

LINC00092 Suppresses the Malignant Progression of Breast Invasive Ductal Carcinoma Through Modulating SFRP1 Expression by Sponging miR-1827

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Abstract

Breast invasive ductal carcinoma (IDC) is a most common kind of breast cancer (BC), yet to date the corresponding effective therapies are limited. Extensive evidence has indicated that lncRNAs are involved in multiple cancers, and the potential mechanism of lncRNAs, such as LINC00092, mentioned in IDC remains elusive. IDC clinical samples from TCGA database were used to analyze the expression levels of LINC00092, miR-1827 and SFRP1. Kaplan-Meier method was applied to plot the overall survival curves. KEGG and GO were employed to screen the pathway that LINC00092 participated in. Pearson's correlation analysis determined the relationship between LINC00092 and SFRP1. Bioinformatics analysis and dual-luciferase reporter assay examined the association among LINC00092, miR-1827, and SFRP1. Cell counting kit-8, colony formation and transwell assays were performed to detect cell viability, colony formation, and migration and invasion, respectively. Quantitative reverse-transcription polymerase chain reaction and western blot were utilized to investigate the expression at RNA and protein levels. LINC00092 expression was down-regulated in IDC tissues and cells, which was correlated with poor prognosis. Down-regulated LINC00092 facilitated cell proliferation, colony formation, and cell migration and invasion, while up-regulated LINC00092 inhibited cell malignant behaviors. LINC00092/SFRP1 physically bound to miR-1827 in IDC. SFRP1 expression was proportional to LINC00092 expression and inversely proportional to miR-1827 expression. The inhibitory effects of LINC00092 on cell aggressive behaviors were partially regulated by miR-1827/SFRP1. In summary, our results indicated that overexpression of LINC00092 inhibited the development of IDC through modulating miR-1827/SFRP1 axis, suggesting new therapeutic targets to treat IDC.

Keywords

breast invasive ductal carcinoma, LINC00092, miR-1827, SFRP1, cell aggressiveness

Introduction

Breast cancer (BC) ranks second in most frequent malignancies, accounting for 11.6% of new tumor cases and is the leading cause of cancer-related deaths in female^{1,2}. Based on the histological structures and development process, breast cancer is broadly partitioned into invasive ductal carcinoma (IDC) and invasive lobular cancer (ILC)³, of which IDC is considered as the most challenging form occupying 80% of BC cases⁴. As we know, the clinicopathological parameters and genetic characteristics of IDC are fairly complex⁵⁻⁷. Thus, despite great advances in fundamental and therapeutic researches of BC as well as IDC, the survival rate of patients with IDC is still unsatisfied mainly due to untimely diagnosis. Consequently, it is urgent to identify efficacious biomarkers and clarify their underlying therapeutic functions in IDC.

Recently, increasing long non-coding RNAs (lncRNAs), a class of RNA molecules composed of > 200 nucleotides with limited protein-encoding ability, have been identified as crucial therapeutic factors for tumor treatment^{8,9}. It is well known that lncRNAs are involved in multiple

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biological processes consisting of interaction with RNAs, miRNA competition, and protein modification at the post-transcriptional level^{10,11}, making profound impacts on the carcinogenesis¹². Heaps of lncRNAs are aberrantly expressed in BC and the altered expressional patterns are related with BC development; however, there is a limited number of lncRNAs explored extensively in IDC. Previous studies have verified that LINC00092 is one of the differentially expressed lncRNAs and correlates with prognosis of tumor patients, such as colon adenocarcinoma¹³, ovarian cancer¹⁴, and BC¹⁵. However, the biological effects of LINC00092 in BC, especially in IDC, remain enigmatic.

Thus, in the present study, we accessed to the TCGA database to detect the expression level of LINC00092 in IDC and determine its prognostic value among IDC patients. We discovered that LINC00092 expression was down-regulated in IDC, which had a bearing on poor outcome of IDC patients. LINC00092 served as a sponge of miR-1827 and SFRP1 can be directly targeted by miR-1827. Functional experiments *in vitro* displayed that LINC00092 could inhibit cell proliferation, migration and invasion through regulating miR-1827/SFRP1 axis. Our findings may shed novel insights on therapies of IDC and expand our visions toward the molecular mechanisms for IDC.

Materials and Methods

Public Data Collection of Clinical Samples

Clinical data of IDC patients from The Cancer Genome Atlas (TCGA; <https://tcga-data.nci.nih.gov/tcga/>) database were collected to determine the expression levels of LINC00092, SFRP1 and miR-1827. Based on the differential expression levels, the overall survival curves were plotted using Kaplan-Meier method with a log-rank test.

GO and KEGG Enrichment

The database for annotation visualization and integrated discovery (DAVID) (<http://david.abcc.ncifcrf.gov/>) was utilized for functional annotation of genes to clearly explain the potential biological functions of genes. Based on data about the gene co-expressed with LINC00092 after screening, functional annotations were carried out using R software clusterProfiler package to perform the GO term and KEGG analyses. False discovery rate (FDR) < 0.05 was established for significant difference.

Cell Lines and Transfection

Three types of human breast cancer cell lines MCF7, MDA-MB-231, and BT549, as well as the normal human breast epithelial cell MCF-10A were obtained from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China) and cultivated in RPMI-1640 medium supplemented with

10 % fetal bovine serum (FBS; Invitrogen, ThermoFisher Scientific, Carlsbad, CA) and antibiotics (100 U/ml penicillin and 0.1 mg/ml streptomycin; Invitrogen). All these cells were maintained in a humidified incubator at 37°C with 5% CO₂.

Sequences of LINC00092 and SFRP1 were amplified by GenePharma Co., Ltd (Shanghai, China) and cloned into the pcDNA3.1 vector to establish the pcDNA3.1-LINC00092 and pcDNA3.1-SFRP1 vectors which were used for up-regulating the expression levels of LINC00092 and SFRP1. In addition, miR-1827 mimic, miR-1827 inhibitor, si-SFRP1, and their corresponding negative controls were also synthesized by Shanghai GenePharma. All agents were separately transfected into tumor cells using Lipofectamine 2000 as per the manufacturer's instructions. After 24 h transfection, cells with over 80% confluence were collected for further experiments.

Quantitative Reverse-Transcription Polymerase Chain Reaction (qRT-PCR)

Isolation of whole RNAs from transfected cells was performed by TRIzol reagents following the protocols of manufacturers. The concentration of RNA was measured by NanoDrop 2000 (Thermo Fisher Scientific, Inc.). Subsequently, PrimeScript RT kit (Takara biomedical Technology Co., Ltd., Beijing, China) or Mir-XTM miRNA first strand synthesis kit (Takara biomedical Technology Co., Ltd.) was used to reversely transcribe RNA into cDNA. The relative mRNA expression was detected using SYBR Premix Ex Taq II (TaKaRa, Japan) or MiScript SYBR-Green PCR kit (Qiagen) on the 7900HT real-time PCR system. The expression levels were calculated by 2^{-ΔΔCt} method and GAPDH or U6 was regarded as the internal control. Sequences of primers were as follows:

LINC00092 F: 5'-CCTATGATTTGGCCTCTGGA-3,'

R: 5'-GAGAGCAGCGTTCAGGAAAC-3';

SFRP1 F: 5'-CAATGCCACCGAAGCCTCCAAG-3,'

R: 5'-CAAACCTCGCTGGCACAGAGATG-3';

GAPDH F: 5'-TGTGTCCGTCGTGGATCTGA-3,'

R: 5'-CCTGCTTACCACCTTCTTGA-3.'

Western Blotting

Total protein of transfected cells was extracted using RIPA buffer supplemented with protease inhibitor. The protein concentration was detected by the BCA method and then boiled at 95 min for denaturation. Next, equal amounts of denatured proteins were loaded in 12% SDS-PAGE and transferred onto PVDF membranes. Afterwards, PVDF membranes were blocked in 5% skimmed milk at room

temperature for 1 h, incubated with primary antibodies (Abcam, Cambridge, MA, USA) against SFRP1 (1:1000; Cell Signaling Technology, Danvers, MA, USA) and GAPDH (1:3000; Cell Signaling Technology) at 4°C overnight, followed by incubation with the horseradish peroxidase (HRP)-conjugated secondary antibody (Abcam) at room temperature for 1 h. After rinsing with phosphate buffered saline (PBS), the protein blots were visualized by enhanced chemiluminescence (ECL) solution (Beyotime; Beijing, China) and the intensity of protein bands was quantified using ImageJ software.

Cell Proliferation Assay

Cell viability was determined using cell counting kit-8 (CCK-8) assay (Beyotime). Briefly, cells were inoculated in 96-well plates with a density of 5×10^4 cells per well. After incubation at 0, 24, 48 and 72 h, 10 μ L of CCK-8 reagents was added into each well for additional 1.5 h incubation at 37°C. Finally, the optical density (OD) value was assessed at a wavelength of 450 nm under a microplate reader (Bio-Rad, Hercules, CA, USA).

Colony Formation Assay

Following 24 h transfection, tumor cells were collected. The collected cells were digested with trypsin and then suspended in the complete medium to prepare cell suspension. The cell suspensions were gradient diluted several times, and the cells (1×10^3) were seeded into the 60 mm dishes containing 10 mL culture medium and maintained at 37°C with 5% CO₂. Three pores were set up for each experiment. Two weeks later, visible colonies observed by naked eyes were fixed with 4% paraformaldehyde for 30 min and dyed in 0.1% crystal violet for 20 min. After washing with PBS, colonies were photographed and the number was counted.

Cell Migration and Invasion Analyses

The 24-well transwell chambers with 8- μ m pore size (BD Biosciences, San Jose, CA, USA) were utilized to examine the migration and invasion capabilities of transfected cells. For cell invasion detection, the Matrigel (1:6 dilution) reagents were used to pre-coat the upper chamber of chambers while 600 μ L culture medium with 10% FBS was placed in the lower chamber, transfected cells (2×10^5 cells/well) were inoculated in the top chamber. After incubation for 24 h, non-invaded cells on the top chamber were wiped out using cotton swabs, and the invaded cells were fixed in 4% paraformaldehyde and stained with 0.1% crystal violet. Following a brief wash, a total of five visual fields were selected to be photographed using microscope. Each experiment was repeated independently for three times. For cell migration test, no Matrigel was coated on the top chamber and the other steps were same as the cell invasion assay.

Dual-Luciferase Reporter Assay

The computational analysis of LncBase Predicted v.2 (<http://www.microrna.gr/LncBase/>) and TargetScan 7.2 (http://www.targetscan.org/vert_72/) revealed a binding site between miR-1827 and LINC00092 as well as SFRP1. The sequences of SFRP1 3'-UTR including wild type (WT) or mutant (MUT) predicted binding sites were cloned into the luciferase vector pmirGLO (Promega, Madison, WI, USA) for SFRP1-WT/MUT construction, respectively. Then, SFRP1-WT/MUT was co-transfected with blank control, si-control, si-LINC00092, miR-1827 inhibitor (inhibitor) and si-LINC00092+inhibitor in MAD-MB-231 or with blank control, pcDNA3.1-empty vector (vector), pcDNA3.1-LINC00092 (LINC00092), miR-1827 mimic (mimic), and LINC00092+mimic in MCF7 cells using Lipofectamine 2000. After the 48-h incubation, cells were harvested for luciferase activity exploration with the Dual-luciferase Assay Kit (Promega).

Statistical Analysis

All the data were exhibited as mean \pm standard deviation (SD), and analyzed by GraphPad Prism 5.0 (GraphPad Software Inc, CA, USA) and SPSS 22.0 software (IBM Corporation, NY, USA). The differences of two groups were determined using Student's *t*-test and the comparison among multiple groups was examined by one-way analysis of variance (ANOVA) with Tukey's post hoc test. Pearson's correlation analysis was conducted to verify the association between LINC00092 and SFRP1. The *P* value less than 0.05 was considered to be statistically significant.

Results

LINC00092 Expression Was Significantly Down-Regulated and Its Down-Regulation Was Associated With Unfavorable Outcome in IDC Cohort From TCGA Database

To investigate whether LINC00092 is associated with the progression of IDC, the IDC cohort composed of 113 normal cases and 1109 tumor samples was harvested from TCGA database. The result showed that the expression level of LINC00092 was conspicuously reduced in IDC tissue samples compared with that in normal controls ($P < 0.0001$, Fig. 1A). Next, these clinical samples were dichotomized into high LINC00092 expression group and low LINC00092 expression group on the basis of the median value. The overall survival curve uncovered that IDC patients with low LINC00092 level had poorer prognosis as compared with those with high LINC00092 level ($P = 3.937e-03$, Fig. 1B). Moreover, the expressional pattern of LINC00092 in tumor cells exhibited the consistent results with that in IDC tissues. As shown in Fig. 1C, LINC00092 expression was markedly

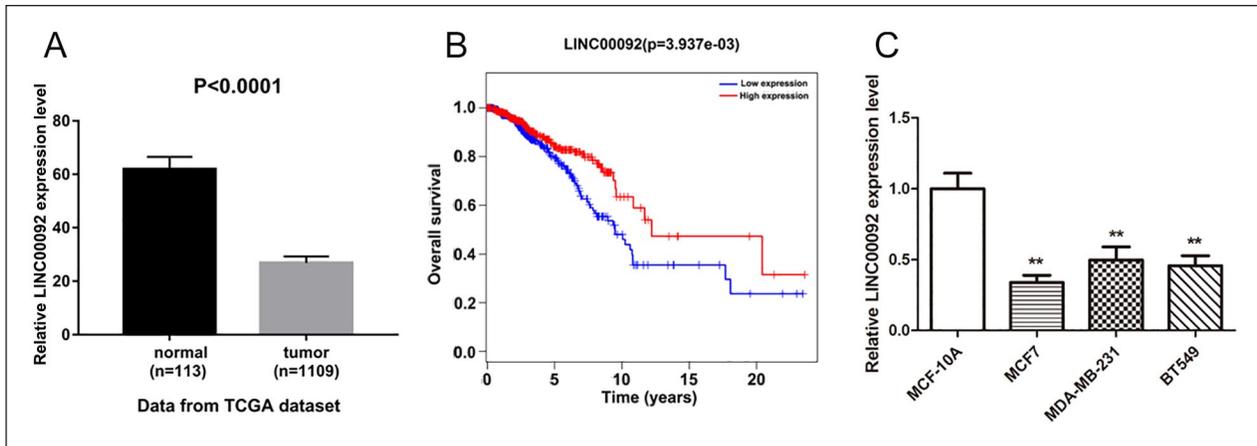


Figure 1. LINC00092 was significantly decreased and associated with unfavorable outcome in IDC cohort from TCGA database. (A) Based on the TCGA-IDC cohort, the expression of LINC00092 in IDC tissues was determined, $P < 0.0001$. (B) Overall survival curve plotted by Kaplan-Meier method showed the positive prognostic value of LINC00092, $P = 3.937e-03$. (C) Expressional pattern of LINC00092 in IDC cells was exhibited using qRT-PCR analysis. IDC: invasive ductal carcinoma; TCGA: The Cancer Genome Atlas; qRT-PCR: Quantitative Reverse-Transcription Polymerase Chain Reaction. $**P < 0.01$ compared with MCF-10A.

down-regulated in three tumor cell lines (MCF7, MAD-MB-231 and BT549) compared with that in human normal cell line MCF-10A ($P < 0.01$). Therefore, these findings supported the view that LINC00092 likely participates in the development of IDC.

LINC00092 Inhibited Cell Proliferation, Colony Formation, and Cell Migration and Invasion in IDC

In order to explore the influence of LINC00092 on IDC, we performed loss/gain-of-function experiments to examine whether LINC00092 can affect cell malignant behaviors in MDA-MB-231 and MCF7. First, after MDA-MB-231 and MCF7 cells were transfected with siLINC00092 and overexpressed LINC00092 plasmids, the expression of LINC00092 was detected, with the results unveiling an up-regulated LINC00092 in LINC00092 group and a down-regulated LINC00092 in siLINC00092 group ($P < 0.01$, Fig. 2A). CCK-8 assay revealed that knockdown of LINC00092 promoted the viability of MDA-MB-231 cells especially at 48 and 72 h, whereas overexpression of LINC00092 suppressed the viability of MCF7 cells ($P < 0.01$, Fig. 2B, C). Subsequently, colony formation assay was conducted to verify the inhibitory effect of LINC00092 on cell growth. Low-expressed LINC00092 elevated clonogenic ability and LINC00092 overexpression inhibited colony formation in corresponding tumor cells ($P < 0.01$, Fig. 2D, E). In addition, the role of LINC00092 in migration and invasion capabilities of MDA-MB-231 and MCF7 cells was determined using Transwell analysis. Fig. 2F signified that compared with si-control group, migration and invasion of MDA-MB-231 cells were increased in si-LINC00092 group ($P < 0.01$). Besides,

up-regulation of LINC00092 remarkably eliminated the invasive and migratory abilities in MCF7 ($P < 0.01$, Fig. 2G). The number of invading and migrating cells also confirmed the above-mentioned findings. Collectively, our results identified that LINC00092 may be an important regulator of malignant behaviors of IDC cells.

Bioinformatics Analysis of Major LINC00092-Mediated Signaling Pathways Using TCGA-IDC Cohort and Downstream Genes of LINC00092

To further identify the key genes and foremost pathways modulated by LINC00092, we performed Kyoto Encyclopedia of Genes and Genomes (KEGG) and gene ontology (GO) analyses to enrich the co-expressed genes of LINC00092. According to the analyses, we enriched six pathways, including basal cell carcinoma, axon guidance, hepatocellular carcinoma, gastric cancer, glycosphingolipid biosynthesis-lacto and neolacto series, and Wnt signaling pathway, with several key genes ($FDR < 0.05$, Fig. 3A, B). These key genes that enriched in these pathways were listed in Table 1 ($P < 0.05$). Besides, the Wnt pathway was one of the six key pathways enriched, which was essential for various biological processes, such as cell survival, proliferation, tissue regeneration, etc¹⁶. SFRP1 that was enriched in this pathway acted as an inhibitor of Wnt pathway¹⁷. Moreover, SFRP1 was identified as a gene that possessed the strongest co-expression relationship with LINC00092 in IDC ($r=0.6539$, $P < 0.0001$, Fig. 3C). Thus, to further detect the underlying mechanism of LINC00092, we employed TCGA-IDC cohort to assess the expression level of SFRP1 in IDC patients, unearthing that the expression level of SFRP1 was dramatically down-regulated in tumor tissues in contrast to that in normal cases ($P <$

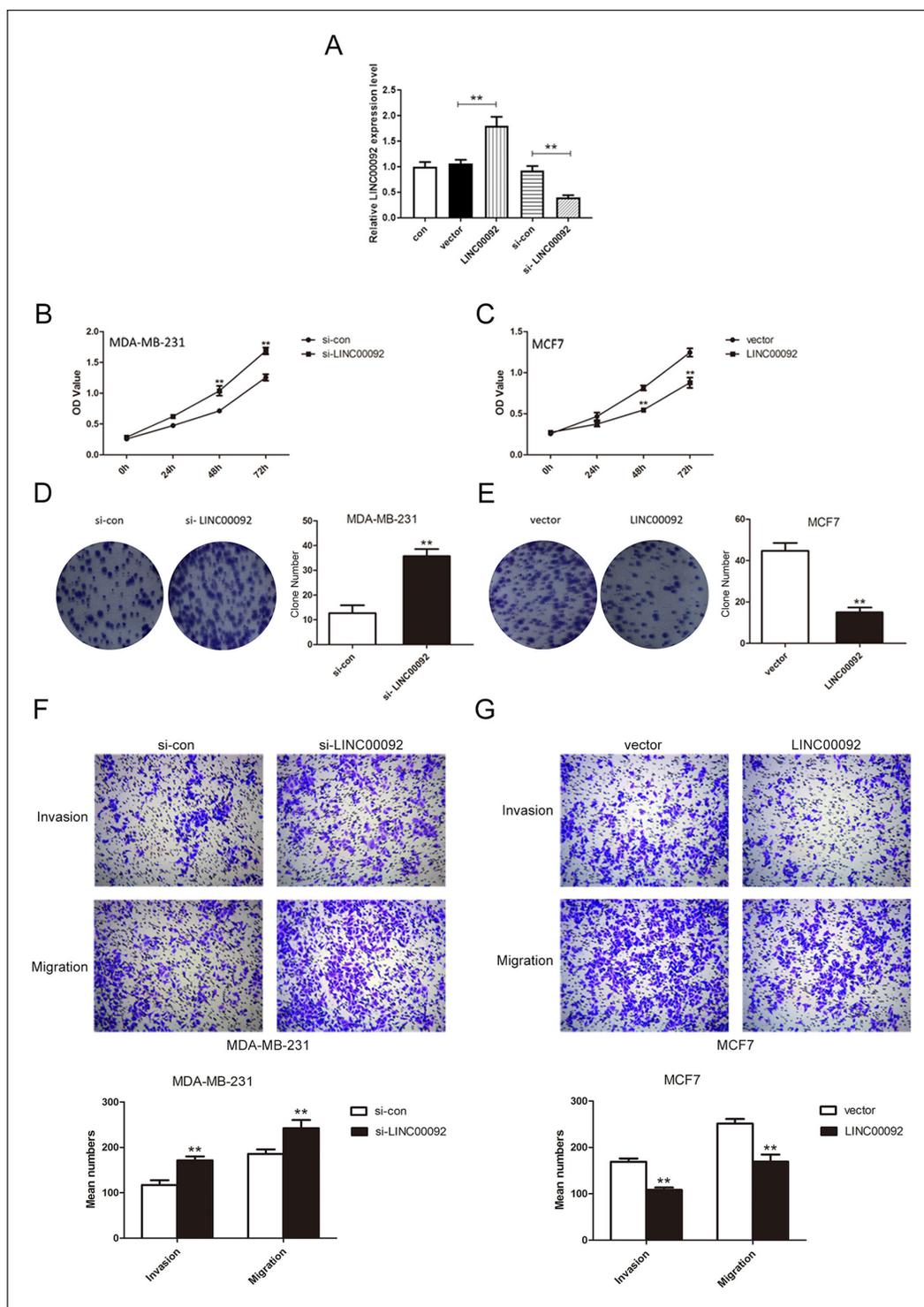


Figure 2. LINC00092 inhibited cell proliferation, colony formation, and cell migration and invasion in IDC. (A) The expression of LINC00092 in MDA-MB-231 and MCF7 cells transfected with si-LINC00092 and pcDNA3.1-LINC00092 was detected using qRT-PCR analysis. (B, C) CCK-8 assays were performed to assess the cell viability of MDA-MB-231 transfected with si-control or si-LINC00092, and MCF7 treated by vector (pcDNA3.1-empty vector) or LINC00092 (pcDNA3.1-LINC00092), $**P < 0.01$ compared with si-control or vector group. (D, E) Colony formation experiments were implemented to measure the clonogenic ability in MDA-MB-231 and MCF7 cells. The cloning percentage was quantified, $**P < 0.01$ compared with si-control or vector group. (F, G) Migration and invasion of IDC cells were investigated by transwell analysis, $**P < 0.01$ compared with si-control or vector group. IDC: invasive ductal carcinoma; OD: optical density; qRT-PCR: Quantitative Reverse-Transcription Polymerase Chain Reaction; CCK-8: cell counting kit-8.

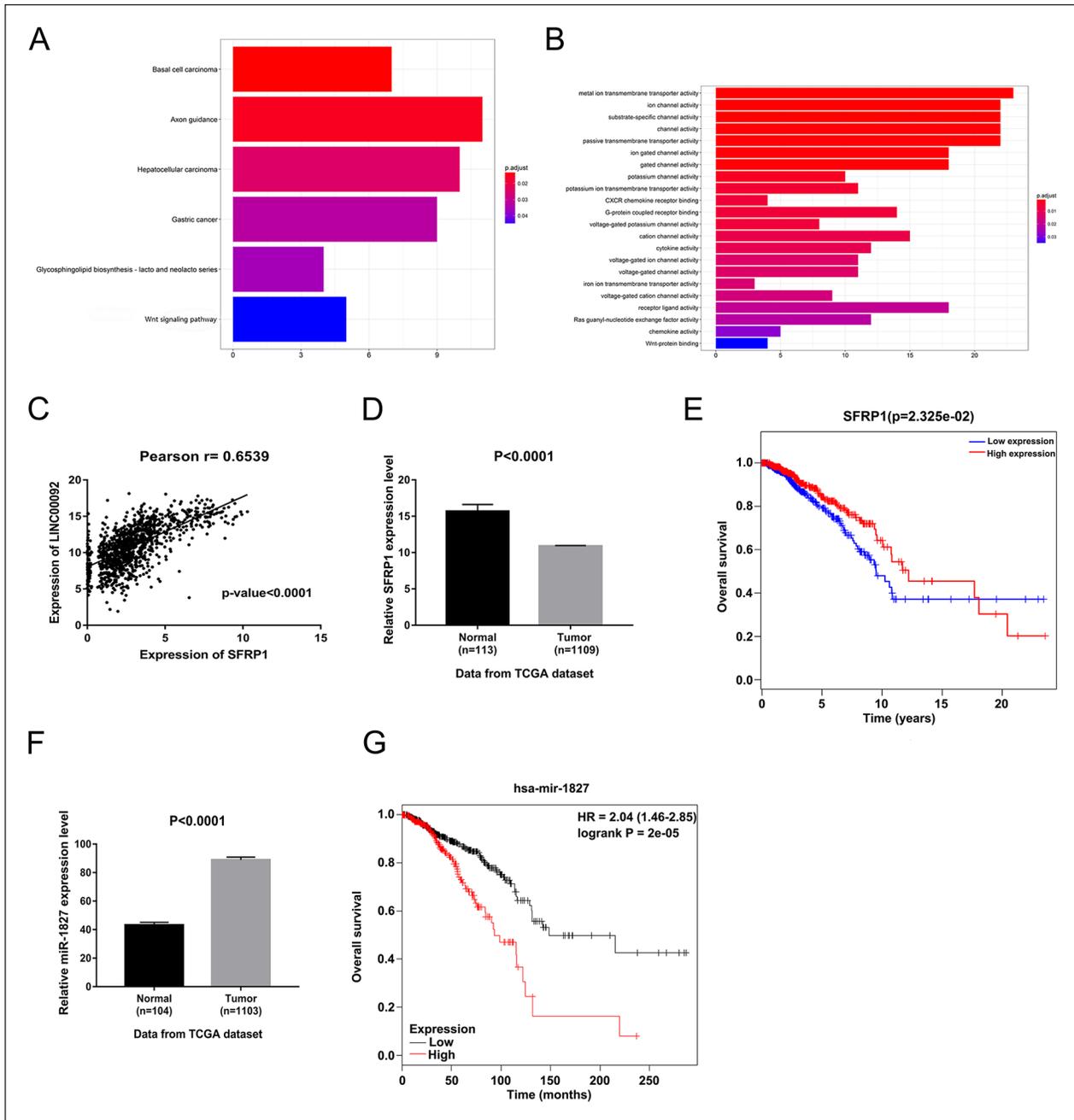


Figure 3. Bioinformatics analysis of major signaling pathways mediated by LINC00092 using TCGA-IDC cohort and downstream genes of LINC00092. (A) KEGG pathway enrichment and (B) GO enrichment analyses. (C) Pearson's correlation analysis was employed to identify the correlation between LINC00092 and SFRP1, $r = 0.6539$, $P < 0.0001$. (D) TCGA datasets suggested that SFRP1 expression was significantly decreased in tumor tissues compared with that in the normal, $P < 0.0001$. (E) Kaplan-Meier survival analysis revealed the prognostic significance of SFRP1, $P = 2.325e-02$. (F) Relative expression of miR-1827 in IDC tissue samples was detected based on the TCGA dataset, $P < 0.0001$. (G) The prognostic potential of miR-1827 was demonstrated, $P = 2e-05$. TCGA: The Cancer Genome Atlas; IDC: invasive ductal carcinoma; KEGG: Kyoto Encyclopedia of Genes and Genomes; GO: gene ontology; CXCR: C-X-C motif chemokine receptor; HR: Hazard ratio.

0.0001, Fig. 3D). The overall survival rate of SFRP1 in IDC patients was similar to that of LINC00092: the low-expressed SFRP1 resulted in poor prognosis ($P = 2.325e-02$, Fig. 3E). Furthermore, LncBase Predicted v.2 was used to predict the target miRNA of LINC00092 and TargetScan was applied to

forecast the upstream miRNA of SFRP1. Finally, miR-1827 was found to bind with both LINC00092 and SFRP1. More importantly, miR-1827 expression was signally increased in IDC, which was associated with poor prognosis ($P < 0.05$, Fig. 3F, G).

Table 1. The Enriched Pathways That Were Related With LINC00092 and Co-Expressed Genes With Statistical Significance Using KEGG Pathway Enrichment and GO Enrichment Analyses.

ID	Description	Gene	P-value	FDR
hsa05217	Basal cell carcinoma	TCF7L1/WNT6/PTCH1/FZD9/PTCH2/FZD7/BMP2	5.76E-05	0.012
hsa04360	Axon guidance	RGMA/EPHB1/PLCG2/PAK3/EPHB6/BOC/PTCH1/DPYSL2/MET/NCK2/ROBO3	1.34E-04	0.015
hsa05225	Hepatocellular carcinoma	PLCG2/TCF7L1/WNT6/EGFR/FZD9/FZD7/MET/DPF3/SHC4/GSTA1	3.26E-04	0.024
hsa05226	Gastric cancer	TCF7L1/WNT6/EGFR/FZD9/FZD7/MET/FGF9/SHC4/RARB	5.87E-04	0.032
hsa00601	Glycosphingolipid biosynthesis—lacto and neolacto series	ST8SIA1/B3GNT5/ST3GAL6/FUT4	8.07E-04	0.035
hsa04310	Wnt signaling pathway	FZD9/SFRP1/WIF1/WNT6/FZD7/	1.23E-03	0.045

KEGG: Kyoto Encyclopedia of Genes and Genomes; GO: gene ontology; FDR: False discovery rate; ID: identification.

LINC00092 Served as a Sponge of miR-1827 and Positively Regulated SFRP1

In accordance with the above data, we found that miR-1827 may be correlated with LINC00092 and SFRP1. To validate the correlation between miR-1827 and LINC00092 or SFRP1 in IDC, we implemented dual-luciferase reporter assays. The binding sites were depicted in Fig. 4A. Results of dual-luciferase reporter assays confirmed that the interference of si-LINC00092 decreased the luciferase activity of WT SFRP1 in MDA-MB-231 cells, while miR-1827 inhibitor elevated the luciferase activity. The luciferase activity of MUT SFRP1 had no significant change ($P < 0.01$, Fig. 4B). In MCF7 cells, up-regulated LINC00092 level improved the luciferase activity of WT SFRP1 and miR-1827 mimic attenuated such luciferase activity ($P < 0.01$, Fig. 4B). More importantly, qRT-PCR and western blot analyses demonstrated that SFRP1 expression was promoted by LINC00092 while reducing by miR-1827. The interference of miR-1827 could abolish the promoting effect of LINC00092 on SFRP1 expression ($P < 0.01$, Fig. 4C, D). These results denoted that LINC00092 might act as a sponge of miR-1827 and SFRP1 is the direct target gene of miR-1827.

LINC00092/miR-1827/SFRP1 Axis Regulated the Development of IDC

To clarify the effects of LINC00092/miR-1827/SFRP1 axis in IDC, a series of rescue experiments was conducted. CCK-8 assay demonstrated that miR-1827 inhibitor and SFRP1 overexpression inhibited MDA-MB-231 cells proliferation, while knockdown of LINC00092 reversed such inhibitory impacts ($P < 0.01$, Fig. 5A). Viability of MCF7 cells was promoted by miR-1827 mimic or si-SFRP1, the promoting effects of which were reversely modulated by pcDNA3.1-LINC00092 ($P < 0.01$, Fig. 5B). Similarly, colony formation assays corroborated that SFRP1 suppressed clonogenic ability of cancer cells while miR-1827 played a positive role on colony formation. LINC00092 strengthened the effects of SFRP1 and eliminated the effect of miR-1827 on colony formation ($P < 0.01$, Fig. 5C, D). We also detected

the biological function of LINC00092/miR-1827/SFRP1 axis using Transwell experiments. As expected, the migration and invasion of MAD-MB-231 cells transfected with miR-1827 inhibitor or pcDNA3.1-SFRP1 were suppressed, which was recovered by down-regulation of LINC00092 ($P < 0.01$, Fig. 6A). On the other hand, miR-1827 mimic and si-SFRP1 all accelerated the migration and invasion of MCF7 cells, the trend of which was overturned by up-regulation of LINC00092 ($P < 0.01$, Fig. 6B). Taken together, these observations indicated that LINC00092/miR-1827/SFRP1 axis might be involved in the IDC progression.

Discussion

BC is the most common gynecological cancer characterized by diverse prognoses and obvious heterogeneity^{3,18}, with IDC and ILC as two main subtypes, of which IDC accounts for approximately 80 % of invasive BC cases. DiCostanzo et al¹⁹ suggested that the outcome of classical IDC is worse than that of ILC. Previous researches demonstrated that IDC has a complicated progression, which generates from hyperplasia of breast, progressing to ductal carcinoma in situ (DCIS) and ultimately to IDC²⁰⁻²². Hence, deciphering the effective targets and examining the possible mechanism are urgently needed for IDC treatment.

Recently, mounting attentions have been paid to lncRNAs, and increasing studies have pointed out that a great deal of lncRNAs such as SPRY4-IT1²³, HOTAIR²⁴, LINC00899²⁵ and LINC01614²⁶ exerted crucial effects on the occurrence and development of BC. In 2019, Wu et al¹⁵ used limma package to identify the differentially expressed lncRNAs (DEs) in BC and found that LINC00092, as well as SLC26A4-AS1 and COLCA1, had significant correlation with the outcomes of BC patients. Considering these above-mentioned publications, we assumed that LINC00092 might be also implicated in the development of IDC. Our results for the first time elucidated that LINC00092 expression was overtly down-regulated in IDC patients compared with normal control. Reduction of LINC00092 was related with unfavorable prognosis of IDC patients. Furthermore, *in vitro* functional

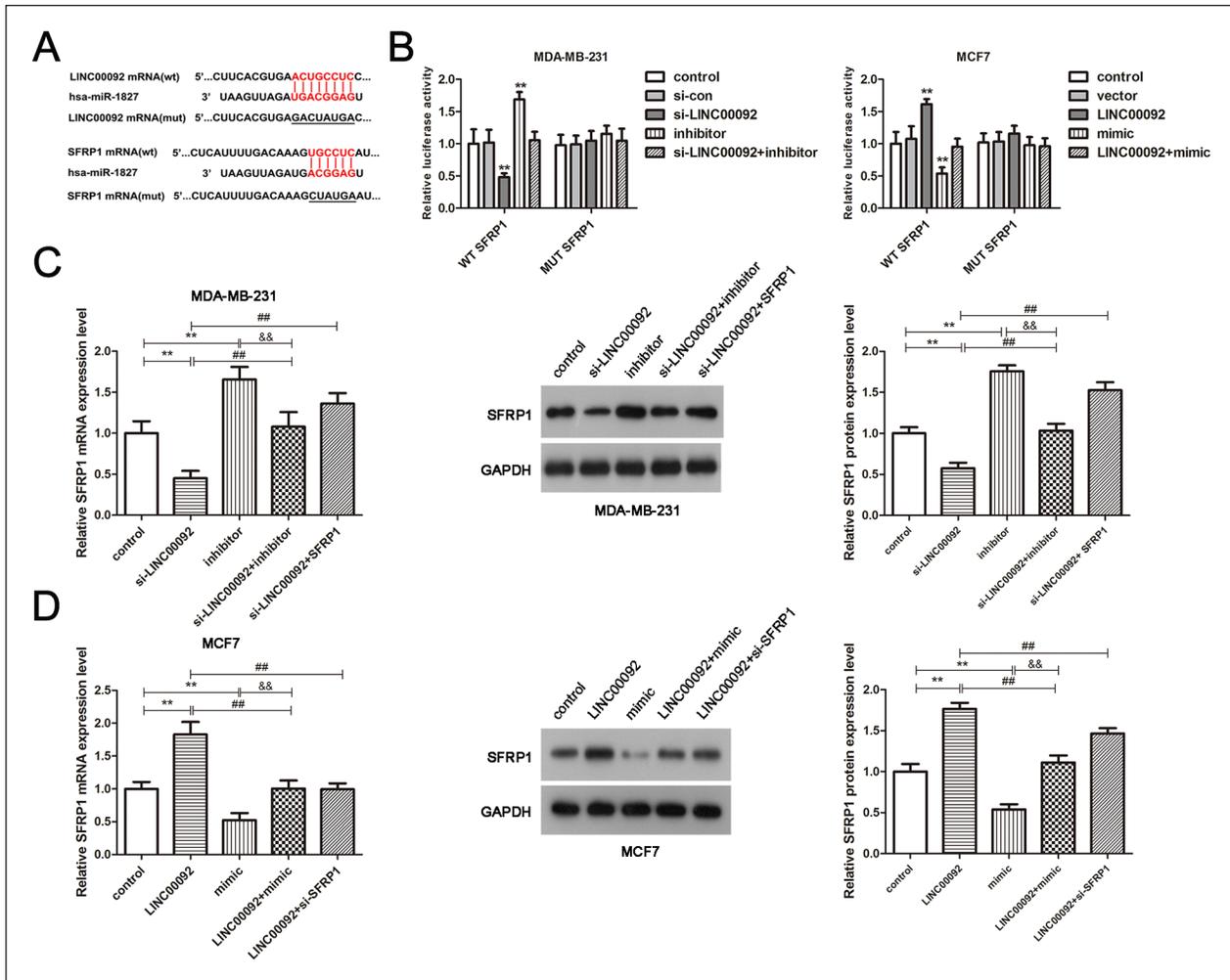


Figure 4. LINC00092 served as a sponge of miR-1827 and positively regulated SFRP1. (A) Sequences of binding sites between miR-1827 and LINC00092 or SFRP1. (B) Dual-luciferase reporter assay was performed to detect the luciferase activity, $**P < 0.01$ compared with si-control, vector or control group. (C, D) The expression of SFRP1 was investigated by qRT-PCR in MDA-MB-231 and MCF7 cells, $**P < 0.01$ compared with control, $##P < 0.01$ compared with si-LINC00092 or LINC00092, $&&P < 0.01$ compared with miR-1827 inhibitor or mimic. qRT-PCR: quantitative reverse-transcription polymerase chain reaction; WT: wild type; MUT: mutant; GAPDH: glyceraldehyde-3-phosphate dehydrogenase.

experiments revealed that LINC00092 played a suppressive role on IDC cells viability, migration and invasion and clonogenic potential. These data demonstrated that LINC00092 maybe act as a pivotal factor for the development of IDC.

To explore the underlying mechanism of LINC00092 in IDC, we performed KEGG and GO analyses to screen the related key genes and major pathways. According to the results, Wnt pathway was selected as one of the six key pathways enriched. Secreted frizzled-related proteins (SFRPs), a family of secretory glycoproteins, can inhibit the activation of Wnt pathway²⁷. Moreover, we also found that SFRP1, a member of SFRPs family, was a gene co-expressed with LINC00092. Extensive publications have unveiled that SFRP1 carries much weight in tumorigenesis. MiR-1254/SFRP1 axis could enhance lung cancer cells

proliferation²⁸. The proliferation and invasion of colon cancer cells were improved by miR-27a/SFRP1 signals²⁹. Interestingly, Vargas et al³⁰ disclosed that the neoplastic epithelium of IDC progression involves amounts of gene expression changes, for example, down-regulation of SFRP1. The result of this research was consistent with our analyses. SFRP1 has been proposed as a promising biomarker for IDC patients due to its frequency of cancer-specific hypermethylation³¹. Thus, we assessed the relationship of LINC00092 and SFRP1 using Pearson's correlation analysis and then observed that LINC00092 was positively correlated with the expression of SFRP1. The survival rate showed that down-regulated SFRP1 was related to poor prognosis of patients with IDC. In addition, microarray analysis established by Wolfson et al³² demonstrated that

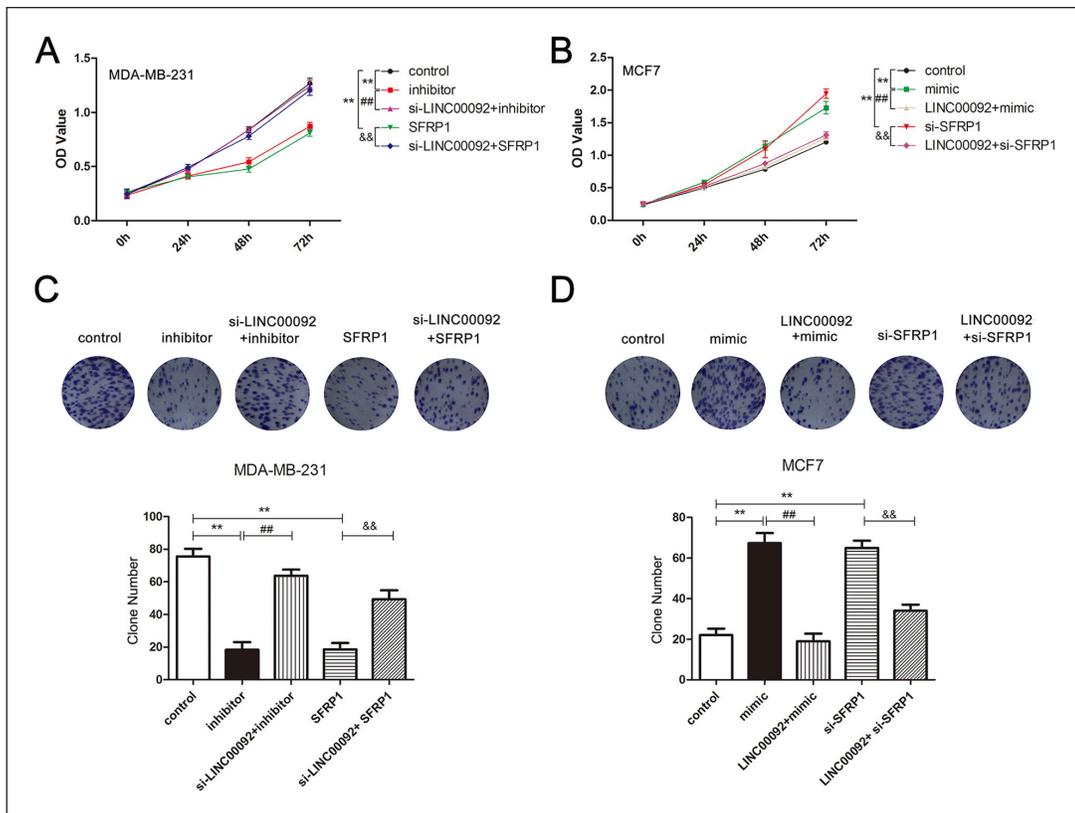


Figure 5. LINC0092 can inhibit cell viability and colony formation via regulating miR-1827/SFRP1 in IDC. (A, B) CCK-8 and (C, D) colony formation assays were conducted to measure the viability and colony formation in MDA-MB-231 and MCF7 cells. IDC: invasive ductal carcinoma; CCK-8: cell counting kit-8; OD: optical density. $^{**}P < 0.01$ compared with control, $^{###}P < 0.01$ compared with miR-1827 inhibitor or mimic, $^{\&\&}P < 0.01$ compared with SFRP1 or si-SFRP1.

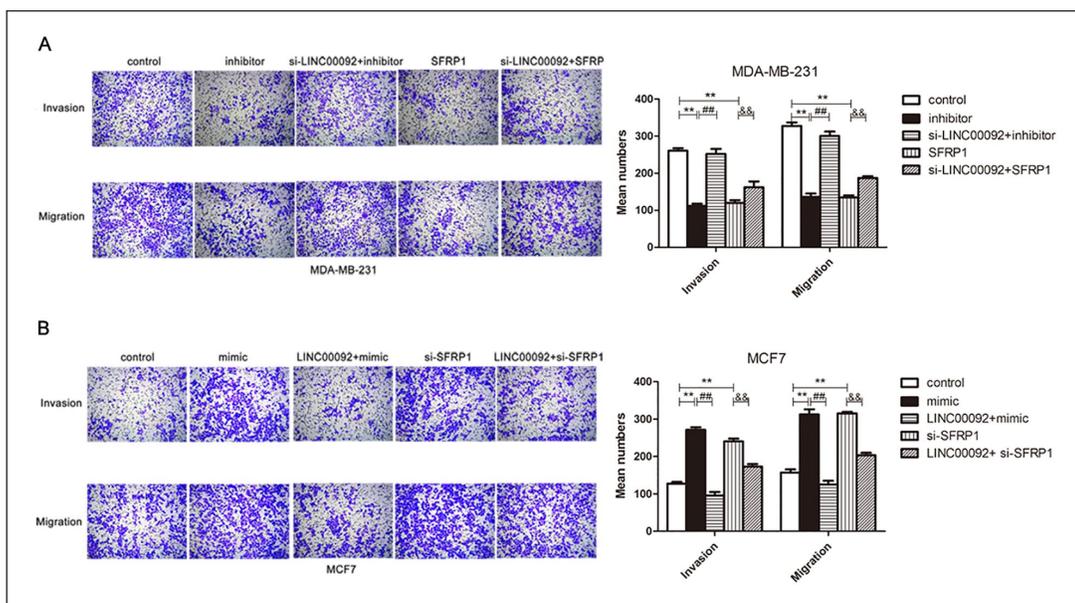


Figure 6. IDC cells invasion and migration were modulated by LINC0092/miR-1827/SFRP1. (A, B) Transwell analysis was carried out to explore the migration and invasion of IDC cells after different treatments. IDC: invasive ductal carcinoma. $^{**}P < 0.01$ compared with control, $^{###}P < 0.01$ compared with miR-1827 inhibitor or mimic, $^{\&\&}P < 0.01$ compared with SFRP1 or si-SFRP1.

loads of abnormally expressed miRNAs were located in IDC specimens, such as miR-671-5p³³, miR-17-5p and miR-106b-5p³⁴. In our study, the prediction tool affirmed that miR-1827 can directly target SFRP1. Zhang et al³⁵ elucidated that miR-1827 could modulate tumor suppressor p53 via repressing MDM2, so as to suppress colorectal cancer development. Additionally, our results illustrated that the expression of miR-1827 in IDC was considerably promoted, which was opposite to SFRP1 as well as LINC00092 expression, and caused poor outcomes of IDC patients. The current theory illustrated that lncRNAs serve as ceRNAs for miRNAs, and they together cooperate with mRNA to regulate the tumorigenesis³⁶. To clarify the correlation among them and possible mechanism in IDC, dual-luciferase reporter assays were implemented and verified the close links. Rescue experiments evidenced that the effects of LINC00092 on cell malignant behaviors were modulated by miR-1827/SFRP1 axis in IDC. However, since we only discussed the role of LINC00092 *in vitro*, further exploring the role of LINC00092 *in vivo* is necessary.

In conclusion, down-regulation of LINC00092 was discovered to be associated with poor outcome of IDC, and up-regulation of LINC00092 inhibited cellular aggressiveness in IDC. Our present study suggested that LINC00092/miR-1827/SFRP1 is a novel signal axis to regulate cell malignant behaviors in IDC, indicating the promising therapy for IDC.

Authors' Contributions

CZ and RJ designed the study. CZ, LL, and JX, performed the experiments. ZL and PS performed bioinformatics analyses. CZ wrote the manuscript. All authors read and approved the final manuscript.

Ethical Approval

Ethical Approval is not applicable for this article.

Statement of Human and Animal Rights

This article does not contain any studies with human or animal subjects.

Statement of Informed Consent

There are no human subjects in this article and informed consent is not applicable.

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