNAs are capable of ting target gene expressions by i acting RNA binding proteins, such as , SUV39H etc.[17-20]. EZH2 is an im nt cata tic subu PRC2, acting as a h e me ransferase to specifically induce hi H3 ine 27 meth- ylation (H3K27 to enes[21]. H2 exerts a key rol tumorige an mor progression[11,22]. also know M1A, is a histone d that specific y demethylates histo H3 (H 2) monomer and lysines 4[23]. A number o ence has shown that LS be a regulato mponent in cellular esses. LSD1 has a high abundance in a vari- of tumors[24] pancreatic cancer, lncRNA IN inhibits tosis and promotes cell pro- li on by bin to LSD1 and EZH2[27].

KLF2 have been identified to be tumo ppressor genes that regulate proliferation and apoptosis of tumor cells[8-10]. In this pa- kdown of LSD1 and EZH2 upregulated on tion-related genes CDKN1A and KLF2 in MCF-7 cells. The silence of KLF2 or CDKN1A elevated the viability of MCF-7 cells. Subsequently, we found that knockdown of RUSC1-AS1 or KLF2/CDKN1A could partially reverse the decreased viability caused by RUSC1-AS1 knockdown. We confirmed that RUSC1-AS1 exerted its biological functions through KLF2 or CDKN1A.

There are still some shortcomings in this work. We mainly elucidated the ontogenetic role of RUSC1-AS1 at the cellular level. Further *in vivo* investigations are required for exploring the potential role of RUSC1-AS1 in BCa.

Conclusions

We showed that RUSC1-AS1 is highly expressed in BCa, which promoted the progression of BCa through mediating CDKN1A and KLF2. RUSC1-AS1 may serve as a potential hallmark for BCa.

Conflict of Interests

The authors declare that they have no conflict of interest.

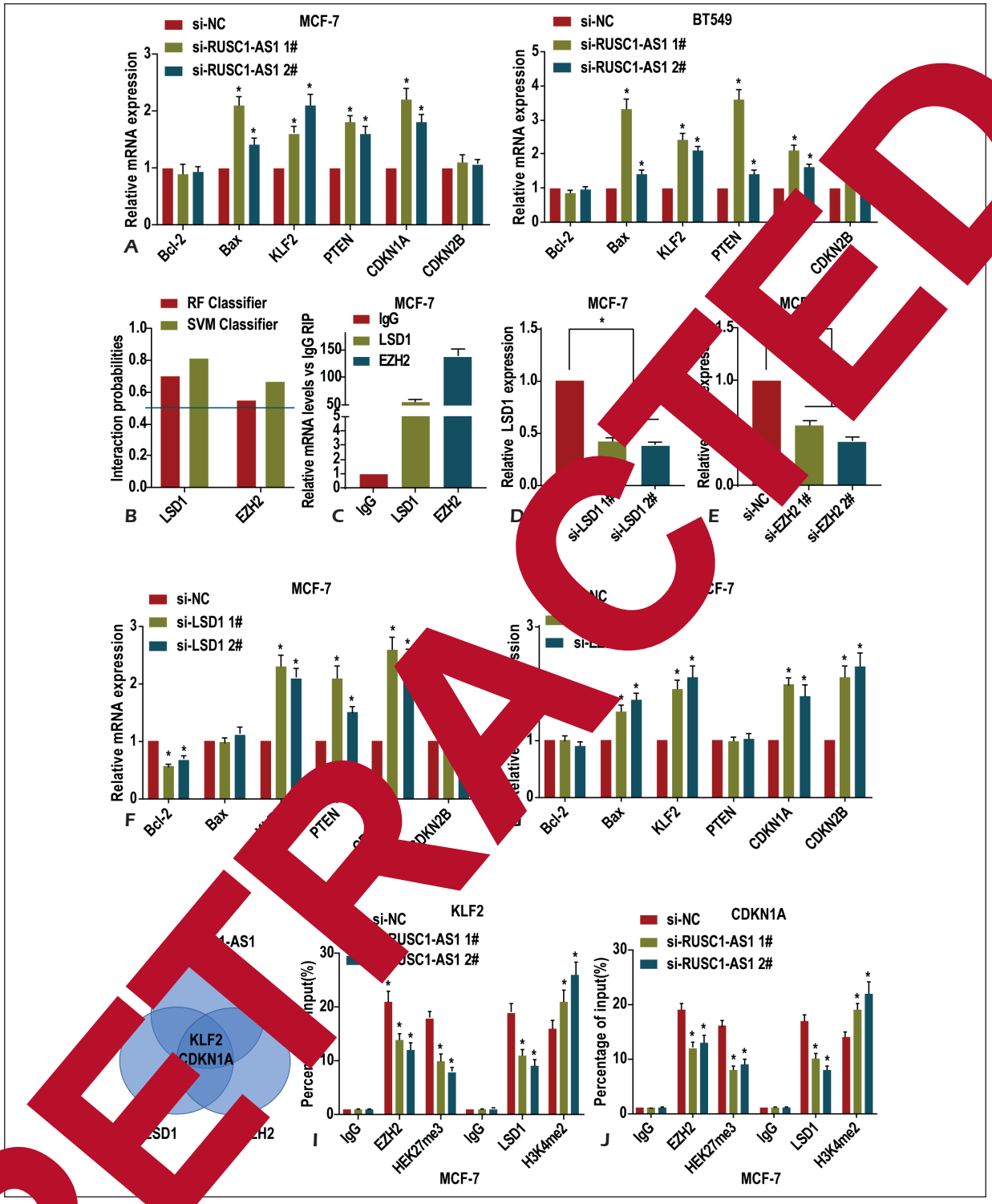


Fig. 4. RUSC1-AS1 silenced KLF2 and CDKN1A by binding to LSD1 and EZH2. ***A,*** RUSC1-AS1 knockdown upregulated KLF2, PTEN, and CDKN1A, while levels of Bcl-2 and CDKN2B did not change. ***B,*** EZH2 and LSD1 were predicted to be the target genes of RUSC1-AS1. ***C,*** RIP assay showed that RUSC1-AS1 directly bound to EZH2 and LSD1 in MCF-7 cells. ***D-E,*** Transfection efficacy of si-LSD1 (1#, 2#) and si-EZH2 (1#, 2#) verified in MCF-7 cells. ***F,*** Knockdown of LSD1 upregulated the mRNA levels of KLF2, PTEN, and CDKNA1A, but downregulated Bcl-2. ***G,*** Knockdown of EZH2 upregulated levels of Bax, KLF2, CDKN1A, and CDKN2B, whereas levels of Bcl-2 and PTEN did not change. ***H,*** Gene intersections of genes with similar expression trends after transfection of si-RUSC1-AS1, si-LSD1 or si-EZH2. ***I-J,*** ChIP assay showed that the enrichment of EZH2, LSD1, and H3K27me3 to the CDKN1A and KLF2 promoter regions was reduced after knockdown of RUSC1-AS1. Conversely, enrichment of H3K4me2 to the CDKN1A and KLF2 promoter regions was elevated.

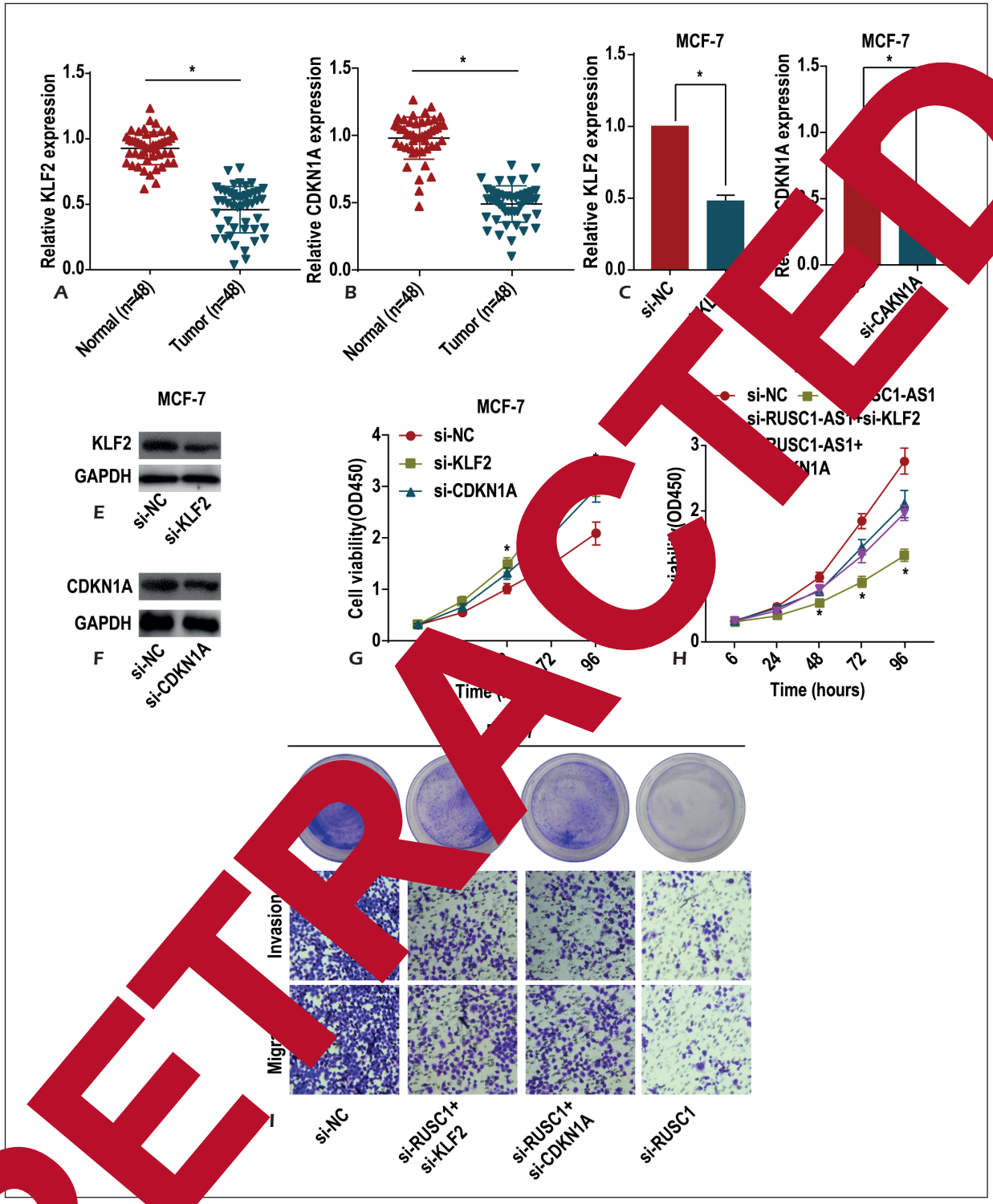


Fig 5. RUSC1-AS1 negatively regulated expressions of KLF2 and CDKN1A. ***A-B,*** KLF2 and CDKN1A were lowly ex issues relative to controls. ***C-F,*** Transfection of si-KLF2 or si-CDKN1A markedly downregulated both A and p in levels in MCF-7 cells. ***G,*** Viability of MCF-7 cells was elevated by the silence of KLF2 or CDKN1A. ***H-I,*** inhibited viability, clonality, invasive and migrated capacity of MCF-7 cells due to RUSC1-AS1 knockdown were partially ed by KLF2/CDKN1A knockdown (magnification × 20).

References

1) Darabi H, Czene K, Zhao W, Liu J, Hall P, Humphreys K. Breast cancer risk prediction and individualised screening based on common genetic variation and breast density measurement. Breast Cancer Res 2012; 14: R25.
2) Zhao J, Jiang GQ. MiR-4282 inhibits proliferation, invasion and metastasis of human breast cancer by targeting Myc. Eur Rev Med Pharmacol Sci 2018; 22: 8763-8771.
3) Pfeiffer RM, Park Y, Kreimer AR, Lacey JJ, Pee D, Greenlee RT, Buys SS, Hollenbeck A, Rosner B, Gail MH, Hartge P. Risk prediction for breast, endometrial, and ovarian cancer in white women aged 50 y or older: derivation and validation from population-based cohort studies. PLoS Med 2013; 10: e1001492.
4) Gutschner T, Hammerle M, Diederichs S. MALAT1 -- a paradigm for long noncoding RNA function in cancer. J Mol Med (Berl) 2013; 91: 791-801.
5) Nakagawa T, Endo H, Yokoyama M, Abe J, Tamai K, Tanaka N, Sato I, Takahashi S, Kondo T, Satoh K. Large noncoding RNA HOTAIR enhances aggressive biological behavior and is associated with short disease-free survival in human non-small cell lung cancer. Biochem Biophys Res Commun 2013; 436: 319-324.
6) Ono H, Motoi N, Nagano H, Miyauchi E, Ushijima M, Matsuura M, Okumura S, Nishio M, H[illegible] T, Inase N, Ishikawa Y. Long noncoding RNA [illegible] is relevant to cellular proliferation, invasi[illegible]ss, and clinical relapse in small-cell lung c[illegible]r. Cancer Med 2014; 3: 632-642.
7) Li H, Li J, Jia S, Wu M, An J, Zheng Q, Zhang W, [illegible] D. miR675 upregulates long n[illegible]g RNA H[illegible] through activating EGR1 in[illegible]r cancer. Oncotarget 2015; 6: 3195[illegible]84.
8) Abbas T, Dutta A. p21 in [illegible]er: intric[illegible]etworks and multiple activities[illegible] Rev C[illegible] 400-414.
9) Xu TP, Liu XX, X[illegible] Yin L[illegible]R, Chen WM, Huang MD, Shu[illegible] SP1-induc[illegible]egulation of the long non[illegible] RNA TINCR[illegible]lates cell proliferation[illegible]ptosis by af[illegible]g KLF2 mRNA sta[illegible]y in ga[illegible]ancer. Oncogene 2015; 34: 564[illegible]5661.
10) Zhan[illegible]Dai Y, Cai Y, Suo[illegible]H, Wang Y, Cheng Z, [illegible]. KLF2 is downreg[illegible]ed in pancreatic d[illegible] adeno[illegible]cinoma and inhibits the growth migrati[illegible] cancer cells. Tumour Biol 2016; [illegible]125[illegible].
11) Yo[illegible]nnighau[illegible] EZH2 methyltransferase and [illegible] met[illegible]ion in breast cancer. Int J [illegible]ol Sci[illegible]-65.
12) [illegible]ova EY, [illegible]-Guzman C, Salzberg AC, Zhang [illegible] Barnstable CJ. LSD1-mediated demethylation [illegible]8 is required for the transition from [illegible]ate p[illegible]itor to differentiated mouse Rod photoreceptor. Mol Neurobiol 2016; 53: 4563-4581.
13) Nagini S. Breast cancer: current molecular therapeutic targets and new players. Anticancer Agents Med Chem 2017; 17: 152-163.
14) Bhan A, Hussain I, Ansari KI, Kasiri S[illegible] Mandal SS. Antisense transcript [illegible]hone[illegible] ing RNA (lncRNA) HOTAIR is [illegible]scriptionally induced by estradiol. J Mol Bio[illegible]3: 425: 3707-3722.
15) Tao S, He H, Chen Q. Estr[illegible]diol inc[illegible]OTAIR levels via GPER-mediat[illegible]R-148a [illegible]n i[illegible] breast cancer. J Trans[illegible]d 2015; 13: 1[illegible]
16) Eades G, Wolfson B, [illegible]g Y, Li [illegible]Yao Y, Zh[illegible] lincRNA-RoR and [illegible]-145 re[illegible]te invasion in triple-negative br[illegible]nce[illegible]targetin[illegible]ARF6. Mol Cancer R[illegible]201[illegible]-338.
17) Sun M, Nie F[illegible]ng C, [illegible] Hou[illegible] C, Li W, He X, Lu K[illegible]e pseudoge[illegible] promotes non-sm[illegible]ng cancer c[illegible]neration and invasi[illegible]y [illegible]netically silen[illegible]g EGR1 and RHO[illegible]Mol The[illegible]7: 25: 739-751.
18) Poliseno L. Pseudo[illegible] newly discovered player[illegible]uman cancer[illegible]ignal 2012; 5: e5.
19) [illegible]rola M, Comisso E, [illegible]scolo R, Chiaradia R, Marion RM, Schneider C, Blasco MA, Schoeftner S, Benetti R. Ep[illegible]etic silencing of Oct4 by a complex containi[illegible]UV39H1 and Oct4 pseudogene lncRNA. Nat [illegible]mmun 2015; 6: 7631.
20) [illegible]W, Xie[illegible]un M, Chen TY, Jin RR, Ma TS, Chen [illegible], He XZ, De W, Zhang ZH. The pseudoge[illegible]erived long noncoding RNA DUXAP8 promotes gastric cancer cell proliferation and [illegible]igration via epigenetically silencing PLEKHO1 [illegible]ssion. Oncotarget 2017; 8: 52211-52224.
21) Yamaguchi H, Hung MC. Regulation and role of EZH2 in cancer. Cancer Res Treat 2014; 46: 209-222.
22) Chase A, Cross NC. Aberrations of EZH2 in cancer. Clin Cancer Res 2011; 17: 2613-2618.
23) Shi Y, Lan F, Matson C, Mulligan P, Whetstine JR, Cole PA, Casero RA, Shi Y. Histone demethylation mediated by the nuclear amine oxidase homolog LSD1. Cell 2004; 119: 941-953.
24) Shi X, Ma C, Zhu Q, Yuan D, Sun M, Gu X, Wu G, Lv T, Song Y. Upregulation of long intergenic noncoding RNA 00673 promotes tumor proliferation via LSD1 interaction and repression of NCALD in non-small-cell lung cancer. Oncotarget 2016; 7: 25558-25575.
25) Wu Y, Zhou BP. Epigenetic regulation of LSD1 during mammary carcinogenesis. Mol Cell Oncol 2014; 1: e963426.
26) Qin Y, Zhu W, Xu W, Zhang B, Shi S, Ji S, Liu J, Long J, Liu C, Liu L, Xu J, Yu X. LSD1 sustains pancreatic cancer growth via maintaining HIF1alpha-dependent glycolytic process. Cancer Lett 2014; 347: 225-232.
27) Lian Y, Wang J, Feng J, Ding J, Ma Z, Li J, Peng P, De W, Wang K. Long non-coding RNA IRAIN suppresses apoptosis and promotes proliferation by binding to LSD1 and EZH2 in pancreatic cancer. Tumour Biol 2016; 37: 14929-14937.