Construction of ceRNA networks reveals differences between distal and proximal colon cancers

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Abstract. Although colon cancer is often referred to as a homogeneous entity, an increasing number of studies have revealed that colon cancer can be divided according to the anatomic site of the cancer. However, few studies have reported the difference between distal and proximal colon cancer with regard to molecular mechanism, and especially non-coding RNA molecules. In the present study, the data of 186 colon tumour tissues and 17 adjacent non-tumour colon tissues in the left colon and 229 colon tumour tissues and 21 adjacent non-tumour colon tissues in the right colon were obtained from The Cancer Genome Atlas (TCGA). A total of 879 lncRNAs, 165 miRNAs and 2,028 mRNAs were identified as left-specific RNAs [$\log_2$(fold change)>2, FDR<0.01]. There were 916 lncRNAs, 227 miRNAs and 2,069 mRNAs identified in right colon cancer. The Kyoto Encyclopaedia of Genes and Genomes (KEGG) pathways were analysed for 2,028 mRNAs from left colon cancer and 2,069 mRNAs from right colon cancer. After removing the elements of the intersection from side-specific lncRNAs of the left and right, we identified specific lncRNAs included exclusively in left or right colon cancer, including 277 lncRNAs in left colon cancer and 314 lncRNAs in right colon cancer. Among these lncRNAs, 20 lncRNAs from the left and 25 lncRNAs from the right were revealed to be associated with overall survival. Then, ceRNA networks were constructed. There were 18 lncRNAs, 22 miRNAs and 57 mRNAs included in the left colon cancer ceRNA network and 21 lncRNAs, 27 miRNAs and 55 mRNAs included in the right ceRNA network. In total, 15 lncRNAs were revealed to be significantly related to clinical features, two of which were ascertained by testing the mRNA expression of tissues. In conclusion, our research aimed to detect the difference between colon cancer in the left and the right colon and to assist in the identification of new potential biomarkers to be used for diagnostic and prognostic purposes.

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Abbreviations: CRC, colorectal cancer; lncRNAs, long non-coding RNAs; DElncRNAs, differentially expressed lncRNAs; RIDElncRNAs, DElncRNAs after removing the elements of the intersection of the left and right colon cancer; miRNAs, microRNAs; KEGG, Kyoto Encyclopaedia of Genes and Genomes; ceRNAs, competing endogenous RNAs

Key words: distal and proximal colon cancer, lncRNA, ceRNA network, bioinformatics analysis

Introduction

Despite the increased understanding of its pathogenic risk and the development of progressive therapeutic strategies, colorectal cancer (CRC) is the third most commonly diagnosed cancer in men and the second most commonly diagnosed cancer in women worldwide (1).

Colon cancer is often referred to as a homogeneous entity, which should be treated accordingly. However, recent advances on CRC that have identified more subgroups of colon cancer may challenge this concept. These studies revealed that colon cancer can be divided into two subgroups (proximal and distal to the splenic flexure), with specific molecular, clinical and pathological characteristics (2-4). Biological differences between the left and right colon may partly explain the significant heterogeneity of the two sides. The difference of embryonic derivation should take into account that proximal and distal colon originate from the midgut and the hindgut, respectively, which may be the initiating factor (5). Additionally, the differential bacterial flora from the left and right colon may contribute to the heterogeneity (6). Thus, the location of the tumour may be an important factor that is worth taking into consideration. However, little is known about the molecular mechanism, especially with regard to non-coding RNA molecules. To further detect the potentially location-related mechanisms, cancer-specific RNAs from left and right colon cancer were identified and a competing endogenous RNA (ceRNA) network was established based on 3 types of RNAs, including long non-coding RNAs (lncRNAs), microRNAs (miRNAs) and mRNAs, which were differentially expressed in the two sides.

Non-coding RNAs (ncRNAs), which play crucial biological roles (7), can be divided into small ncRNAs (<200 bp) and long ncRNAs (>200 bp) based on the number of base pairs. Numerous studies have revealed that lncRNAs play important roles in the process of tumourigenesis (8-10). The competing endogenous RNA (ceRNA) hypothesis presented by Salmena *et al* in 2011 indicated a regulatory RNA network (11). All types of RNA transcripts, including mRNAs, lncRNAs and pseudogene transcripts in the network, could communicate with each other and compete for the binding of miRNA response elements (MREs). This competition exerts a crucial role in tumourigenesis by affecting the expression levels of various RNAs through MREs.

Several studies on the differences between the proximal and distal colon in pathway activation and their clinical implications have been reported (12,13). However, there is still a lack of large sample size studies and cancer-specific lncRNA biomarkers concerning the differences of the colon sides, and almost none of the studies focused on the potential ceRNA network. To detect the relationship between RNAs of these two sides, data was downloaded from The Cancer Genome Atlas (TCGA) (http://cancergenome.nih.gov), which contains mRNA, miRNA and lncRNA data of 186 samples of tumour tissues and 17 adjacent non-tumour colon tissues in the left colon and 229 samples of tumour tissues and 21 adjacent non-tumour colon tissue in the right colon. To the best of our knowledge, our study is the first to use a large-scale sequencing database to explore the side-specific lncRNA expression profiles and ceRNA co-expression network in the proximal and distal colon. The present study may further our insight into the potentially location-related mechanisms and help clarify the functions of lncRNAs in colon cancer.

Materials and methods

Patients and samples. RNA expression and clinical data were downloaded, including sex, TNM stage, survival information, from TCGA database. The criteria of exclusion were set as follows: i) Histologic diagnosis was not colon cancer; ii) tissue samples without enough data for analysis; iii) patients who suffered other malignancies; and iv) patients who had received preoperative chemotherapy. A total of 186 colon tumour tissues and 17 adjacent non-tumour colon tissues in the left colon and 229 colon tumour tissues and 21 adjacent non-tumour colon tissues in the right colon were collected in the present study. Our study fully abided by the publication guidelines of TCGA, and thus the approval of an Ethics Committee was not required.

A total of 116 paired colon cancer tissue samples (58 pairs from both sides) were surgically obtained between June 2005 and June 2018 at The First Affiliated Hospital of Nanjing Medical University (Jiangsu, China). Our study was approved by the Research Ethics Committee of Nanjing Medical University, and informed consent was obtained from all patients. All the tissues were frozen in liquid nitrogen immediately after surgical excision and stored at -80˚C. The TNM stage was classified on the basis of the National Comprehensive Cancer Network (NCCN) guidelines. Patients who received any preoperative treatments were not included in the present study.

RNA sequence data sets and computational analysis. The colon cancer (COAD) RNA expression prolife data (level 3) was downloaded from TCGA database (September 2017) (http://cancergenome.nih.gov). We obtained the normalized count data of RNA sequencing, including lncRNA and mRNA expression profiles. Level 3 miRNAseq data was obtained from TCGA by an Illumina HiSeq 2000 miRNA sequencing (miRNAseq) platform (Illumina, Inc., Hayward, CA, USA). First, the tumour samples were divided into 2 groups (left and right colon cancer). Then, we compared the differentially expressed lncRNAs (DElncRNA), mRNAs (DEmRNA) and miRNAs (DEmiRNA) between tumour tissues and adjacent non-tumour tissues using the Empirical Analysis of Digital Gene Expression Data package in R (edgeR, R version 3.4.1, http://www.bioconductor.org/packages/) [absolute $\log_2$(fold change)>2.0, FDR<0.01] in these 2 groups. In the next step, the intersecting lncRNAs were identified in the aforementioned 2 groups. After removing the elements of the intersection from DElncRNAs (RIDElncRNAs) of the left and right side, the DElncRNAs included exclusively in left or right colon cancer were obtained. Fig. 1 displays the bioinformatics analysis process.

Construction of the ceRNA network. According to the relationship among lncRNAs, miRNAs and mRNAs and the theory that lncRNAs can regulate miRNAs by binding them and further regulate mRNAs, a ceRNA network was constructed. miRcode (http://www.mircode.org/) was used to predict the differentially expressed miRNA targets to find the lncRNA-miRNA interactions. TargetScan (http://www.TargetScan.org/), miRDB (http://www.mirdb.org/) and miRanda (http://www.microrna.org/microrna/home.do) were used to predict miRNA-targeted mRNA. Then, the intersection with the differentially expressed lnRNAs and mRNAs was retained. Finally, the lncRNA/miRNA/mRNA ceRNA network was constructed using Cytoscape v3.0 (http://www.cytoscape.org/). Fig. 2. reveals the flowchart of the ceRNA network.

GO and pathway analysis. Differentially expressed mRNAs included in the ceRNA network were entered into the Database for Annotation, Visualization, and Integrated Discovery (DAVID) bioinformatics resource (https://david.ncifcrf.gov/) for functional enrichment analysis.

Clinical feature analysis of key lncRNAs. Based on the bioinformatics analysis and the ceRNA network, the relationship between the clinical features, including sex, age, tumour staging, TNM staging and lymphatic metastasis, and the expression of key lncRNAs was analysed. In addition, the association between side-specific lncRNAs and colon cancer patient survival time was analysed.

RNA extraction and reverse transcription-quantitative polymerase chain reaction (RT-qPCR) validation. RNA from tissue samples was extracted by TRIzol reagent (Invitrogen; Thermo Fisher Scientific, Inc., Waltham, MA, USA). A PrimeScript RT kit (Takara Biotechnology Co., Ltd., Dalian, China) was used to synthesize complementary DNA (cDNA). qPCR was performed in a 20-μl volume system (2 μl cDNA; 1.2 μl primers;

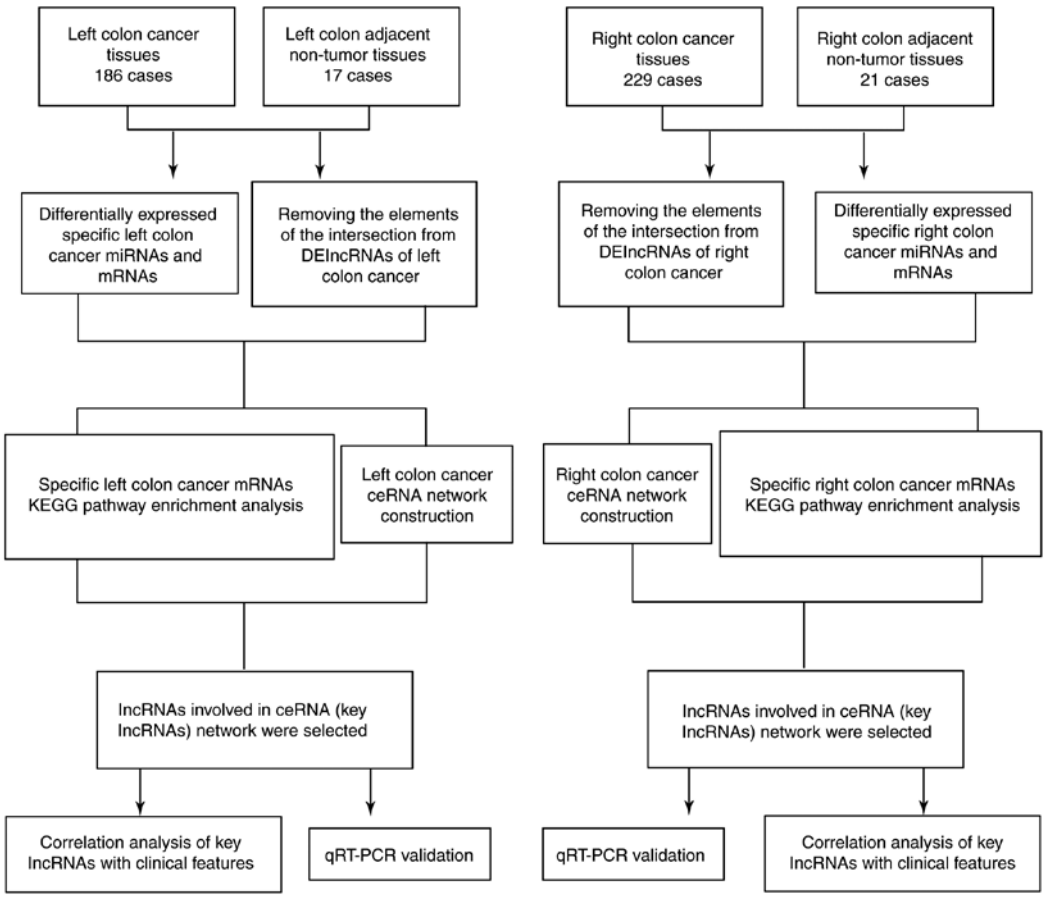

Figure 1. Flowchart of bioinformatics analysis. lncRNAs, long non-coding RNAs; DElncRNAs, differentially expressed lncRNAs; miRNAs, microRNAs; KEGG, Kyoto Encyclopaedia of Genes and Genomes; ceRNAs, competing endogenous RNAs.

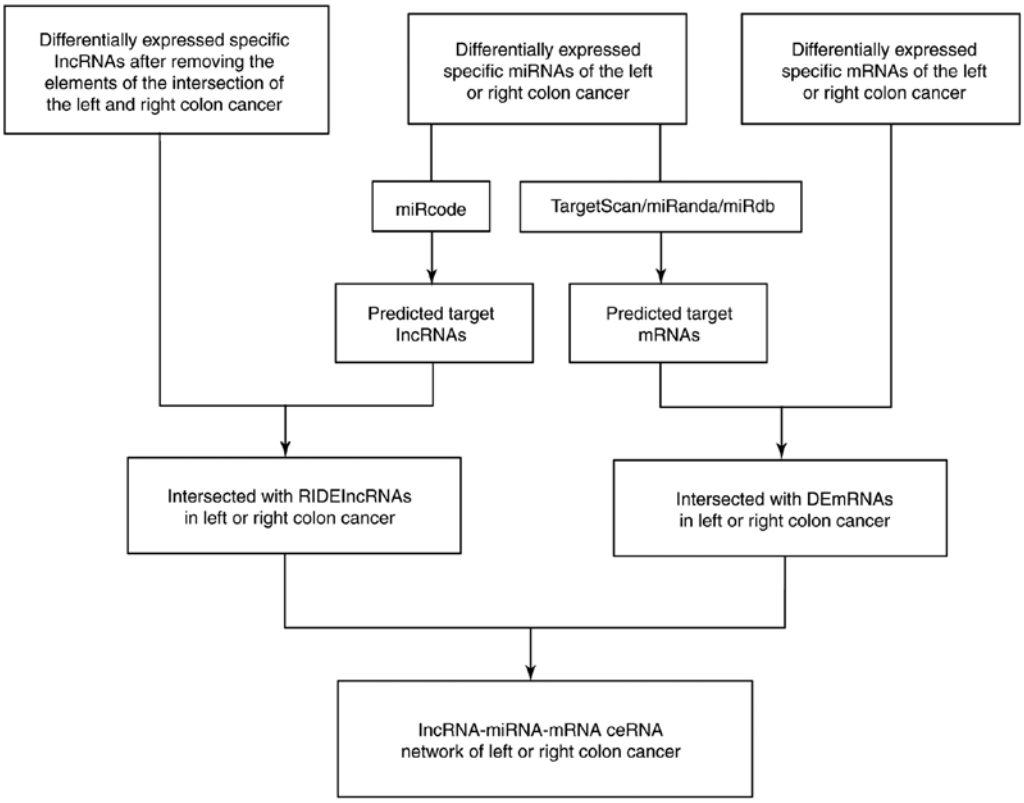

Figure 2. Flowchart of the construction of a ceRNA network. lncRNAs, long non-coding RNAs; DElncRNAs, differentially expressed lncRNAs; RIDElncRNAs, DElncRNAs after removing the elements of the intersection of left and right colon cancer; miRNAs, microRNAs; ceRNAs, competing endogenous RNA.

Table I. Key lncRNAs involved in the ceRNA network.

lncRNAs from left colon cancer	Log_2 (fold change)	-Log (FDR)	lncRNAs from right colon cancer	Log_2 (fold change)	-Log (FDR)
CMAHP	-2.31	20.48	LINC00483	-2.15	17.50
PRSS30P	2.05	4.64	LINC00488	-3.20	15.36
GRIK1-AS1	-2.66	16.87	LPP-AS1	3.94	2.06
MIR7-3HG	-3.05	15.69	COL4A2-AS2	2.53	2.92
WT1-AS	3.21	2.76	ST7-AS2	3.47	2.38
MIR22HG	-2.17	38.57	MIR205HG	5.31	2.26
MUC19	3.33	2.53	WASIR2	3.06	6.24
LY86-AS1	-2.12	5.68	OSBPL10-AS1	2.75	2.59
LINC00473	-2.79	16.34	ERVH48-1	3.12	2.77
LINC00393	3.54	2.23	DSCAM-AS1	4.55	2.11
STEAP2-AS1	2.88	4.84	EGOT	2.28	4.27
ATP11A-AS1	3.72	2.40	THOC7-AS1	2.27	2.14
CYP1B1-AS1	-2.28	25.21	ZBTB20-AS1	2.37	2.07
BOK-AS1	5.57	3.63	AC007731.1	3.47	3.56
LINC00402	-3.18	19.67	ITGB5-AS1	2.50	2.40
AC112721.1	2.84	3.18	ARHGEF38-IT1	2.21	4.51
GDNF-AS1	-2.59	17.19	ANO1-AS2	2.02	2.28
ITPK1-AS1	2.38	2.01	C8orf49	3.46	2.29
			RMST	-2.01	3.57
			NOVA1-AS1	-2.01	3.48
			KCNQ1OT1	2.33	6.52

lncRNAs, long non-coding RNAs; ceRNA, competing endogenous RNA.

6.8 μl dH_2O; 10 μl SYBR-Green) using FastStart Universal SYBR-Green Master Kit (Roche Diagnostics, Indianapolis, IN, USA) and a StepOnePlus Real-time PCR System (Applied Biosystems; Thermo Fisher Scientific, Inc.). Thermocycling conditions were as follows: Hot-start DNA polymerase activation at 95˚C for 10 min, 40 cycles at 95˚C for 15 sec and 60˚C for 1 min, followed by one cycle of melt curve analysis at 95˚C for 15 sec, 60˚C for 1 min, and 95˚C for 15 sec. The data were analysed using the $2^{-\Delta\Delta Cq}$ (14) method and the mRNA expression levels were normalized to GAPDH. The rimer sequences were as follows: LINC00402 forward, 5'-TAGGCAGGAAAG AGGTTG-3' and reverse, 5'-TGGTAGGTAGCAGGTGGT-3'; KCNQ1OT1 forward, 5'-AGGGTGACAGTGTTTCATAGG CT-3' and reverse, 5'-GAGGCACATTCATTCGTTGGT-3'; GAPDH forward, 5'-ACAGTCAGCCGCATCTTCTT-3' and reverse, 5'-GACAAGCTTCCCGTTCTCAG-3'. All RT-qPCR reactions were performed in triplicate.

Statistical analysis. All the results were expressed as the mean ± SD. R Studio (R version 3.4.1; https://www.rstudio.com), Statistical Programme for Social Sciences 20.0 (IBM Corp., Armonk, NY, USA) and GraphPad Prism 5.0 software (GraphPad Software, Inc., La Jolla, CA, USA) were used to analyse the data. The prognostic characteristics of lncRNAs based on the univariate Cox proportional hazards regression model were detected. Then, multivariate Cox regression analysis was applied for further study. The differences in the qRT-PCR results were compared by paired Student's t-test. $P<0.05$ was considered to indicate a statistically significant difference.

Results

DElncRNAs that are included exclusively in left or right colon cancer. It was determined that 879 lncRNAs (DElncRNA) were differentially expressed between tumour samples and adjacent tissues in left colon cancer from TCGA database [absolute log_2(fold change)>2.0, FDR<0.01]. There were 916 in right colon cancer. After removing the elements of the intersection from DElncRNAs (RIDElncRNAs) of left and right colon cancer, the DElncRNAs included exclusively in left or right colon cancer were obtained, including 277 DElncRNAs (RIDElncRNAs) in the left colon cancer and 314 DElncRNAs (RIDElncRNAs) in the right colon cancer. Finally, 18 RIDElncRNAs were used to construct the ceRNA network of left colon cancer (Tables I and II), and 21 RIDElncRNAs were used to construct the network of right colon cancer (Tables I and III).

Prediction of lncRNA-targeted miRNAs. For further investigation, 165 and 227 side-specific miRNAs that were differentially expressed between tumour tissues and adjacent tissues from left and right colon cancer, respectively, were determined. These miRNAs were identified as side-specific

Table II. lncRNAs that may target specific miRNAs in left colon cancer.

lncRNAs	miRNAs
CMAHP	hsa-mir-96, hsa-mir-141, hsa-mir-144, hsa-mir-145, hsa-mir-150, hsa-mir-424, hsa-mir-182, hsa-mir-183, hsa-mir-187, hsa-mir-192, hsa-mir-215, hsa-mir-375
PRSS30P	hsa-mir-143, hsa-mir-150, hsa-mir-424, hsa-mir-21
GRIK1-AS1	hsa-mir-145, hsa-mir-375
MIR7-3HG	hsa-mir-145, hsa-mir-150
WT1-AS	hsa-mir-96, hsa-mir-141, hsa-mir-145, hsa-mir-424, hsa-mir-17, hsa-mir-182, hsa-mir-98, hsa-mir-193b, hsa-mir-429, hsa-mir-22, hsa-mir-32, hsa-mir-375
MIR22HG	hsa-mir-375, hsa-mir-32
MUC19	hsa-mir-375, hsa-mir-22, hsa-mir-32, hsa-mir-429, hsa-mir-193b, hsa-mir-215, hsa-mir-192, hsa-mir-187, hsa-mir-98, hsa-mir-182, hsa-mir-17, hsa-mir-424, hsa-mir-150, hsa-mir-152, hsa-mir-14, hsa-mir-144, hsa-mir-143, hsa-mir-96, hsa-mir-454
LY86-AS1	hsa-mir-375, hsa-mir-187, hsa-mir-183, hsa-mir-182, hsa-mir-424, hsa-mir-150, hsa-mir-145, hsa-mir-141, hsa-mir-96, hsa-mir-454
LINC00473	hsa-mir-424, hsa-mir-150, hsa-mir-145
LINC00393	hsa-mir-215, hsa-mir-192
STEAP2-AS1	hsa-mir-375, hsa-mir-424, hsa-mir-152, hsa-mir-143
ATP11A-AS1	hsa-mir-22, hsa-mir-215, hsa-mir-192, hsa-mir-187, hsa-mir-424, hsa-mir-152, hsa-mir-143, hsa-mir-96
CYP1B1-AS1	hsa-mir-21, hsa-mir-429, hsa-mir-193b, hsa-mir-150, hsa-mir-152, hsa-mir-145, hsa-mir-454
BOK-AS1	hsa-mir-150
LINC00402	hsa-mir-22, hsa-mir-429, hsa-mir-193b, hsa-mir-182, hsa-mir-17, hsa-mir-150, hsa-mir-143, hsa-mir-141
AC112721.1	hsa-mir-424
GDNF-AS1	hsa-mir-187, hsa-mir-424, hsa-mir-145, hsa-mir-143
ITPK1-AS1	hsa-mir-22, hsa-mir-17, hsa-mir-150, hsa-mir-144, hsa-mir-141

lncRNAs, long non-coding RNAs; miRNAs, microRNAs.

miRNAs. Then, it was determined whether these 165 miRNAs could target the aforementioned 277 RIDElncRNAs in left colon cancer and whether the 227 miRNAs could target the 314 RIDElncRNAs in right colon cancer. Based on miRcode (http://www.mircode.org/), 22 miRNAs targeting 18 lncRNAs were predicted in left colon cancer (Table II), and there were 27 miRNAs and 21 lncRNAs predicted in the ceRNA of right colon cancer (Table III).

Prediction of miRNA-targeted mRNAs. First, 2,028 and 2,069 differentially expressed mRNAs between tumour tissues and adjacent tissues [absolute $\log_2$(fold change)>2, FDR<0.01] in left and right colon cancer, respectively, were identified. These mRNAs were identified as side-specific mRNAs (DEmRNAs). The 2,028 and 2,069 DEmRNAs were analysed with DAVID bioinformatics resources. According to the P-values, the top 21 KEGG pathways of the DEmRNAs were revealed (Figs. 3 and 4). Among these pathways, the transcriptional dysregulation in cancer, cAMP, Wnt and PPAR signalling pathways, which were related to cancer-associated pathways, were revealed to be more important in left colon cancer. However, the chemical carcinogenesis pathway was more involved in right colon cancer. For further investigation, the miRNAs included in the ceRNA network were then used to predict the targeted mRNAs using miRanda, TargetScan and miRDB. The intersection of the predicted mRNAs and DEmRNAs was obtained. Finally, 57 mRNAs were included in the left colon cancer ceRNA network, and 55 were included in the right (Tables IV and V).

According to the relationship between RNAs revealed by Tables II-V, lncRNA-miRNA-mRNA ceRNA networks were constructed. Cytoscape 3.0 was used to draw the ceRNA network. In our results, there were 18 lncRNAs, 22 miRNAs and 57 mRNAs included in the left colon cancer ceRNA network and 21 lncRNAs, 27 miRNAs and 55 mRNAs included in the right ceRNA network (Figs. 5 and 6).

Key lncRNAs and clinical feature association. The clinical features of the key lncRNAs in the ceRNA network were then further analysed. The clinical features, including sex, tumour grade, TNM stage and lymphatic metastasis status, were provided by TCGA data sets. In total 15 lncRNAs were revealed, including 3 in left colon cancer and 12 in right colon cancer, which were significantly related to clinical features (Table VI). The results revealed that CYP1B1-AS1, LPP-AS1, MIR205HG, DSCAM-AS1, RMST and NOVA1-AS1 were related to age, LPP-AS1, WASIR2, OSBPL10-AS1 and DSCAM-AS1 were related to sex, AC112721.1, DSCAM-AS1, ARHGEF38-IT1, C8orf49 and KCNQ1OT1 were related to lymphatic metastasis, LINC00402 and ZBTB20-AS1 were related to tumour grade, and AC112721.1, DSCAM-AS1, ARHGEF38-IT1, C8orf49 and KCNQ1OT1 were related to TNM stage (Table VI).

Table III. lncRNAs that may target specific miRNAs in right colon cancer.

lncRNAs	miRNAs
LINC00483	hsa-mir-223, hsa-mir-21, hsa-mir-215, hsa-mir-192, hsa-mir-183, hsa-mir-182, hsa-mir-17, hsa-mir-150, hsa-mir-144, hsa-mir-96, hsa-mir-106a
LINC00488	hsa-mir-21, hsa-mir-215, hsa-mir-192, hsa-mir-98, hsa-mir-144, hsa-mir-96
LPP-AS1	hsa-mir-338, hsa-mir-143
COL4A2-AS2	hsa-mir-338, hsa-mir-424, hsa-mir-150, hsa-mir-152
ST7-AS2	hsa-mir-22, hsa-mir-429, hsa-mir-215, hsa-mir-192, hsa-mir-145
MIR205HG	hsa-mir-22, hsa-mir-215, hsa-mir-192, hsa-mir-183, hsa-mir-150, hsa-mir-152, hsa-mir-145, hsa-mir-143, hsa-mir-454, hsa-mir-301b
WASIR2	hsa-mir-338, hsa-mir-193b, hsa-mir-150
OSBPL10-AS1	hsa-mir-375, hsa-mir-182, hsa-mir-424, hsa-mir-145, hsa-mir-96
ERVH48-1	hsa-mir-338, hsa-mir-223, hsa-mir-22, hsa-mir-21, hsa-mir-187, hsa-mir-98, hsa-mir-182, hsa-mir-145, hsa-mir-144, hsa-mir-141, hsa-mir-96, hsa-mir-454, hsa-mir-301b
DSCAM-AS1	hsa-mir-338, hsa-mir-150, hsa-mir-143, hsa-mir-141
EGOT	hsa-mir-375, hsa-mir-21, hsa-mir-183, hsa-mir-424, hsa-mir-143, hsa-mir-141
THOC7-AS1	hsa-mir-215, hsa-mir-192, hsa-mir-187
ZBTB20-AS1	hsa-mir-217, hsa-mir-152, hsa-mir-454, hsa-mir-301b
AC007731.1	hsa-mir-215, hsa-mir-192, hsa-mir-183, hsa-mir-150, hsa-mir-152
ITGB5-AS1	hsa-mir-21, hsa-mir-193b
ARHGEF38-IT1	hsa-mir-338, hsa-mir-150, hsa-mir-143
ANO1-AS2	hsa-mir-375, hsa-mir-98, hsa-mir-17, hsa-mir-152, hsa-mir-106a
C8orf49	hsa-mir-375, hsa-mir-338, hsa-mir-32, hsa-mir-429, hsa-mir-17, hsa-mir-424, hsa-mir-150, hsa-mir-152, hsa-mir-143, hsa-mir-106a, hsa-mir-454, hsa-mir-301b
RMST	hsa-mir-375, hsa-mir-338, hsa-mir-32, hsa-mir-429, hsa-mir-193b, hsa-mir-182, hsa-mir-17, hsa-mir-424, hsa-mir-150, hsa-mir-145, hsa-mir-144, hsa-mir-96, hsa-mir-454, hsa-mir-301b
NOVA1-AS1	hsa-mir-223, hsa-mir-22, hsa-mir-217
KCNQ1OT1	hsa-mir-375, hsa-mir-338, hsa-mir-32, hsa-mir-223, hsa-mir-22, hsa-mir-217, hsa-mir-429, hsa-mir-193b, hsa-mir-215, hsa-mir-192, hsa-mir-187, hsa-mir-98, hsa-mir-183, hsa-mir-301b, hsa-mir-454, hsa-mir-106a, hsa-mir-96, hsa-mir-141, hsa-mir-143, hsa-mir-145, hsa-mir-152, hsa-mir-150, hsa-mir-182, hsa-mir-17, hsa-mir-424

lncRNAs, long non-coding RNAs; miRNAs, microRNAs.

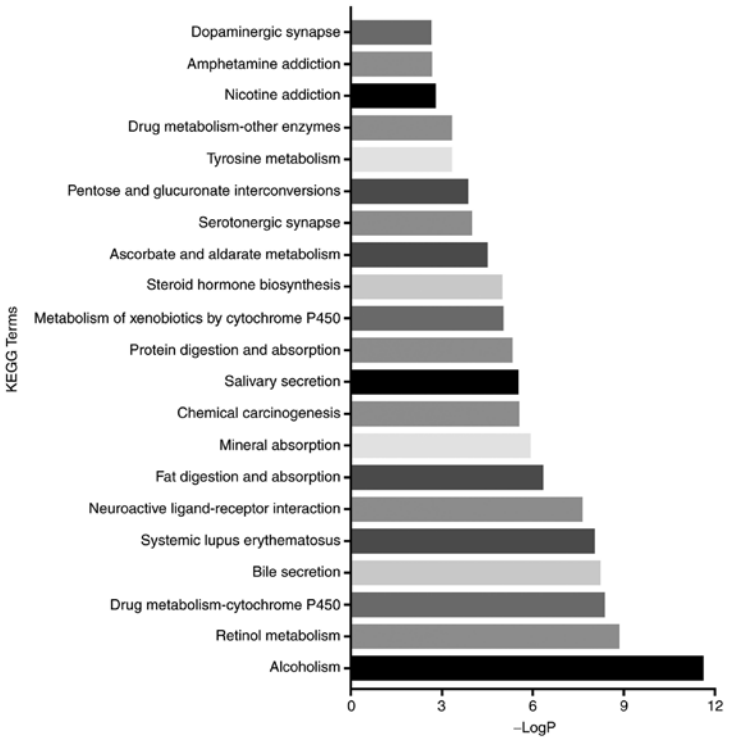

Figure 3. Top 21 KEGG terms for mRNAs from the left colon cancer. -LogP, -Log(P-value); KEGG, Kyoto Encyclopaedia of Genes and Genomes.

Table IV. miRNAs that may target specific mRNAs in left colon cancer.

miRNAs	mRNAs
hsa-mir-141	PHLPP2, ELAVL4, MACC1, KIAA1549, EPHA7
hsa-mir-143	COL1A1, SERPINE1
hsa-mir-144	GRIK3, TBX18
hsa-mir-145	SERPINE1
hsa-mir-150	HILPDA, SLC7A11, ZNF460, EREG
hsa-mir-152	NPTX1, BMP3, KLF4
hsa-mir-17	E2F1, FOXQ1, CADM2, FJX1, CFL2, SALL3, FAM129A, SMOC1, CLIP4, FAM46C, ANKRD33B
hsa-mir-182	CHL1, NPTX1, TCEAL7, FOXF2, ULBP2
hsa-mir-183	KIF5C
hsa-mir-192	TCF7
hsa-mir-193b	PLAU, DCAF7
hsa-mir-21	EDIL3, OSR1, ATP2B4, TGFBI
hsa-mir-215	TCF7
hsa-mir-22	RGS2
hsa-mir-32	UGP2, CCDC113, PHLPP2, ATP2B4
hsa-mir-375	ELAVL4
hsa-mir-424	PSAT1, PHLPP2, ZNRF3, TPM2, TMEM100, AXIN2, CBX2
hsa-mir-454	RBM20, SALL3, CFL2, SMOC1
hsa-mir-96	ALK, TRIB3
hsa-mir-98	TRIM71, CPA4, IGF2BP3, HAND1, SLC5A6, PRSS22, IGF2BP1

miRNAs, microRNAs.

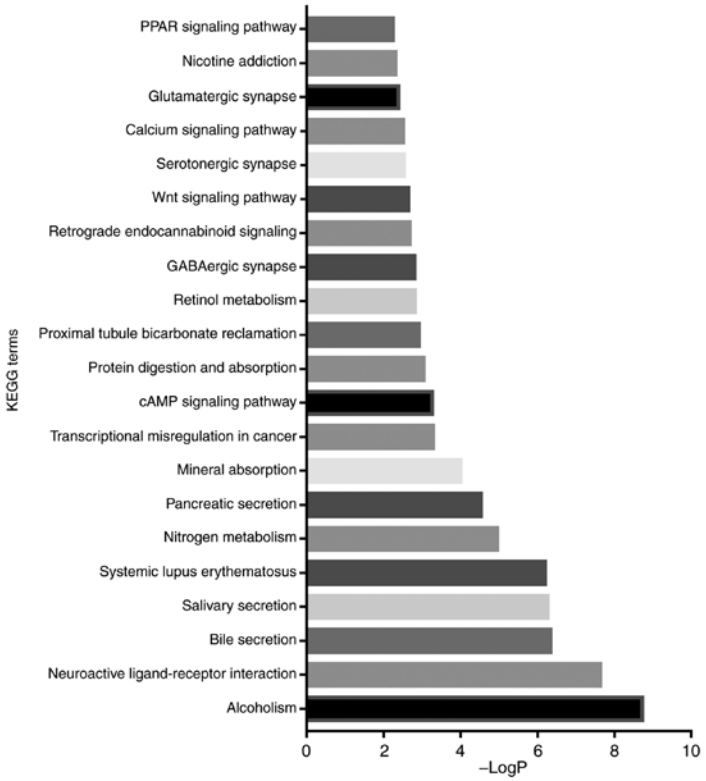

Figure 4. Top 21 KEGG terms for mRNAs from the right colon cancer. -LogP, -Log(P-value); KEGG, Kyoto Encyclopaedia of Genes and Genomes.

Furthermore, the prognostic characteristics of RIDElncRNAs from both sides were detected. Based on the univariate Cox proportional hazards regression model, the association of the overall survival of patients with the expression

Table V. miRNAs that may target specific mRNAs in right colon cancer.

miRNAs	mRNAs
hsa-mir-106a	CFL2, FAM129A, FJX1, CADM2, FOXQ1
hsa-mir-141	KIAA1549, PHLPP2, ELAVL4, EPHA7, DPY19L1
hsa-mir-143	COL1A1, SERPINE1
hsa-mir-144	GRIK3, KCNQ5
hsa-mir-145	SERPINE1
hsa-mir-150	ZNF460, PDCD4, SLC7A11, HILPDA, EREG
hsa-mir-152	KLF4, NPTX1, BMP3
hsa-mir-17	FOXQ1, CADM2, FJX1, FAM129A, CFL2, SLC16A9, CYBRD1
hsa-mir-182	ULBP2, CHL1, NPTX1, FOXF2
hsa-mir-183	PDCD4
hsa-mir-192	GRHL1
hsa-mir-193b	PLAU
hsa-mir-21	PDCD4, TGFBI, JPH1
hsa-mir-217	DACH1
hsa-mir-223	ECT2, EPB41L3
hsa-mir-32	PAX9, PBLD, MIER3, PHLPP2, UGP2
hsa-mir-338	NOVA1
hsa-mir-375	ELAVL4
hsa-mir-424	TPM2, TMEM100, PHLPP2, CBX2, E2F7, PSAT1, AXIN2
hsa-mir-429	PMAIP1
hsa-mir-454	CFL2, RBM20
hsa-mir-96	TRIB3
hsa-mir-98	RGS16, IGF2BP3, TRIM71, PRSS22, HAND1, IGF2BP1, FMO4, CPA4

miRNAs, microRNAs.

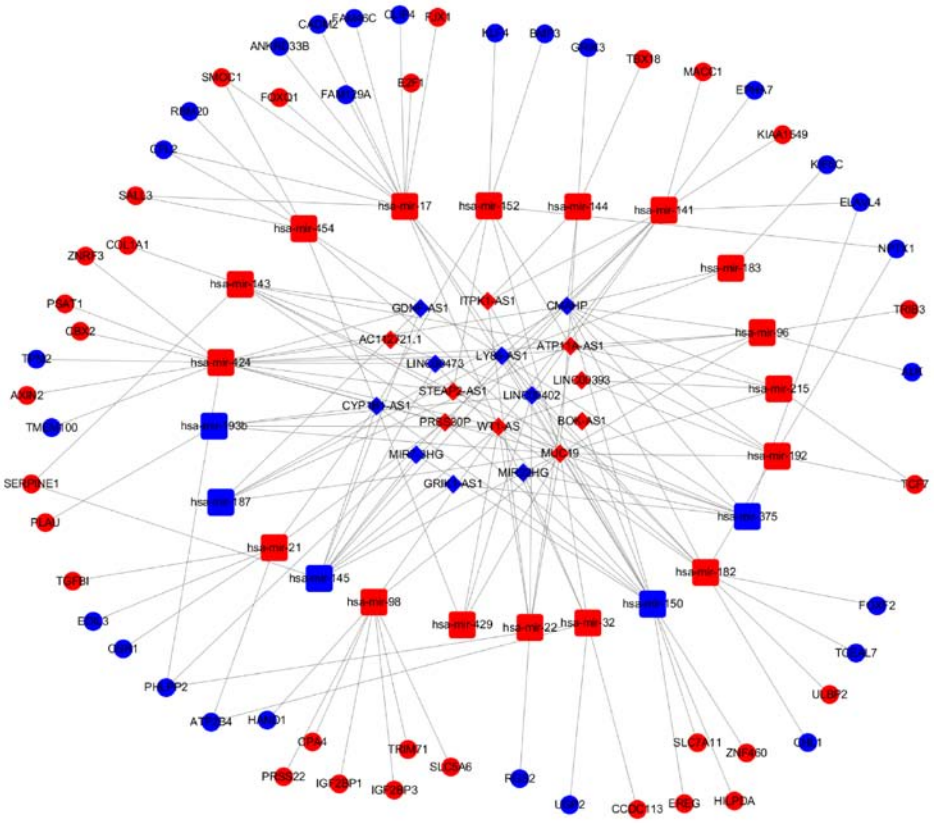

Figure 5. The lncRNA/miRNA/mRNA ceRNA network of left colon cancer. Red balls, upregulated mRNAs; blue balls, downregulated mRNAs; red squares, upregulated miRNAs; blue squares, downregulated miRNAs; red diamonds, upregulated lncRNAs; blue diamonds, downregulated lncRNAs. lncRNAs, long non-coding RNAs; miRNAs, microRNAs; ceRNA, competing endogenous RNA.

Table VI. Correlation between key lncRNAs involved in the ceRNA network and their clinical features.

Comparisons	Left colon cancer	Right colon cancer
Age (<60 vs. >60 years)	CYP1B1-AS1	LPP-AS1, MIR205HG, DSCAM-AS1, RMST, NOVA1-AS1
Sex (female vs. male)		LPP-AS1, WASIR2, OSBPL10-AS1, DSCAM-AS1
Lymphatic metastasis (no vs. yes)	AC112721.1	DSCAM-AS1, ARHGEF38-IT1, C8orf49, KCNQ1OT1
TNM staging system (T1+T2 vs. T3+T4)	LINC00402	ZBTB20-AS1
Tumour stage (stage I, II vs. stage III, IV)	AC112721.1	DSCAM-AS1, ARHGEF38-IT1, C8orf49, KCNQ1OT1
MSI status (MSI-H vs. other status)		LINC00483, LINC00488, WASIR2, DSCAM-AS1, NOVA1-AS1

lncRNAs, long non-coding RNAs; ceRNA, competing endogenous RNA.

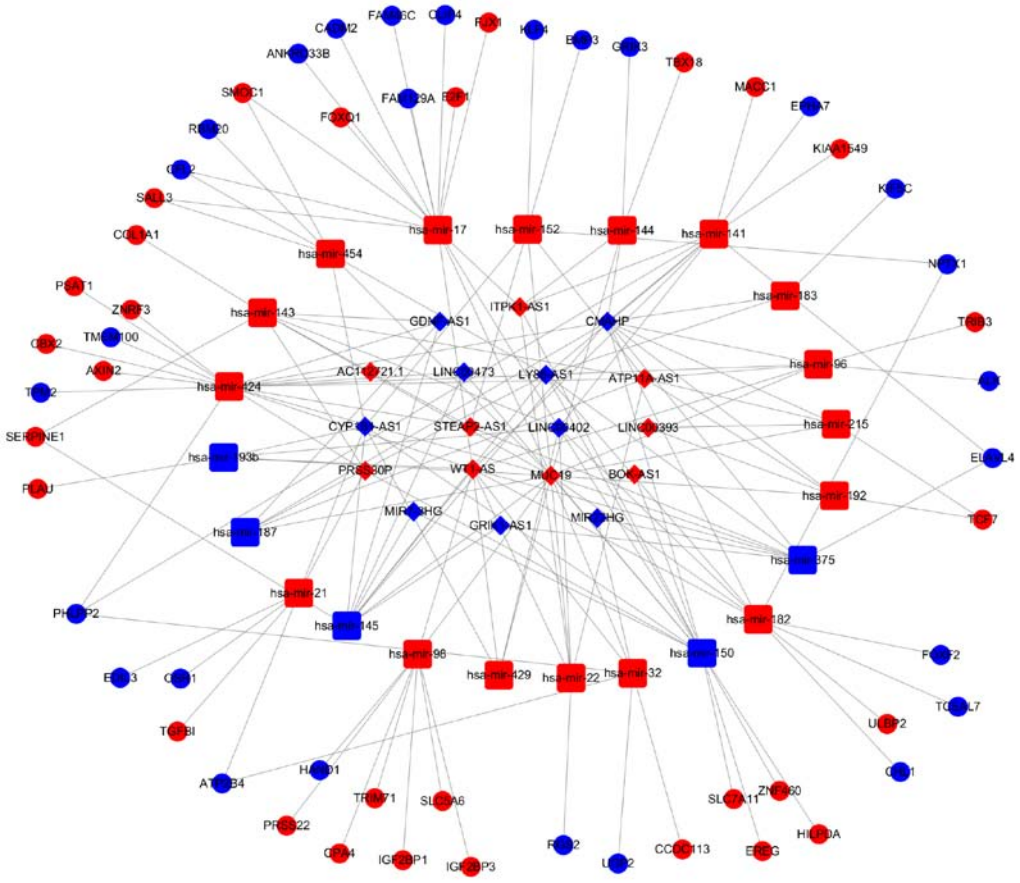

Figure 6. The lncRNA/miRNA/mRNA ceRNA network of right colon cancer. Red balls, upregulated mRNAs; blue balls, downregulated mRNAs; red squares, upregulated miRNAs; blue squares, downregulated miRNAs; red diamonds, upregulated lncRNAs; blue diamonds, downregulated lncRNAs. lncRNAs, long non-coding RNAs; miRNAs, microRNAs; ceRNA, competing endogenous RNA.

level of RIDElncRNAs was analysed. Finally, 20 lncRNAs from left colon cancer that were significantly associated with overall survival (log-rank P<0.05) (Fig. S1 and Table VII) were revealed, and 25 lncRNAs from right colon cancer were determined to be related to overall survival (log-rank P<0.05) (Fig. S2 and Table VII). Multivariate Cox regression analysis was then performed with these lncRNAs. Fifteen lncRNAs from the left colon cancer were revealed to be independent factors of survival time and 12 in the right colon cancer. The results are presented in Table VIII.

Subsequently, to confirm the reliability of the bioinformatics results, 2 key lncRNAs (LINC00402 from the left side and KCNQ1OT1 from the right side) were randomly selected from the networks and their RNA expression level was determined in 58 paired colon cancer tissues from left colon cancer and right colon cancer. The bioinformatics results revealed that LINC00402 presented lower expression in left colon cancer tissues than adjacent non-tumour tissues, and KCNQ1OT1 was significantly expressed higher in tumour tissues. Fig. 7 revealed that the qRT-PCR results were consistent with the

Table VII. Kaplan-Meier survival analysis for lncRNAs that were associated with overall survival.

lncRNAs from the left	P-value	lncRNAs from the right	P-value
AC019118.4	0.003	AC003991.3	0.005
CTB-181H17.1	0.01	AC011288.2	0.003
CTD-2308L22.1	0.04	AC012531.25	0.01
FGF13-AS1	0.006	B4GALT1-AS1	0.0005
HORMAD2-AS1	0.03	CTC-428G20.6	0.02
IGBP1-AS2	0.03	CTC-573N18.1	0.05
LINC01990	0.02	CTD-2591A5.2	0.05
RP1-10C16.1	0.03	CTD-3064M3.7	0.05
RP11-108K3.1	0.04	LINC00513	0.005
RP11-205M3.3	0.03	LINC01630	0.03
RP11-281P23.2	0.002	LINC01633	0.008
RP11-342A23.2	0.01	RP11-126H7.4	0.04
RP11-354P11.4	0.02	RP11-138A9.1	0.04
RP11-475B2.1	0.03	RP11-138A9.2	0.01
RP11-515O17.2	0.01	RP11-157F20.3	0.03
RP11-674E16.4	0.02	RP11-277P12.20	0.05
RP11-686O6.1	0.04	RP11-304L19.12	0.03
RP11-713P17.3	0.01	RP11-495P10.5	0.002
RP3-453C12.15	0.04	RP11-619I22.1	0.03
RP6-114E22.1	0.02	RP11-661A12.9	0.04
		RP11-67K19.3	0.05
		RP1-29C18.10	0.05
		RP4-676L2.1	0.05
		RP4-811H24.9	0.05
		SSTR5-AS1	0.03

lncRNAs, long non-coding RNAs.

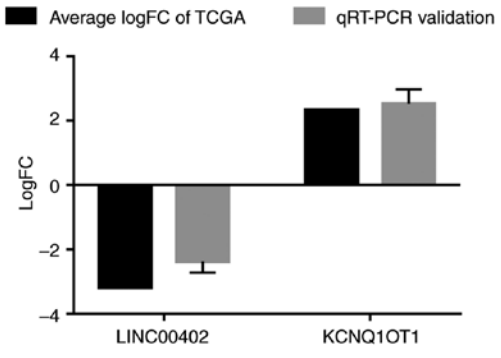

Figure 7. Comparison of logFC (-ΔΔCq) of lncRNAs between TCGA and qRT-PCR results. LogFC, $\log_2$(fold change).

bioinformatics results. The relationship between the expression of the 2 lncRNAs and clinicopathological characteristics was then further analysed. The results revealed that LINC00402 was related to TNM staging (Table IX), and KCNQ1OT1 was significantly associated with lymphatic metastasis and tumour stage (Table IX), which were almost the same as the bioinformatics results (Table VI).

Discussion

In terms of clinical behaviour and response to therapy, colorectal cancer (CRC) is a heterogeneous disease (15,16). Patients can benefit from this heterogeneity when stratified for their response to therapeutic strategies. However, CRC development involves the complex transformation of molecular events of which we lack enough knowledge. CRC still has a high incidence and mortality. It is our hope that the study of the molecular difference between left and right side colon cancer will help with the exploration of CRC heterogeneity.

In the present study, we determined side-specific lncRNAs, miRNAs and mRNAs based on the differential expression between tumour tissues and adjacent non-tumour tissues in the two sides. Through KEGG analysis, we analysed the pathways that the side-specific mRNAs may be involved in. Combining the bioinformatics resources, we established ceRNA networks with side-specific DEmRNAs, DEmiRNAs and RIDElncRNAs. We then further analysed the clinical features of the key lncRNAs belonging to the ceRNA network. Side-specific RIDElncRNAs were further analysed to determine whether they were correlated with overall survival. To check the reliability of the bioinformatics results, we randomly selected 2 key lncRNAs (LINC00402 from the left and KCNQ1OT1 from the right) and determined their expression by qRT-PCR.

There were several cancer-related roles in the RIDElncRNA group from the two sides. For example, MIR22HG was reported to suppressed hepatocellular and endometrial carcinoma (17,18) and linc00483 was reported to promoted gastric cancer (19). We conducted univariate and multivariate Cox regression analyses and found that 15 lncRNAs from the left and 12 lncRNAs from the right were found to be independent factors of survival time (Table VIII). In KEGG analysis, we identified the top 21 pathways of the DEmRNAs (Figs. 3 and 4). After removing the same KEGG terms, the results revealed that different cancer-related pathways were involved in the two sides, i.e., the transcriptional dysregulation in cancer, cAMP, Wnt and PPAR signalling pathways were more important in the left colon cancer, and the chemical carcinogenesis pathway played a more important role in the right colon cancer. Some of these cancer-related pathways, such as cAMP, Wnt and PPAR signalling pathways, have been reported to play important roles in the CRC. Lu *et al* reported that the cAMP pathway could be activated to inhibit angiogenesis and vasculogenic mimicry in CRC (20). As previous studies reported (21,22), the Wnt pathway could reduce apoptosis, stimulate cell proliferation and promote metastasis in CRC. Zarkou *et al* revealed that lncRNAs can modulate the WNT pathway by affecting gene expression through diversified mechanisms, from the transcriptional to the post-translational level (23). As for the PPAR signalling pathway, it has been revealed to be inhibited in CRC (24). However, few studies have examined the performance of these pathways in left or right colon cancer.

By constructing the ceRNA network, our research focused on the potentially different mechanisms of distal and proximal colon cancers. Several previous studies have already reported the interactions between RNAs in the ceRNA network. For example, MIR22HG, an lncRNA from the left ceRNA network, was reported to interact with miR-141-3p

Table VIII. Results of multivariate cox regression analysis.

lncRNAs from the left	β	OR (95% CI)	P-value	lncRNAs from the right	β	OR (95% CI)	P-value
RP6-114E22.1	-1.037	0.354 (0.158-0.793)	0.012	AC003991.3	0.676	1.966 (1.142-3.387)	0.015
RP3-453C12.15	-0.515	0.597 (0.273-1.306)	0.197	AC011288.2	-0.706	0.494 (0.286-0.851)	0.011
RP11-713P17.3	-0.886	0.412 (0.188-0.904)	0.027	AC012531.25	-0.672	0.511 (0.287-0.908)	0.022
RP11-686O6.1	0.809	2.245 (1.055-4.778)	0.036	B4GALT1-AS1	0.879	2.408 (1.347-4.305)	0.003
RP11-674E16.4	0.684	1.982 (0.899-4.369)	0.09	CTC-428G20.6	0.413	1.511 (0.869-2.628)	0.144
RP11-515O17.2	1.404	4.072 (1.591-10.424)	0.003	CTC-573N18.1	-0.543	0.581 (0.335-1.007)	0.053
RP11-475B2.1	-0.931	0.394 (0.179-0.865)	0.02	CTD-2591A5.2	-0.708	0.492 (0.280-0.866)	0.014
RP11-354P11.4	1.049	2.854 (1.252-6.506)	0.013	CTD-3064M3.7	-0.541	0.582 (0.336-1.009)	0.054
RP11-342A23.2	-0.916	0.400 (0.182-0.877)	0.022	LINC00513	0.641	1.898 (1.079-3.337)	0.026
RP11-281P23.2	1.023	2.783 (1.213-6.384)	0.016	LINC01630	0.462	1.587 (0.896-2.811)	0.113
RP11-205M3.3	0.79	2.204 (1.016-4.780)	0.045	LINC01633	-0.702	0.496 (0.285-0.863)	0.013
RP11-108K3.1	-0.545	0.580 (0.255-1.318)	0.193	RP11-126H7.4	-0.372	0.689 (0.398-1.194)	0.184
RP1-10C16.1	-0.921	0.398 (0.180-0.879)	0.023	RP11-138A9.1	0.421	1.524 (0.887-2.619)	0.127
LINC01990	-0.761	0.467 (0.208-1.051)	0.066	RP11-138A9.2	0.728	2.070 (1.150-3.729)	0.015
IGBP1-AS2	0.737	2.089 (0.904-4.828)	0.085	RP11-157F20.3	-0.495	0.610 (0.349-1.064)	0.081
HORMAD2-AS1	-1.334	0.263 (0.114-0.607)	0.002	RP11-277P12.20	-0.478	0.620 (0.358-1.073)	0.088
FGF13-AS1	-1.154	0.315 (0.143-0.695)	0.004	RP11-304L19.12	-0.47	0.625 (0.365-1.069)	0.086
CTD-2308L22.1	-1.085	0.338 (0.147-0.774)	0.01	RP11-495P10.5	-0.693	0.500 (0.282-0.886)	0.017
CTB-181H17.1	-1.03	0.357 (0.160-0.795)	0.012	RP11-619I22.1	0.578	1.783 (1.048-3.035)	0.033
AC019118.4	-0.991	0.371 (0.170-0.810)	0.013	RP11-661A12.9	-0.32	0.726 (0.417-1.265)	0.259
				RP11-67K19.3	0.655	1.926 (1.103-3.363)	0.021
				RP1-29C18.10	-0.455	0.634 (0.370-1.086)	0.097
				RP4-676L2.1	0.505	1.656 (0.924-2.969)	0.09
				RP4-811H24.9	-0.385	0.681 (0.394-1.176)	0.168
				SSTR5-AS1	0.615	1.850 (1.061-3.225)	0.03

lncRNAs, long non-coding RNAs.

and therefore inhibited endometrial carcinoma cell proliferation (18). Additionally, KCNQ1OT1, which belonged to the right ceRNA network, modulated CCNE2 by sponging miR-145 in BRCA (25). Another study reported that KCNQ1OT1 could ameliorate particle-induced osteolysis by inhibiting miR-21a-5p (26). These previous studies strongly demonstrated that our analysis was reliable. Therefore, there may be some internal contact between lncRNA/miRNA/mRNA in the progression and development of CRC. Based on significant differences in lncRNA, miRNA and mRNA expression data, a ceRNA network was constructed by bioinformatics prediction and correlation analysis. The relationship between the key lncRNAs and clinical features was then further analysed. In the left ceRNA network, 3 key lncRNAs (CYP1B1-AS1, LINC00402, and AC112721.1) were confirmed to be related to clinical features. AC112721.1 has been reported to be correlated with bladder cancer patient survival. In the right ceRNA network, 12 key lncRNAs (LINC00483, LPP-AS1, MIR205HG, WASIR2, OSBPL10-AS1, DSCAM-AS1, ZBTB20-AS1, ARHGEF38-IT1, C8orf49, RMST, NOVA1-AS1, and KCNQ1OT1) were identified to be associated with clinical features. Among these 12 lncRNAs, LINC00483 was reported to regulate proliferation and apoptosis in gastric cancer (19); Di Agostino *et al* reported that MIR205HG led to unrestrained proliferation in head and neck squamous cell carcinoma by depleting miR-590-3p (27); DSCAM-AS1 could interact with miR-137 to enhance tamoxifen resistance in breast cancer (28). Microarray data revealed that lncRNA RMST was differentially expressed in cervical cancer, and the *in vitro* assay revealed that RMST played the role of tumour suppressor in TNBC by inhibiting cell proliferation, invasion and migration (29); some studies reported that KCNQ1OT1 played an important role in multiple malignancies by interacting with several miRNAs, such as miR-140-5p, miR-384b, miR-145, miR-211-5p, miR-7-5p and miR-504 (25,30-34). However, more research still needs to be carried out to confirm and understand the relationship between these RNAs and clinical features.

Our analysis has ramifications for the ceRNA network in colon cancer, and some results were confirmed by qRT-PCR. However, there are still several limitations in our study. First, the present study was derived only from the data of The Cancer Genome Atlas (TCGA) database, and the conclusion is relatively preliminary and requires validation from large-scale clinical trials. Second, our confirmation experiment is limited to qRT-PCR of two lncRNAs,

Table IX. Expression of lncRNAs related to clinical features according to the clinicopathological characteristics of patients.

		LINC00402		
Characteristics	No.	Low group	High group	P-value
Age (years)				0.581
<60	20	11	9	
≥60	38	18	20	
Sex				0.597
Female	26	12	14	
Male	32	17	15	
Lymphatic metastasis				0.185
No	33	14	19	
Yes	25	15	10	
TNM staging system				0.03
T1+T2	22	7	15	
T3+T4	36	22	14	
Tumor stage				0.293
Stage I, II	30	13	17	
Stage III, IV	28	16	12	
MSI status				0.066
MSI-H	14	5	9	
Other status	44	22	12	

		KCNQ1OT1		
Characteristics	No.	Low group	High group	P-value
Age (years)				0.279
<60	22	9	13	
≥60	36	20	16	
Sex				0.430
Female	27	12	15	
Male	31	17	14	
Lymphatic metastasis				0.007
No	36	23	13	
Yes	22	6	16	
TNM staging system				0.426
T1+T2	25	11	14	
T3+T4	33	18	15	
Tumour stage				0.033
Stage I, II	34	21	13	
Stage III, IV	24	8	16	
MSI status				0.557
MSI-H	16	9	7	
Other status	42	20	22	

lncRNAs, long non-coding RNAs.

and further research is also required on the functions of key lncRNAs *in vivo* and *in vitro*, and the relationship of expression and function in the RNAs also requires further validation.

In conclusion, our research aimed to detect the difference between left and right colon cancer. By constructing ceRNA networks and analysing the relationship between the key lncRNAs and the clinical features, the putatively different

mechanism of the two sides and the relationship among these 3 types of RNAs was partially revealed. The present study may further our insight into the difference between left and right colon cancer at the genetic level.

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Availability of data and materials

The data sets used and/or analysed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

YS conceptualized and designed the research. WQ and YF performed the experiments, analysed and interpreted the results, made the figures and wrote the manuscript. JL, WP and QG performed the experiments and analysed the data. JL, ZZ and DJ provided the patient tissues. QG, ZZ and DJ also helped design the experimental studies and edited the manuscript. QW and DZ interpreted the results and wrote the manuscript. All authors read and approved the manuscript and agree to be accountable for all aspects of the research in ensuring that the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Ethics approval and consent to participate

The present study was approved by the Research Ethics Committee of The First Affiliated Hospital of Nanjing Medical University, and written informed consent was obtained from all patients prior to enrolment in the study.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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