

Linc01194 acts as an oncogene in colorectal carcinoma and is associated with poor survival outcome

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Background: The incidence of colorectal cancer ranks among the top three malignant tumors, attributing to more than 50,000 deaths in the United States every year. Survival rate is directly correlated with TNM stage at diagnosis, and identifying the molecules involved in the cancer development process will provide directions to better investigate the mechanisms of colorectal cancer.

Materials and methods: Bioinformatics analysis of differentially expressed long noncoding RNAs (lncRNAs), survival analysis, cell proliferation assay, migration assay, and Western blot analysis were performed.

Results: Fifty-one lncRNAs were identified between the early stage and late-stage groups. In the survival analysis, we found that Linc01194 is correlated with poor survival of colon cancer patients. In addition, by suppressing the expression of Linc01194 in colon cancer cell lines, cell proliferation and migration were inhibited. Western blot showed that N-cadherin and vimentin were downregulated, whereas E-cadherin was upregulated indicating that the process of epithelial–mesenchymal transition (EMT) was restrained.

Conclusion: *Linc01194* promotes the proliferation and migration ability of colon cancer cells by activating EMT. It acts as an oncogene in colorectal carcinoma and is associated with worse survival outcome.

Keywords: colorectal cancer, TCGA, lncRNA, Linc01194, EMT

Introduction

The incidence of colorectal cancer ranks among the top three malignant tumors, attributing to more than 50,000 deaths in the United States every year.^{1,2} Survival rate correlates directly with TNM stage at diagnosis, and the 5-year survival rate sharply decreases from 90% at the early stage to 10% at the late stage.³ The molecular mechanism of colorectal cancer has been investigated well, but its development process remains unknown. Identifying the molecules involved therein will provide directions to better investigate the mechanisms of colorectal cancer.

Previous studies have revealed that long noncoding RNA (lncRNA) has a nonnegligible function in colorectal cancer.^{4,5} PVT1 is over expressed in colon cancer tissues compared to adjunct tissues and promotes cell proliferation and migration through the miR-30d-5p/runt-related transcription factor 2 (RUNX2) axis.^{6,7} In addition, lncTCF7 could activate the Wnt/ β -catenin signaling pathway to promote metastasis in colorectal cancer.^{8,9} The Cancer Genome Atlas (TCGA) supplies genome information including sequence information, methylation data, copy number variations, and corresponding

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clinical parameters.¹⁰ Taking advantage of these data, the metastasis of colon cancer can be studied thoroughly to provide directions for future investigation.

Bioinformatics analysis identified 51 lncRNAs between the early stage and late-stage groups, and Kaplan–Meier analysis identified four lncRNAs with prognostic value in colorectal cancer. Combined with the above analysis, we hypothesized that *Linc01194* functions as an oncogene in colon cancer. To verify our hypothesis, assays for cell proliferation, migration, and invasion were performed. In addition, epithelial–mesenchymal transition (EMT) markers were detected. These findings suggest that *Linc01194* could promote the migration of colorectal cancer cells by activating EMT, providing new insights to further study the metastasis of colon cancer.

Materials and methods

Sequence data and clinical parameters

Sequence data and clinical parameters of colorectal cancer were acquired from the TCGA, which is a comprehensive public data bank for research purposes. In this data bank, 467 colorectal cancer samples including 268 stage I/II and 199 stage III/IV samples were acquired as of August 1, 2018 with the use of Genomic Data Commons tool according to the guidelines. In terms of clinical parameters, vital status, and survival days (days to death plus days to follow-up) of corresponding patients were collected.

Differentially expressed lncRNAs

lncRNA data were derived from sequence data based on CRCh38.87 mapping. lncRNA expression was normalized using the calcNormFactors function in the edgeR R package within and among samples.¹¹ In addition, to avoid low-abundance lncRNA, lncRNAs with an average expression of less than one in all samples were excluded. To identify differentially expressed lncRNAs, sequence data from stage III/IV samples were compared with those of stage I/II samples. In this procedure, the edgeR R package was utilized again. Differentially expressed lncRNAs were defined with a cutoff of false discovery rate (FDR)-adjusted $P < 0.01$ and $|\log FC| \geq 1.5$ (FC = fold change).

Survival analysis

To discover candidate lncRNAs with prognostic value, Kaplan–Meier analysis was conducted to compare the overall survival of the top ten differentially expressed lncRNAs. lncRNAs were divided into two groups: low expression (expression lower than

median) and high expression (expression higher than median). Log-rank test was employed to identify key lncRNAs, and $P < 0.05$ was considered statistically significant.

Cell culture

The colorectal cancer cell lines HT29, HCT116 SW620, and LoVo were purchased from the Cell Bank of Typical Culture Collection (Chinese Academy of Sciences, Shanghai, People's Republic of China). All cells were grown in DMEM (Thermo Fisher Scientific, Waltham, MA, USA) supplemented with 10% FBS, 100 $\mu\text{g/mL}$ streptomycin sulfate, and 100 U/mL penicillin. Cells were cultured in a humidified incubator at 37°C with 5% CO_2 .

Small interfering RNA (siRNA) transfection

HCT116 cells were transfected with siRNA targeting *Linc01194* or negative control siRNA (Shanghai GenePharma Co., Ltd., Shanghai, People's Republic of China) using X-treme GENE siRNA transfection reagent (Roche Applied Science, Penzberg, Germany) according to the protocol. The siRNA sequences were as follows: si-*Linc01194*, ACAUAUUUACCCAUGUAGCAA, and GCUACAUGGGUAAUAUGUGC.

RNA extraction

RNA was extracted following the protocol of TRIzol reagent. Briefly, cells at 80%–90% confluence were washed thrice with cold PBS. Then, the cells were lysed with cold TRIzol for 1 minute on ice. Chloroform was added into the lysate and left standing for 10 minutes at room temperature following centrifugation at 12,000 rpm at 4°C for 15 minutes. Thereafter, the transparent liquid was extracted carefully, mixed with an equal volume of isopropanol, and left standing for 10 minutes at room temperature followed by centrifugation at 12,000 rpm at 4°C for 15 minutes. After centrifugation, the supernatant was discarded and the precipitate was washed with diethyl pyrocarbonate (DEPC) for 5 minutes. Then, the precipitate was dissolved in DEPC and reverse transcribed into complementary DNA (cDNA) using transcriptor first strand cDNA synthesis kit (Takara Bio Inc., Kusatsu, Japan).

Real-time (RT)-PCR

The cDNA was diluted at a ratio of 1:10, and the reaction system was constructed based on the protocol of the RT-PCR kit (Takara Bio Inc.) using the 7500 Fast RT-PCR system (Thermo Fisher Scientific). The primers of GAPDH

were: forward, CCTGCACCACCAACTGCTTA and reverse, GGCCATCCACAGTCTTCTGAG. The primers of Linc01194 were: forward, AGACTGCTCTTGAGGCTG-GAGT and reverse, AGGCTGAGGCTGGAGGATCTCT.

Cell proliferation assay

HCT116 cells transfected with negative control or si-Linc01194 were seeded into 96-well plates. The Cell Counting Kit-8 assay (Dojindo Laboratories Co. Ltd., Kumamoto, Japan) was used to assess the proliferative capacity of the cells according to the protocol provided by the manufacturer. The absorbance was detected using a microplate reader (MTX LAB SYSTEMS INC, Vienna, VR, USA) at 450 nm after 24, 48, 72, and 96 hours. The average absorbance in each group was calculated from six wells.

Cell migration and invasion assay

For the cell migration assay, HCT116 cells transfected with negative control or si-Linc01194 were seeded onto the upper chamber in medium containing 1% FBS, and full growth medium was loaded in the lower chamber. After 24 hours, the cells were washed and fixed in 4% paraformaldehyde for half an hour. Then the cells in the upper layer were removed carefully. The membranes were stained with crystal violet for approximately half an hour. Cell counts were performed on five random fields in each well. Cell invasion assay was performed following a similar procedure as the cell migration assay, except that the membrane was pre-coated with Matrigel.

Cell cycle assay

After transfecting with negative control or si-Linc01194 for 72 hours, HCT116 cells were harvested and fixed with 75% ethanol and resuspended in reagent A (cell cycle staining kit, Hangzhou Multi Sciences (Lianke) Biotech Co., Ltd., Hangzhou, People's Republic of China) according to the protocol supplied. About half an hour later, cells were analyzed by flow cytometry. Each experiment was carried out thrice.

Western blot analysis

Proteins from HCT116 cells transfected with negative control or si-Linc01194 were extracted by RIPA buffer containing proteinase inhibitor cocktail and phosphatase inhibitor cocktail. The proteins were separated using 10% SDS-PAGE and transferred to polyvinylidene fluoride membranes. After blocking with BSA for 2 hours, the membranes were incubated with primary antibodies at 4°C overnight followed by incubation with secondary antibody for 2 hours. After washing thrice with tris-buffered saline/Tween 20, the membranes

were incubated with Super Signal West Pico chemiluminescent substrate and analyzed using the GeneGnome HR Image Capture System.

Gene ontology analysis

mRNAs that may be involved in the competing endogenous RNA (ceRNA) network of Linc01194 were acquired from the StarBase database.¹² These mRNAs were sequenced in the Gene Ontology function enrichment analysis using the clusterProfiler R package downloaded from the Bioconductor website.¹³ Terms with $P < 0.05$ were considered as statistically significant.

Statistical analysis

The correlation between Linc01194 expression and corresponding clinical parameters including outcome details were analyzed by SPSS software using chi-squared test. $P < 0.05$ was considered statistically significant. Other statistical analyses were performed as stated above.

Results

Differentially expressed lncRNAs

A total of 7,469 lncRNAs were identified in colorectal cancer samples. Among them, after comparing the stage III/IV samples with stage I/II samples, 51 lncRNAs were defined as differentially expressed according to the FDR cutoff < 0.01 and $|\log FC| \geq 1.5$, as shown in Figure 1 and Table S1. The expression levels of these differentially expressed lncRNAs are illustrated in Figure 2. The top ten differentially expressed lncRNAs were BX322234.2, Linc00400, Linc01419, FP671120.3, AC011840.1, AC105243.1, Linc01468, Linc01790, Linc02476, and Linc01194.

Survival analysis of lncRNAs

Kaplan–Meier analysis was performed on the top ten differentially expressed lncRNAs, revealing four lncRNAs with prognostic value. As shown in Figure 3, their high expression correlated directly with poor outcome of colorectal carcinoma patients.

Expression of Linc01194 in colorectal cancer cell lines

To elucidate the possible function of Linc01194, we detected the expression level of Linc01194 in four colorectal cancer cell lines: HT29, HCT116, SW620, and LoVo. Using RT-PCR, we found that Linc01194 is highly expressed in HCT116 cells. Then, we transfected HCT116 cells with Linc01194 siRNA to detect the interference efficiency. As

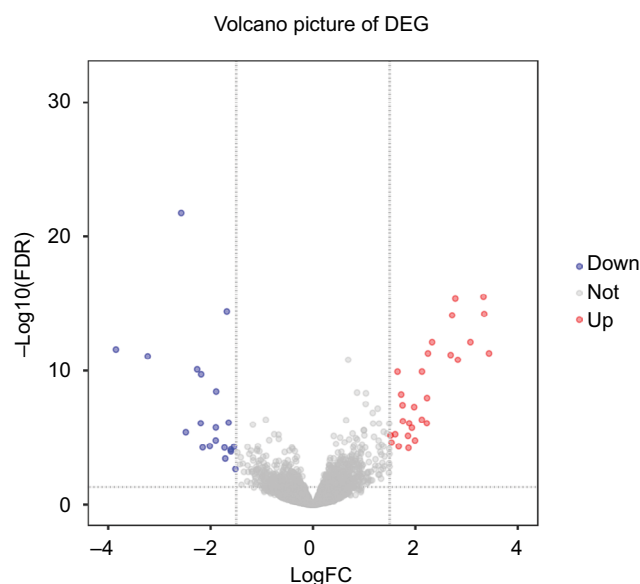


Figure 1 Volcano plot for lncRNAs between early and late stages of colorectal cancer samples.

Notes: Each gene is marked as a dot; those genes that were significantly upregulated are highlighted in red, the downregulated genes are highlighted in blue, and the nonsignificant genes are labeled as gray dots. In the figure, the gray lines indicate the marginal lines separating differently expressed lncRNAs from nondifferently expressed lncRNA, with the horizontal lines denoting the *P*-value threshold ($FDR < 0.01$) and the vertical lines denoting the FC cutoff ($|\log FC| \geq 1.5$).

Abbreviations: DEG, differentially expressed gene; FC, fold change; FDR, false discovery rate; lncRNAs, long noncoding RNAs.

shown in Figure 4, the inhibition rate of siRNA was greater than 70%. Thus, the siRNA sequence could be used for the subsequent experiments.

Linc01194 promoted the proliferation, migration, and invasion abilities of HCT116 cells

After HCT116 cells were transfected with Linc01194 siRNA, assays for proliferation, migration, and invasion were performed. As shown in Figure 5A, after 48 hours, the proliferation of HCT116 cells transfected with si-Linc01194 decreased significantly, and this phenomenon continued for another 48 hours. Then, the migration and invasion abilities were examined following the procedures mentioned above. As shown in Figure 5B and C, the number of migrating or invading cells were sharply decreased compared to that in the control group. As shown in Figure 5D, compared to the control group, the cells transfected with si-Linc01194 were arrested in G0/G1 phase.

Linc01194 promoted EMT in colorectal cancer

Western blot was performed on HCT116 cells treated with Linc01194 siRNA, using β -actin as the reference gene. In

Figure 6, the expressions of N-cadherin and vimentin, which are markers of EMT, were decreased significantly, whereas E-cadherin expression was increased.

Correlation between Linc01194 and clinical parameters

Patients were ranked by Linc01194 expression from low-est to highest. The first quarter was defined as the low-expression group, whereas the last quarter was defined as the high-expression group. In addition, clinical parameters including age, gender, body mass index, location of carcinoma, TNM staging, histological type, neoplasm status, and risk level were divided into two groups. The criteria are shown in Table 1 in detail. To investigate the correlation between Linc01194 and clinical factors, the chi-squared test was performed using SPSS 23.0 software. As described in Table 2, the expression level of Linc01194 had a statistically significant correlation with stage ($P < 0.001$), N stage ($P < 0.001$), and neoplasm status ($P < 0.001$). Patients with higher expression of Linc01194 seemed to have increased possibility of aggressive cancer stage, lymph node metastasis, and survival with tumor.

Gene Ontology analysis

As shown in Table S2, a total of 69 mRNAs that may be involved in the ceRNA network of Linc01194 were identified using the following criteria: common miRNA family ≥ 3 , *P*-value < 0.01 , and $FDR < 0.01$. Then, these genes were sequenced into the Gene Ontology function enrichment analysis. Of note, as shown in Table 3, only two terms, namely ubiquitin-protein transferase activity and ubiquitin-like protein transferase activity, were significantly enriched.

Discussion

lncRNAs are a type of noncoding RNAs that affect various biological processes, such as stemness maintenance, cell proliferation, and cell differentiation.^{14,15} Cancer is a complex disease, and lncRNAs play important roles in its progression and development.^{16,17} lncRNAs can exert the effects of ceRNA at cancer and act as either oncogenes or anti-oncogenes.^{18–20} In colon cancer research, it is important to identify biomarkers for early diagnosis and prognosis to provide recommendations for clinicians.

In order to explore the potential lncRNA biomarkers in colorectal cancer diagnosis and their associated biological functions, a bioinformatics assay was performed by comparing lncRNA sequencing data for the early stage (I and II) and late stage (III and IV) acquired from TCGA database.

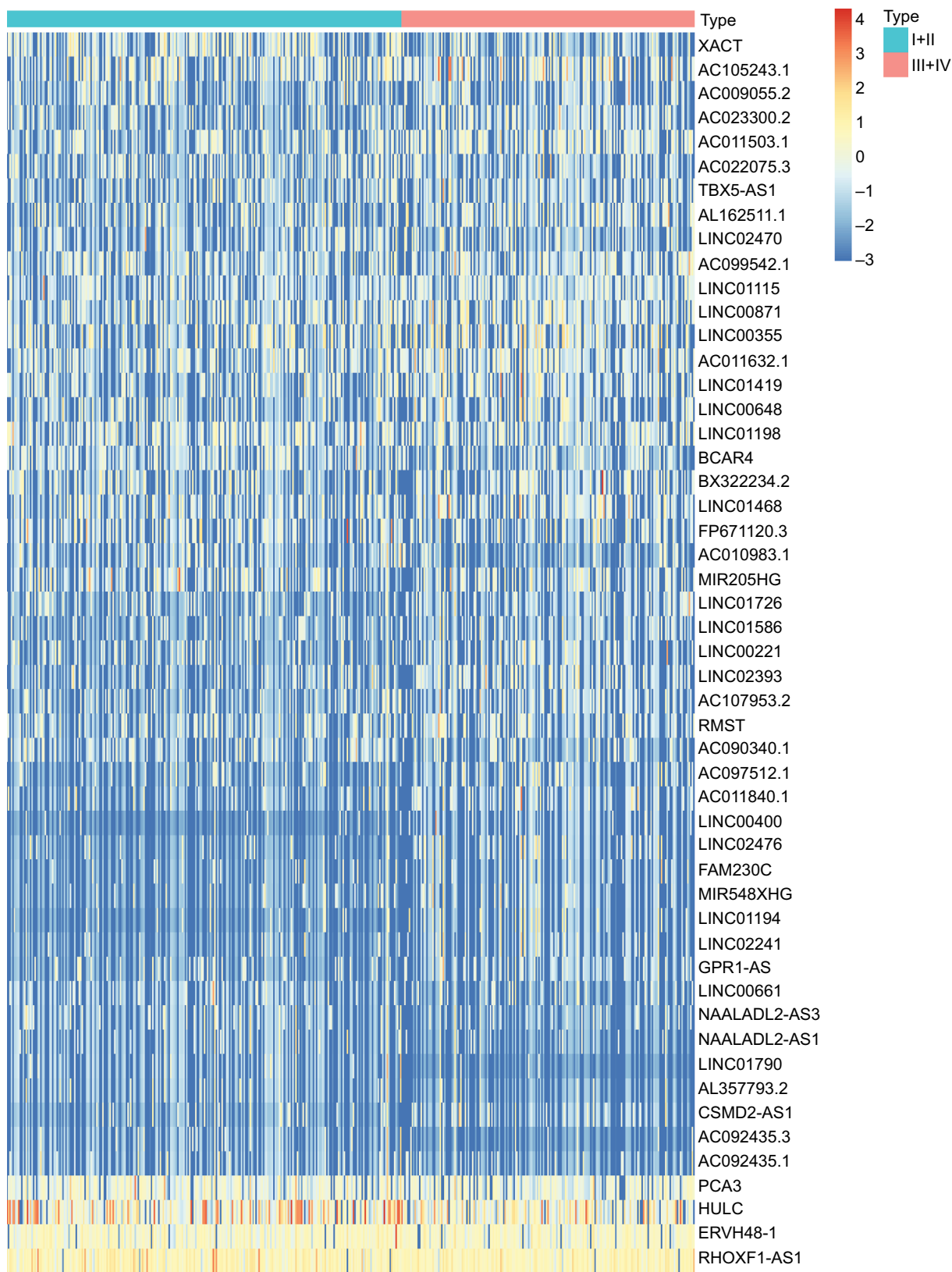


Figure 2 Heat map of differentially expressed lncRNAs in colorectal cancer samples.
Notes: Each block represents the color-coded expression levels of differentially expressed lncRNAs in colorectal cancer samples. Orange represents the relatively higher expression, whereas the blue represents the relatively lower expression.
Abbreviation: lncRNAs, long noncoding RNAs.

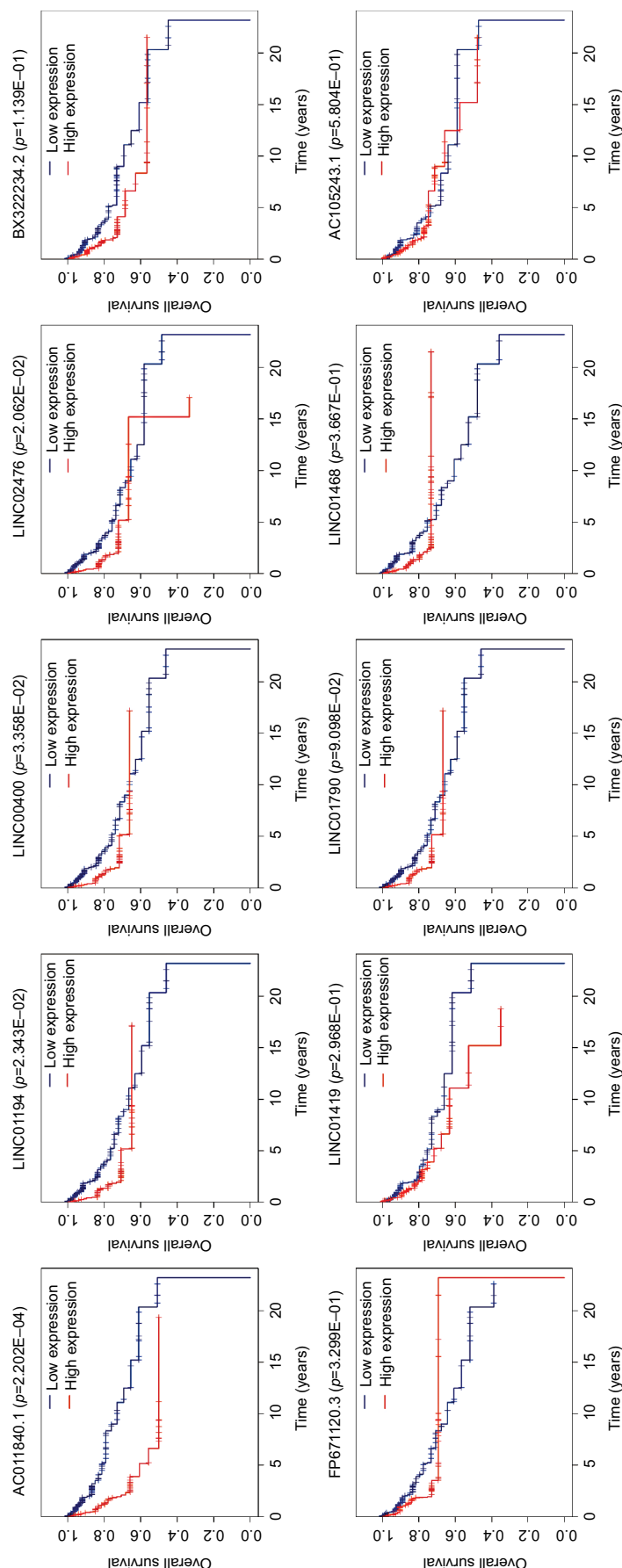


Figure 3 Survival analysis of ten lncRNAs.

Notes: The red line of each image represents the samples with the high expression (expression higher than median) of the corresponding lncRNA, whereas blue line represents the samples with the low expression (expression lower than median) of the corresponding lncRNA. Log-rank test was utilized to perform the statistical analysis.

Abbreviation: lncRNAs, long noncoding RNAs.

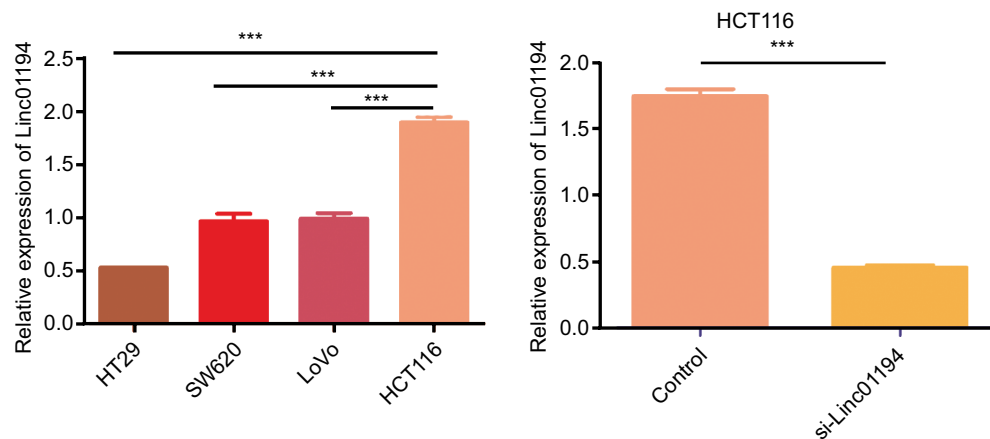


Figure 4 Expression of Linc01194 in colorectal cancer cell lines by real-time-PCR.

Notes: (A) Expression of Linc01194 in colorectal cancer cell lines. (B) Interference efficiency of si-Linc01194 in HCT116 cells. *** $P < 0.001$.

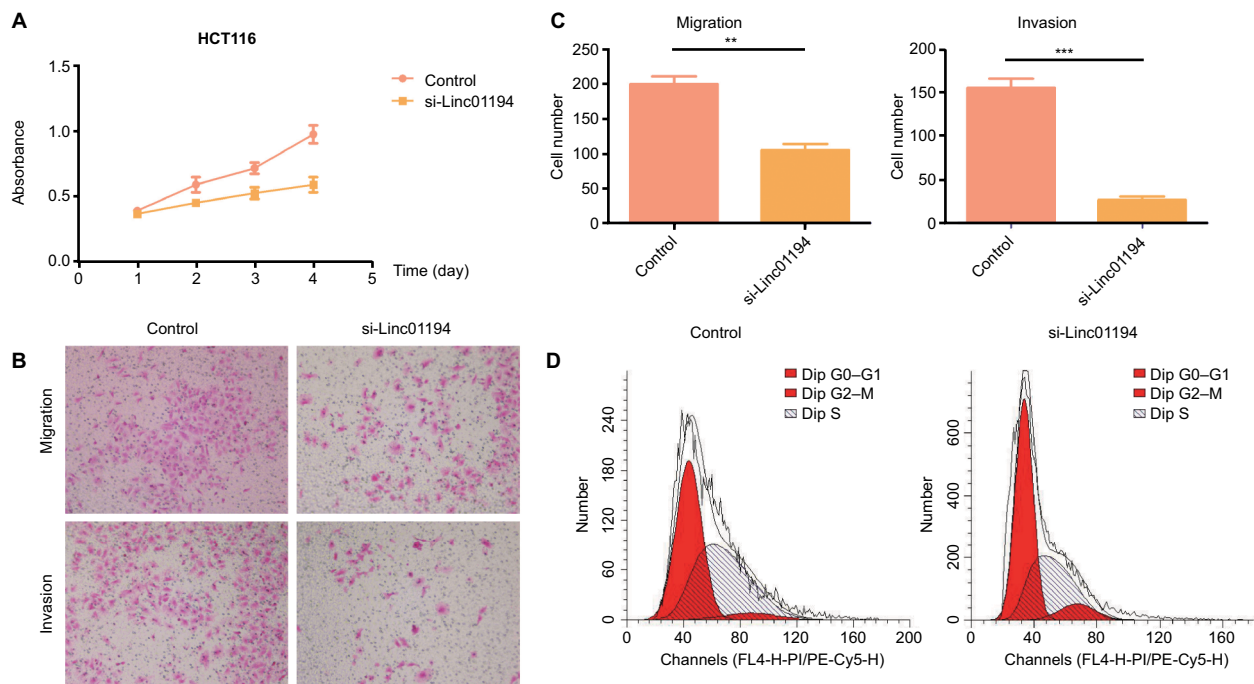


Figure 5 Impact of Linc01194 on HCT116 cells.

Notes: (A) Proliferation activity of HCT116 cells measured by CCK-8 assay. After transfecting with negative control or si-Linc01194, the absorbance at 450 nm was detected at 24, 48, 72, and 96 hours. (B) Migration and invasion of HCT116 cells. After transfecting with negative control or si-Linc01194, cells that crossed the membrane were stained with crystal violet and observed under a microscope (200 $\times$). (C) Quantification of the invasion and migration assays. Significant reduction was observed in si-Linc01194 group compared to negative control group migration and invasion assays (Student's *t*-test). (D) HCT116 transfected with si-Linc01194 cells were significantly arrested in G0/G1 phase in cell cycle. ** $P < 0.01$; *** $P < 0.001$.

Abbreviation: CCK-8, cell counting-kit-8.

We found four lncRNAs, namely Linc01194, Linc00400, Linc02476, and AC011840.1, that were related to the prognosis of colorectal cancer. Among these, Linc00400 may have a relationship with coronary artery disease in the Japanese population.²¹ No research has reported the biological functions of the three other lncRNAs.

Cancer metastasis is closely related to cancer progression. In the first half of the investigation, Linc01194 was upregulated in late-stage colorectal carcinoma. Thus, we explored the biological function of Linc01194 by various methods. First, the expression levels of Linc01194 in HT29, HCT116, SW620, and LoVo colon cancer cells were evaluated, and

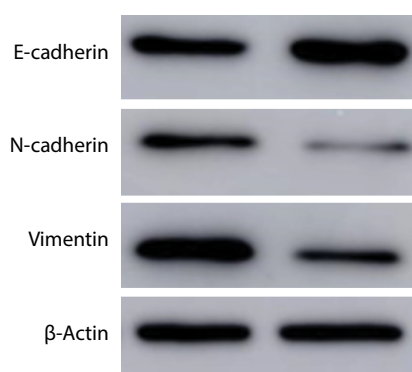


Figure 6 Western blot of N-cadherin, vimentin, E-cadherin, and β -actin.

Notes: After transfecting with negative control or si-Linc01194, protein of HCT116 cells were extracted for Western blot analysis.

Table 1 Clinical parameters of colorectal cancer patients

Subgroup	Frequency	Percent	Valid percent
Age, years			
<60	69	31.4	31.4
≥ 60	151	68.6	68.6
Gender			
Male	115	52.3	52.3
Female	105	47.7	47.7
Body mass index			
<25	33	15	29.5
≥ 25	79	35.9	70.5
Location			
Left	98	44.5	45.2
Right	89	40.5	54.8
Histology			
Adenocarcinoma	186	84.5	85.3
Mucinous adenocarcinoma	32	14.5	14.7
Stage			
I+II	84	38.2	38.2
III+IV	136	61.8	61.8
T stage			
Tis, T1–3	189	85.9	85.9
T4	31	14.4	14.4
N stage			
N0	91	41.4	41.4
N1+N2	129	58.6	58.6
M stage			
M0	140	63.6	74.5
M1	48	21.8	25.5
Neoplasm status			
Tumor free	91	41.4	46.2
With tumor	106	48.2	53.8
Vital status			
Alive	194	88.2	88.2
Dead	26	11.8	11.8
Risk level			
Low	110	50	50
High	110	50	50

Linc01194 expression was found to be the highest in HCT116 cells. Then, RNA interference experiments were performed in HCT116 cells to examine the impact of Linc01194 on colorectal cancer cells. Linc01194 knockdown had significant effects on the proliferation of colorectal cancer cells, which indicated that it may influence cell cycle progression. Besides fast proliferation, metastasis is another biological characteristic of cancer cells, and the highly metastatic characteristic of cancer are also correlated with poor prognosis. In our previous bioinformatics analysis, Linc01194 could be used as a biomarker to predict the prognosis of colorectal cancer patients, which indicated that there may be a correlation between Linc01194 and metastasis. In order to evaluate the biological effects of Linc01194 on metastasis, Transwell assays were performed to investigate cancer cell migration and invasion. By knocking down Linc01194, the number of cells moving through the membrane during the Transwell assays decreased significantly, which suggested that Linc01194 promoted cell movement. Transwell is a 3D cell culture method that can reliably simulate the *in vivo* microenvironment. The Transwell assays also showed that Linc01194 may greatly contribute to colon cancer metastasis. EMT is the central biological event that regulates metastasis,^{22,23} and it is necessary to explore the function of Linc01194 in EMT. E-cadherin, N-cadherin, and vimentin are the key markers in analyzing EMT. Linc01194 knockdown induced a decrease in the mesenchymal markers N-cadherin and vimentin, but increased the expression of E-cadherin, which is an epithelial marker. Moreover, lncRNAs can regulate microRNAs and decrease or increase the expression of genes via the ceRNA mechanism.²⁴

Because Linc01194 is a relatively new lncRNA, we took advantage of databases to predict vital molecules influenced by Linc01194. Table S2 shows the mRNAs that may be involved in the ceRNA network of Linc01194. To investigate gene ontology in which these genes may be involved, we perform function enrichment analysis of these genes. Of note, GO:0004842 and GO:0019787 are involved in ubiquitin-protein transferase and ubiquitin-like protein transferase activity, respectively. These results indicated that changes in the ubiquitin system may be related to the progression and development of colorectal cancer. The ubiquitin system has complex biological functions and is a posttranslational modification that plays a role in several cellular processes including cell cycle progression, cell proliferation, DNA replication, and apoptosis.^{25,26} Research has shown that the ubiquitin system is frequently altered in various cancers and

Table 2 Relationship between Linc01194 expression and clinical parameters

Subgroup	Low expression (%)	High expression (%)	Total	P-value
Age, years				0.467
<60	37 (16.82)	32 (14.55)	69	
≥60	73 (33.18)	78 (35.45)	151	
Gender				0.5
Male	60 (27.27)	55 (25)	115	
Female	50 (22.73)	55 (25)	105	
Body mass index				0.55
<25	26 (23.21)	7 (6.25)	33	
≥25	58 (51.79)	21 (18.75)	79	
Location				0.712
Left	48 (25.67)	50 (26.74)	98	
Right	46 (24.60)	43 (22.99)	89	
Histology				0.444
Adenocarcinoma	91 (41.74)	95 (43.58)	186	
Mucinous adenocarcinoma	18 (8.26)	14 (6.42)	32	
Stage				<0.001*
I+II	60 (27.27)	24 (10.90)	84	
III+IV	50 (22.73)	86 (39.10)	136	
T stage				0.561
Tis, T1–3	96 (43.64)	93 (42.28)	189	
T4	14 (6.36)	17 (7.72)	31	
N stage				<0.001*
N0	64 (29.09)	27 (12.27)	91	
N1+N2	46 (20.91)	83 (37.73)	129	
M stage				0.213
M0	67 (35.64)	73 (38.83)	140	
M1	18 (9.57)	30 (15.96)	48	
Neoplasm status				<0.001*
Tumor free	68 (34.52)	23 (11.68)	91	
With tumor	25 (12.68)	81 (41.12)	106	
Vital status				0.676
Alive	96 (43.64)	98 (44.55)	194	
Dead	14 (6.36)	12 (5.45)	26	

Note: *P<0.05.

Table 3 Gene Ontology analysis of mRNA involved in the ceRNA network of Linc01194

ID	Description	Gene ratio	Bg ratio	P-value	P adjust	q-value	Gene ID	Count
GO:0004842	Ubiquitin-protein transferase activity	10/65	430/16982	5.03E-06	0.000727	0.000689	BACH1/TRIM2/BIRC6/FBXL5/FBXO11/RNF2/RBBP6/KLHL2/RNF128/LNX1	10
GO:0019787	Ubiquitin-like protein transferase activity	10/65	451/16982	7.65E-06	0.000727	0.000689	BACH1/TRIM2/BIRC6/FBXL5/FBXO11/RNF2/RBBP6/KLHL2/RNF128/LNX1	10

Abbreviations: Bg ratio, background ratio; ceRNA, competing endogenous RNA.

takes part in the vital movement of cancer, and can be used as a therapeutic target of cancer.^{27–29} Using bioinformatics, Linc01194 was predicted to upregulate the expression of BIRC6, BACH1, RNF2, etc, all of which play roles in the ubiquitination of proteins, via the ceRNA mechanism. BIRC6 is upregulated in various cancers, such as colon, liver, prostate, and lung cancer.^{30–33} In colon cancer, BIRC6 can be used as

a biomarker for the prediction of prognosis, like Linc01194, which suggests that Linc01194 plays a central role in colon cancer. *BACH1* is also an important gene regulated by Linc01194 that takes part in the development of breast, renal, colon, and ovarian cancers.^{34,35} It can induce the migration of colon cancer cells through reduction of metastasis-related genes. In our research, Linc01194 regulated the migration,

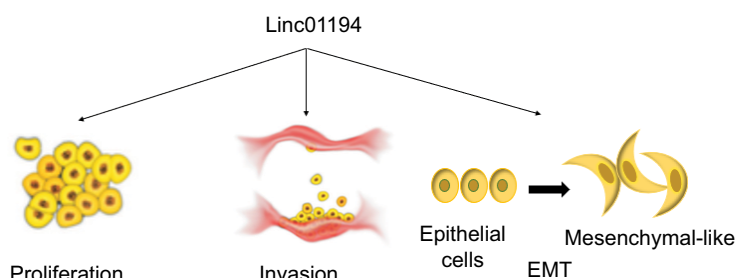


Figure 7 The role Linc01194 plays in colorectal cancer.

Notes: Linc01194 could promote proliferation, invasion, and EMT in colon cancer.

Abbreviation: EMT, epithelial–mesenchymal transition.

invasion, and EMT progression of colon cancer cells. At the same time, Linc01194 may also regulate the expression of BACH1 through microRNA sponge function, indicating that Linc01194 may regulate colon cancer metastasis through the *BACH1* gene. RNF2 is a type of RING-Type E3 ubiquitin transferase that is important for the function of the ubiquitination system and regulates cell cycle progression, apoptosis, DNA damage repair, and senescence.³⁶ It is over expressed in many types of cancer, including melanoma, gastric, breast, and prostate cancers. However, the relationship between the *RNF2* gene and colon cancer is unclear. Analysis of the ceRNA network gave us a promising direction, but the mechanisms of Linc01194 and the abovementioned genes need to be further investigated in future research.

Conclusion

In this study, we revealed that Linc01194 was over expressed in advanced colorectal cancer cells, predicting poor clinical outcome. As shown in Figure 7, Linc01194 induced proliferation and migration of colon cancer cells and regulated the expression of EMT-associated genes. The ceRNA network in which Linc01194 may be involved needs to be further investigated.

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Disclosure

The authors report no conflicts of interest in this work.

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Supplementary materials

Table S1 Fifty-one differently expressed lncRNAs in colon cancer

lncRNA	logFC	P-value
FP671120.3	-3.84936	4.43E-15
LINC01790	-3.22837	1.86E-14
ERVH48-1	-2.57414	7.29E-26
MIR205HG	-2.48432	2.75E-08
LINC01115	-2.26202	2.08E-13
HULC	-2.1929	4.40E-09
BCAR4	-2.18462	5.53E-13
LINC00661	-2.15479	6.20E-07
AC092435.3	-2.01592	4.57E-07
NAALADL2-AS1	-1.89972	1.49E-07
AC010983.1	-1.89776	1.11E-08
PCA3	-1.89108	1.16E-11
LINC02470	-1.72825	6.65E-07
AC092435.1	-1.71363	6.38E-06
RHOXF1-AS1	-1.68307	3.62E-18
XACT	-1.64822	3.90E-09
AC090340.1	-1.60672	1.01E-06
NAALADL2-AS3	-1.6034	1.49E-06
LINC01198	-1.54383	5.35E-07
AL357793.2	-1.51568	7.30E-05
LINC00871	1.514383	5.39E-08
AL162511.1	1.534812	2.29E-07
TBX5-AS1	1.605736	4.09E-08
AC011503.1	1.651576	3.36E-13
LINC02393	1.674402	4.83E-07
LINC01586	1.722765	2.15E-11
AC022075.3	1.749524	1.62E-10
RMST	1.75569	2.95E-09
LINC00355	1.855968	5.85E-08
FAM230C	1.866044	7.22E-07
AC097512.1	1.879261	4.65E-09
CSMD2-AS1	1.932389	1.18E-08
GPR1-AS	1.976878	2.26E-10
MIR548XHNG	1.993145	1.53E-07
AC009055.2	2.126523	2.17E-09
AC099542.1	2.128981	3.18E-13
LINC02241	2.223141	4.63E-09
LINC00648	2.227592	4.05E-11
AC023300.2	2.246047	9.29E-15
AC011632.1	2.327388	1.13E-15
LINC00221	2.689394	1.41E-14
AC107953.2	2.718111	9.20E-18
LINC01726	2.780815	3.55E-19
LINC01194	2.829866	3.85E-14
LINC02476	3.075124	1.17E-15
LINC01468	3.330697	2.19E-19
AC105243.1	3.345494	6.47E-18
AC011840.1	3.4388	9.90E-15
LINC01419	4.225451	7.33E-31
LINC00400	4.687224	2.91E-25
BX322234.2	5.870644	3.73E-36

Abbreviations: FC, fold change; lncRNAs, long noncoding RNAs.

Table S2 mRNA involved in the ceRNA network of Linc01194

LincRNA	mRNA	HitNum	P-value	FDR	Pan-cancer
LINC01194	RAB6C	8	9.66E-07	8.41E-05	11
LINC01194	AGTR2	4	8.43E-06	3.67E-04	7
LINC01194	PPP1R8P1	3	1.32E-05	3.82E-04	11
LINC01194	LINC00484	3	1.32E-05	3.82E-04	4
LINC01194	MAP3K7	10	1.39E-05	3.82E-04	15
LINC01194	AL357140.2	4	1.66E-05	3.82E-04	9
LINC01194	C5orf30	10	2.32E-05	3.82E-04	11
LINC01194	EIF5A2	11	2.58E-05	3.82E-04	8
LINC01194	AC112496.1	3	5.19E-05	5.02E-04	4
LINC01194	AL606760.1	3	5.19E-05	5.02E-04	10
LINC01194	AL355355.1	3	5.19E-05	5.02E-04	12
LINC01194	PAF1	5	7.02E-05	5.09E-04	10
LINC01194	TVP23B	8	8.16E-05	5.46E-04	8
LINC01194	LHX6	5	8.97E-05	5.58E-04	8
LINC01194	POLK	9	9.44E-05	5.58E-04	5
LINC01194	TSC22D2	9	1.03E-04	5.58E-04	10
LINC01194	ASB9	4	1.11E-04	5.70E-04	7
LINC01194	MYNN	8	1.22E-04	5.88E-04	4
LINC01194	NPM1P10	3	1.28E-04	5.88E-04	10
LINC01194	AC073188.4	3	1.28E-04	5.88E-04	3
LINC01194	RGL1	9	1.31E-04	5.88E-04	10
LINC01194	YTHDC2	9	1.42E-04	5.88E-04	6
LINC01194	BIRC6	8	1.47E-04	5.88E-04	7
LINC01194	E2F5	7	1.74E-04	6.30E-04	13
LINC01194	STEAP4	7	1.74E-04	6.30E-04	7
LINC01194	CDKN2AIP	8	2.13E-04	7.12E-04	8
LINC01194	RBM22P2	3	2.52E-04	8.13E-04	8
LINC01194	LINC01798	3	2.52E-04	8.13E-04	5
LINC01194	LAMP5	3	2.52E-04	8.13E-04	10
LINC01194	UR11	8	2.54E-04	8.13E-04	13
LINC01194	METTL7A	8	2.77E-04	8.13E-04	7
LINC01194	FLT1	6	2.83E-04	8.13E-04	8
LINC01194	LAMA3	4	2.95E-04	8.13E-04	6
LINC01194	ZNF367	8	3.01E-04	8.13E-04	7
LINC01194	TEX30	5	3.11E-04	8.13E-04	14
LINC01194	PKIA	8	3.56E-04	8.61E-04	9
LINC01194	SLAIN1	7	3.66E-04	8.61E-04	6
LINC01194	FBXL5	8	3.87E-04	8.86E-04	8
LINC01194	ZNF292	9	4.29E-04	9.57E-04	6
LINC01194	AL731537.1	3	4.35E-04	9.57E-04	7
LINC01194	AL645728.1	3	4.35E-04	9.57E-04	10
LINC01194	PLCXD3	8	4.92E-04	1.02E-03	7
LINC01194	AIDA	8	4.92E-04	1.02E-03	5
LINC01194	C6orf120	9	5.26E-04	1.04E-03	14
LINC01194	RBBP6	7	5.38E-04	1.04E-03	6
LINC01194	TMX3	10	5.60E-04	1.06E-03	6
LINC01194	WASF3	9	5.62E-04	1.06E-03	7
LINC01194	ASAP2	9	6.00E-04	1.09E-03	8
LINC01194	OXR1	9	6.40E-04	1.14E-03	5
LINC01194	FNBP1L	10	6.66E-04	1.16E-03	8
LINC01194	DYRK2	11	6.66E-04	1.16E-03	12
LINC01194	BTBD7P1	3	6.86E-04	1.16E-03	5
LINC01194	FGD5	3	6.86E-04	1.16E-03	8

(Continued)

Table S2 (Continued)

LincRNA	mRNA	HitNum	P-value	FDR	Pan-cancer
LINC01194	FBXO11	8	7.21E-04	1.16E-03	6
LINC01194	LNXI	4	7.93E-04	1.25E-03	6
LINC01194	GPR180	9	8.27E-04	1.28E-03	8
LINC01194	RNF128	6	8.31E-04	1.28E-03	8
LINC01194	BACH1	10	8.34E-04	1.28E-03	8
LINC01194	ERII	8	8.34E-04	1.28E-03	10
LINC01194	MDFIC	8	8.34E-04	1.28E-03	6
LINC01194	TBC1D15	7	8.43E-04	1.28E-03	10
LINC01194	FNIP2	9	8.80E-04	1.28E-03	10
LINC01194	RAP2C	9	8.80E-04	1.28E-03	7
LINC01194	SLC16A6	7	9.18E-04	1.28E-03	6
LINC01194	CAV2	8	9.63E-04	1.29E-03	5
LINC01194	SMCHD1	9	9.96E-04	1.31E-03	5
LINC01194	KLHL2	7	9.99E-04	1.31E-03	7
LINC01194	REVI	7	9.99E-04	1.31E-03	5
LINC01194	OSER1-AS1	3	1.01E-03	1.31E-03	13
LINC01194	IL6R-AS1	3	1.01E-03	1.31E-03	6
LINC01194	AC002310.2	3	1.01E-03	1.31E-03	11
LINC01194	AL807752.1	3	1.01E-03	1.31E-03	8
LINC01194	RNF2	8	1.03E-03	1.31E-03	16
LINC01194	SASS6	8	1.27E-03	1.49E-03	17
LINC01194	COQ10B	7	1.28E-03	1.49E-03	4
LINC01194	PDE5A	7	1.28E-03	1.49E-03	8
LINC01194	TRIM2	10	1.29E-03	1.49E-03	8
LINC01194	RAB21	9	1.34E-03	1.50E-03	5
LINC01194	MPC1	6	1.38E-03	1.53E-03	6
LINC01194	ZFYVE16	8	1.66E-03	1.80E-03	8
LINC01194	ACSL4	9	1.69E-03	1.82E-03	6
LINC01194	B4GALT6	8	1.77E-03	1.87E-03	11
LINC01194	CCPI10	8	1.77E-03	1.87E-03	8
LINC01194	VNIR28P	2	1.82E-03	1.89E-03	13
LINC01194	GLS	9	1.89E-03	1.94E-03	7
LINC01194	SLC4A7	10	1.94E-03	1.97E-03	3
LINC01194	CBFB	9	2.00E-03	2.00E-03	7

Abbreviations: ceRNA, competing endogenous RNA; FDR, false discovery rate; HitNum, number of miRNAs that may be involved; Pan-cancer, number of cancers that may be involved.

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