Screening key IncRNAs for human rectal adenocarcinoma based on IncRNA-mRNA functional synergistic network

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Abstract

Background: Rectal adenocarcinoma (READ) is one of the deadliest malignancies, and the molecular mechanisms underlying the initiation and development of READ remain largely unknown. In this study, we aimed to find key long noncoding RNAs (lncRNAs) and mRNAs in READ by RNA sequencing.

Methods: RNA sequencing was performed to identify differentially expressed mRNAs (DEmRNAs) and lncRNAs (DElncRNAs) between READ and normal tissue. READ-specific protein-protein interaction (PPI), DElncRNA-DEmRNA coexpression, and DElncRNA-nearby DEmRNA interaction networks were constructed. DEmRNAs and DEmRNAs coexpressed with DElncRNAs were functionally annotated.

Results: A total of 2113 DEmRNAs and 150 DElncRNAs between READ and normal tissue were identified. The PPI network identified several hub proteins, including CDK1, AURKB, CDC6, FOXQ1, NUF2, and TOP2A. The DElncRNA-DEmRNA coexpression and DElncRNA-nearby DEmRNA interaction networks identified some hub lncRNAs, including CCAT1, LOC105374879, GAS5, and B3GALT5-AS1. The colorectal cancer pathway, the intestinal immune network for IgA production and the p53 signaling pathway were three pathways significantly enriched in DEmRNAs and DEmRNAs coexpressed with DElncRNAs. MSH6 coexpressed with two DElncRNAs (LOC105374879 and CASC15) and BCL2 coexpressed with B3GALT5-AS1 were significantly enriched in the colorectal cancer signaling pathway. TNFRSF17 coexpressed with B3GALT5-AS1 was enriched in the intestinal immune network for IgA production. CCNB2 coexpressed with LOC105374879 was enriched in the p53 signaling pathway.

Conclusion: A total of four DEmRNAs (MSH6, BCL2, TNFRSF17, and CCNB2) and three DElncRNAs (LOC105374879, CASC15, and B3GALT5-AS1) may be involved in the pathogenesis of READ; this data may contribute to understanding the mechanisms of READ and the development of therapeutic strategies for READ.

KEYWORDS
DElncRNA-DEmRNA coexpression, DElncRNAs, DEmRNAs, rectal adenocarcinoma, RNA sequencing

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1 | INTRODUCTION

Colorectal cancer is one of the most common malignant tumors causing cancer-related deaths and has one of the highest incidence rates among all types of cancer worldwide. Rectal adenocarcinoma (READ) is a common type of colorectal cancer. Although advancements in treatments and the prognosis and diagnosis of READ have been achieved through research, its mortality remains high, which may be due to the lack of efficient biomarkers for READ and the unclear mechanisms underlying READ. Hence, identifying efficient biomarkers and deciphering the detailed molecular mechanisms underlying READ are urgently required.

In the field of gene-gene network analysis, the construction of coexpression networks has opened up enormous possibilities for exploring the role of genes in biological processes. Coexpression analysis of lncRNAs-mRNAs is the most commonly used approach to screen potential target genes of lncRNAs and further research on the biological functions of lncRNAs in many kinds of diseases.

The advent of high-throughput genetic analysis means that a large portion of the genome can be transcribed, resulting in the discovery of the extensive transcription of large RNA transcripts named long noncoding RNAs (lncRNAs). Accumulating numbers of reports of aberrant lncRNA expression have demonstrated that lncRNAs may potentially serve as novel independent biomarkers for the early diagnosis and prognosis of and metastasis prediction in various cancer types. Recently, lncRNA profiling has been performed in several other types of colorectal cancer, which identified novel candidate diagnostic and prognostic biomarkers, such as SNHG6, PVT1, ZFAS1, LINC01555, RP11-610P16.1, RP11, 108K3.1, and LINC01207. However, research on lncRNA biomarkers in READ is rare.

Owing to the limited research linking lncRNAs with READ, this study aimed to further investigate this issue. In this study, RNA sequencing was performed to identify DEmRNAs and DElncRNAs between READ and normal tissue. READ-specific protein-protein interaction (PPI), DElncRNA-DEmRNA coexpression, and DElncRNA-nearby DEmRNA interaction networks were constructed. The functional annotation of DEmRNAs and DEmRNAs coexpressed with DElncRNAs was performed. Our study identified potential key genes and lncRNAs in READ and provides further insights into the mechanisms and predictive capacity of lncRNAs in READ.

2 | MATERIALS AND METHODS

2.1 | Patients

Three patients with READ were enrolled in our study. Three tissue samples and three paired adjacent normal samples were selected from three cases of READ. The tissue samples were biopsy samples obtained from surgery. The detailed characteristics of the patients are displayed in Table 1. All the participants submitted signed informed consent forms, and the protocols were approved by the ethical committee of our hospital.

2.2 | RNA isolation, library construction, and sequencing

Total RNA was extracted from the samples using TRIzol reagent (Invitrogen, Carlsbad, CA). A Nanodrop ND-2000 spectrophotometer (Thermo Scientific, Wilmington, DE) was applied to check the RNA concentration and purity. The integrity of the RNA was detected by agarose gel electrophoresis. The RIN value was obtained by an Agilent 2100 Bioanalyzer. The criteria for cDNA library construction were as follows: (a) total RNA >5 μg; (b) concentration of RNA ≥200 ng/μL; and (3) an OD 260/280 value of 1.8-2.2.

Ribosomal RNA was removed with a Ribo-Zero Magnetic kit (EpiCentre, Madison, WI), and the RNA was purified and fragmented into 200-500-base pair fragments. The RNA fragments were primed with random hexameric primers, and the first cDNA strand was synthesized, with the second cDNA strand synthesized with dUTP instead of dTTP. After purification with AMPure XP Beads (Beckman Coulter, Brea, CA), end repair, adenylation of the 3' ends and adapter ligation were performed. Polymerase chain reaction (PCR) was performed to construct a library for the high-throughput sequencing of lncRNA, and the mRNA from the second cDNA strand was digested using UNG enzyme (Illumina, Inc, San Diego, CA). All libraries used for the high-throughput

| Case 1    | Case 2    | Case 3    |
|-----------|-----------|-----------|
| Age (years) | 83        | 82        | 52        |
| Gender     | Male      | Female    | Male      |
| Diagnostic method | Colonoscopy | Surgery    | Colonoscopy |
| TNM stage  | T3N1M0    | T4N2M1    | T4N2M1    |
| Tumor infiltration | Serosa    | Serosa    | Serosa    |
| Tumor differentiation | Medium-grade | Medium low-grade | Medium low-grade |

| TABLE 1 | Patient characteristics |
### Table 2
The top 20 DEmRNAs and in READ

| ID     | Symbol | log₂FC  | P-value | FDR    | Up/down |
|--------|--------|---------|---------|--------|---------|
| 3854   | KRT6B  | 7.73832 | 5.00E-05| 0.002611 | Up      |
| 342667 | STAC2  | 6.74383 | 5.00E-05| 0.002611 | Up      |
| 282344 | SLCO1B3| 6.69561 | 5.00E-05| 0.002611 | Up      |
| 5655   | KLK10  | 5.91643 | 5.00E-05| 0.002611 | Up      |
| 221416 | C6orf223| 5.08019| 5.00E-05| 0.002611 | Up      |
| 90161  | HS6ST2 | 5.06794 | 5.00E-05| 0.002611 | Up      |
| 1800   | DPEP1  | 5.02634 | 5.00E-05| 0.002611 | Up      |
| 1767   | DNAH5  | 4.95572 | 5.00E-05| 0.002611 | Up      |
| 990    | CDC6   | 4.9442  | 5.00E-05| 0.002611 | Up      |
| 9271   | PIWIL1 | 4.94295 | 5.00E-05| 0.002611 | Up      |
| 55532  | SLC30A10| −4.3053| 5.00E-05| 0.002611 | Down    |
| 229    | ALDOB  | −4.02838| 5.00E-05| 0.002611 | Down    |
| 2346   | FOLH1  | −4.01874| 5.00E-05| 0.002611 | Down    |
| 374569 | ASPG   | −3.97546| 5.00E-05| 0.002611 | Down    |
| 10022  | INSL5  | −3.84557| 5.00E-05| 0.002611 | Down    |
| 5320   | PL2A2G2A| −3.76694| 5.00E-05| 0.002611 | Down    |
| 6689   | SPIB   | −3.67292| 5.00E-05| 0.002611 | Down    |
| 8115   | TCL1A  | −3.46401| 5.00E-05| 0.002611 | Down    |
| 1380   | CR2    | −3.36749| 5.00E-05| 0.002611 | Down    |
| 266675 | BEST4  | −3.34166| 5.00E-05| 0.002611 | Down    |

### Table 3
The top 20 DElncRNAs in READ

| ID     | Symbol     | log₂FC     | P-value | FDR    | Up/down |
|--------|------------|------------|---------|--------|---------|
| 503638 | LINC01296  | 5.37396    | 5.00E-05| 0.002611 | Up      |
| 652995 | UCA1       | 4.39663    | 5.00E-05| 0.002611 | Up      |
| 105369370| LOC105369370| 4.23597   | 5.00E-05| 0.002611 | Up      |
| 102723961| LOC102723961| 3.53756   | 5.00E-05| 0.002611 | Up      |
| 100507056| CCAT1       | 3.34382    | 5.00E-05| 0.002611 | Up      |
| 105374879| LOC105374879| 2.59434   | 5.00E-05| 0.002611 | Up      |
| 407975 | MIR17HG    | 2.18303    | 5.00E-05| 0.002611 | Up      |
| 105370108| LOC105370108| 4.57119   | 0.0001  | 0.004682 | Up      |
| 105376380| LOC105376380| 3.45882   | 0.0002  | 0.007904 | Up      |
| 60674  | GAS5       | 1.20787    | 0.0002  | 0.007904 | Up      |
| 105377567| LOC105377567| −2.77552  | 0.0001  | 0.004682 | Down    |
| 283422 | LINC01559  | −1.28123   | 0.00015 | 0.006442 | Down    |
| 283663 | LINC00926  | −2.10879   | 0.00035 | 0.011778 | Down    |
| 114041 | B3GALT5-AS1| −1.89195   | 0.0004  | 0.012989 | Down    |
| 284185 | LINC00482  | −1.90215   | 0.00055 | 0.016205 | Down    |
|       | LOC101926893| −3.61245  | 0.0006  | 0.017319 | Down    |
|       | LOC1005070616| −2.896    | 0.0007  | 0.019391 | Down    |
| 149837 | LINC00654  | −1.49029   | 0.0007  | 0.019391 | Down    |
| 100289019| SLC25A25-AS1| −1.13489  | 0.0008  | 0.021382 | Down    |
|       | LOC389332  | −1.9268    | 0.00095 | 0.023864 | Down    |
sequencing of lncRNAs and mRNAs were amplified by 15 cycles of PCR. The quality of the library was assessed using the Agilent 2100 Bioanalyzer and ABI StepOnePlus Real-Time PCR System. The sequencing of lncRNAs and mRNAs was performed on an Illumina HiSeq Xten platform (Illumina, San Diego, CA).

2.3 | Quality control of raw sequences and mapping of clean reads

FASTQ sequence data were obtained from the RNA-seq data using Base Calling V 0.11.4 (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/). Low-quality reads, including adaptor sequences, sequences with a quality score <20, and sequences with an N base percentage of the raw reads >10% were removed using Cutadapt V 1.9.1 (https://cutadapt.readthedocs.io/en/stable/) with TopHat (http://tophat.cbcb.umd.edu/) and Ensembl gene annotation. The clean reads were aligned with the human reference genome, Ensembl GRCh38.p7 (ftp://ftp.ncbi.nlm.nih.gov/genomes/Homo_sapiens). The expression of mRNAs and lncRNAs was determined using Cuffquant V 2.2.1.

2.4 | Differential expression analysis of mRNAs and lncRNAs

The mRNAs and lncRNAs were quantified using Cuffquant V 2.2.1. Cuffdiff (http://cufflinks.cbcb.umd.edu/) uses the quantitative results of Cuffquant to compare differences in the expression of each mRNA and lncRNA in READ and normal tissue. mRNAs and lncRNAs with a P-value <0.05 and |log2 fold change| >1 were significantly differentially expressed mRNAs (DEmRNAs) and differentially expressed lncRNAs (DElncRNAs), respectively. A heat map of the DEmRNAs and DElncRNAs in READ was obtained by heatmap.2 (http://127.0.0.1:28428/library/gplots/html/heatmap.2.html).
2.5 | Functional annotation

GeneCodis 3 (http://genecodis.cnb.csic.es/analysis) is an online software tool for functional annotation analysis used to reveal the biological functions related to large lists of genes. Gene Ontology (GO) classification (biological process, cellular component, and molecular function) is a major bioinformatics analysis method for annotating genes. The Kyoto Encyclopedia of Genes and Genomes (KEGG) is a database used to determine the biological systems associated with the output of high-throughput experimental technologies. GO classification and KEGG pathway enrichment analyses were performed using GeneCodis 3. An false discovery rate (FDR) <0.05 was used to indicate statistical significance.

2.6 | PPI network construction

The top 100 upregulated or downregulated DEmRNAs in READ were used to build a PPI network using Biological General Repository for Interaction Datasets (BioGRID) (http://thebiogrid.org/) and Cytoscape 3.5.0 (http://www.cytoscape.org/). We used nodes to represent proteins and edges to represent the interactions between two proteins.

2.7 | DEmRNA-DElncRNA interaction analysis

To identify DEmRNAs near DElncRNAs with cis-regulatory effects, DEmRNAs transcribed within a 100 kb window up- or downstream of DElncRNAs in READ and normal controls
were identified. In addition, DEmRNAs coexpressed with DElncRNAs were identified. Pairwise Pearson correlation coefficients between DEmRNAs and DElncRNAs were calculated. DElncRNA-DEmRNA pairs with \( P < 0.001 \) and \( |r| \geq 0.98 \) were defined as significant mRNA-lncRNA coexpression pairs.

3 | RESULTS

3.1 | DEmRNAs and DElncRNAs in READ

The raw data has been uploaded to Gene Expression Omnibus (GEO) (GSE128969, https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE128969). A total of 2113 DEmRNAs (809 downregulated and 1304 upregulated mRNAs) and 150 DElncRNAs (81 downregulated and 69 upregulated lncRNAs) between READ and normal tissue were identified with an FDR < 0.05 and \( |\log_{2}\text{fold change}| > 1 \). The top 20 most significant DEmRNAs and DElncRNAs are displayed in Tables 2 and 3, respectively.

Heatmaps of the top 100 DEmRNAs and all of DElncRNAs between READ and normal tissue are shown in Figure 1A,B, respectively. Circos plots representing the distribution of DElncRNAs and DEmRNAs on chromosomes are shown in Figure 1C.

3.2 | Functional annotation of DEmRNAs in READ

DEmRNAs were used for GO and KEGG enrichment analyses. GO enrichment analysis showed that the DEmRNAs were significantly enriched in the mitotic cell cycle (FDR = 3.62E-38), cell division (FDR = 4.10E-28), cytoplasm (FDR = 1.70E-75), nucleus (FDR = 5.13E-75), protein binding (FDR = 2.82E-72), and ATP binding (FDR = 4.42E-51) terms. The top 15 GO terms for the DEmRNAs in READ are displayed in Figure 2A-C. KEGG pathway enrichment analysis revealed that the p53 signaling pathway (FDR = 2.05E-08), intestinal immune network for
IgA production (FDR = 9.91E-04), and colorectal cancer (FDR = 3.49E-03) pathway were three significantly enriched pathways in READ. The top 15 most significantly enriched KEGG pathways for the DEmRNAs in READ are shown in Figure 2D.

3.3 | READ-specific PPI network construction

A PPI network of the top 100 up- and downregulated DEmRNAs consisted of 464 nodes and 591 edges (Figure
3. CDK1 (degree = 67), AURKB (degree = 34), CDC6 (degree = 20), FOXQ1 (degree = 20), NUF2 (degree = 19), and TOP2A (degree = 18) were considered hub proteins.

3.4 | DElncRNA-DEmRNA coexpression network

A total of 5122 DElncRNA-DEmRNA coexpression pairs including 150 DElncRNAs and 2110 DEmRNAs were identified with an absolute value of the Pearson correlation coefficient $|r| \geq 0.98$ and a $P$-value $<0.001$. We obtained a total of 3293 lncRNA-mRNA pairs that were positively coexpressed and 1829 lncRNA-mRNA pairs that were negatively coexpressed. The positively coexpressed DElncRNA-DEmRNA network (Figure 4) consisted of 1364 nodes and 3293 edges, and its hub lncRNAs were CCAT1 (degree = 87), LOC105374879 (degree = 164), MIR17HG (degree = 72), UCA1 (degree = 35), and B3GALT5-AS1 (degree = 141).

The negatively coexpressed DElncRNA-DEmRNA network (Figure 5) consisted of 1049 nodes and 1829 edges, and its hub lncRNAs were LOC105374879 (degree = 33), LINC00482 (degree = 42), B3GALT5-AS1 (degree = 31), and MIR17HG (degree = 55).

3.5 | Functional annotation of DEmRNAs coexpressed with DElncRNAs

According to the GO enrichment analysis of DEmRNAs with an FDR < 0.05, the mitotic cell cycle (FDR = 8.66E-21), DNA replication (FDR = 8.36E-19), nucleus (FDR = 5.30E-60), cytoplasm (FDR = 6.70E-53), protein binding (FDR = 2.47E-50), and ATP binding (FDR = 5.58E-41) terms were the most significantly enriched GO terms. The top 15 GO terms of the DEmRNAs in READ are displayed in Figure 6A-C. After KEGG pathway enrichment analysis (FDR < 0.05), we found that the cell cycle (FDR = 1.36E-12), purine metabolism (FDR = 2.74E-12), and DNA replication (FDR = 5.16E-12) pathways were the three most significantly enriched pathways in READ. The top 15 most significantly enriched KEGG pathways for DEmRNAs in READ are shown in Figure 6D. The p53 signaling pathway (FDR = 0.0023), intestinal immune network for IgA production (FDR = 0.0084), and colorectal

**Figure 5** Negatively coexpressed DElncRNA-DEmRNA network. Ellipses and inverted triangles represent DEmRNAs and DElncRNAs, respectively. Red and blue colors represent up- and downregulation, respectively. The black border indicates the top 10 up- and downregulated DElncRNAs and DEmRNAs.
cancer pathway (FDR = 0.0014) were three READ-related pathways. The p53 signaling pathway, intestinal immune network for IgA production and colorectal cancer pathway are displayed in Figure 7.

3.6 | DElncRNA-nearby DEmRNA interaction network

The functions of most lncRNAs remain unknown. We hypothesized that lncRNAs may exert their functions by regulating nearby genes. A total of 75 DElncRNA-nearby target DEmRNA pairs were obtained that consisted of 54 DElncRNAs and 69 DEmRNAs (Figure 8A). Ten DElncRNAs with the closest DEmRNAs were CCAT1, LOC102723961, LOC105369370, MIR17HG, UCA1, GAS5, LINC00926, B3GALT5-AS1, and LINC00482, which were nearby 1, 2, 1, 2, 1, 1, 1, 1, and 2 DEmRNAs, respectively. The DElncRNA-nearby DEmRNA pairs in which the DEmRNA was coexpressed with the DElncRNA are displayed in Table 4. After looking for overlaps in the DElncRNA-DEmRNA coexpression network and the DElncRNAs-nearby DEmRNAs interaction network, we obtained a total of five lncRNA-mRNA pairs including five DElncRNAs and five DEmRNAs (Figure 8B). Among these, LOC105369370 was within the top 10 DElncRNAs. Moreover, MYEOV was not only an DEmRNA nearby LOC105369370 but was also coexpressed with LOC105369370.

4 | DISCUSSION

READ is one of the deadliest malignancies, and the molecular mechanisms underlying the initiation and development of READ remain largely unknown. Hence, comprehensive detailing of its mechanisms is critical. An increasing number of studies have explored the important regulatory effects of lncRNAs on tumor formation and metastasis. Here, DEmRNAs and DElncRNAs in READ were studied using RNA sequencing. A total of 2113 DEmRNAs (809 downregulated and 1304 upregulated mRNAs) and 150 DElncRNAs (81 downregulated and 69 upregulated lncRNAs) between READ and normal tissue were identified. Additionally, we constructed a READ-specific PPI network, a DElncRNA-DEmRNA coexpression network and a DElncRNA-nearby DEmRNA interaction network. In addition, DEmRNAs and DEmRNAs coexpressed with DElncRNAs were functionally annotated.

Coexpression networks have been used in other studies to identify important modules associated with cancer and the
FIGURE 7  READ pathways (p53 signaling pathway, intestinal immune network for IgA production, and colorectal cancer pathway) enriched in DEmRNAs during READ. The red and green rectangles represent components regulated by DEmRNAs that are enriched in READ.
functions of the lncRNAs involved within them. Herein, construction of the DElncRNA-nearby DEmRNA interaction network showed that the top ten DElncRNAs with the closest DEmRNAs were CCAT1, LOC102723961, LOC105369370, LOC105374879, MIR17HG, UCA1, GAS5, LINC00926, B3GALT5-AS1, and LINC00482. To our knowledge, besides CCAT1, MIR17HG, UCA1, and GAS5, three upregulated DElncRNAs (LOC102723961, LOC105369370, and LOC105374879) and three downregulated DElncRNAs (LINC00926, B3GALT5-AS1, and LINC00482) in READ have been reported for the first time, and their biological functions remain unclear.

Most network construction techniques can only address positive correlations in gene expression data, whereas biologically significant genes exhibit both positive and negative correlations. In this study, positively correlated DEmRNAs and DElncRNAs in READ were defined as positively coexpressed DElncRNA-DEmRNA pairs, and negatively correlated DEmRNAs and DElncRNAs were defined as negatively coexpressed DElncRNA-DEmRNA pairs. CDK1, AURKB, CDC6, FOXQ1, NUF2, and TOP2A were the hub proteins of the READ-specific PPI network. CDK1, a member of the CDKs, is a serine/threonine kinase that promotes the G2-M transition and regulates G1 progression and G1-S transition. CDK1 is overexpressed in human colorectal cancers and relevant to the clinical behavior of human colorectal cancers, which was shown by the association between a high ratio of CDK1 nuclear to cytoplasmic expression and poor overall survival and that CDK1 was an independent risk factor for outcome. AURKB, a member of the aurora kinase family, is an important diagnostic and prognostic marker involved in the carcinogenesis of colorectal cancers. FOXQ1 is frequently upregulated in colorectal cancers, and FOXQ1 knockdown suppressed cell proliferation and the migration and invasion of colorectal cancers. TOP2A is a potential predictive biomarker for anthracycline and irinotecan treatment in colorectal cancer, and high frequency of gene gains for the TOP1 and TOP2A genes were reported in colorectal cancer.
Elevated NUF2 expression was associated with poor prognosis in colorectal cancer, and the knockdown of NUF2 expression suppressed the growth of tumor cells. Therefore, we speculated that CDK1, AURKB, FOXQ1, NUF2, and TOP2A might play important roles in READ. Interaction network analysis showed that AURKB was co-expressed with SNHG5 and that FOXQ1 was co-expressed with LOC105374879. Hence, we further hypothesized that SNHG5 and LOC105374879 might play important roles in READ by regulating AURKB and FOXQ1, respectively.

CCAT1 is upregulated in colorectal cancer but not in normal tissue. A CCAT1-specific peptide nucleic acid-based molecular beacon was reported to serve as a powerful diagnostic tool for the specific identification of colorectal cancer. GAS5 is associated with not only susceptibility to colorectal cancer but also the metastasis of colorectal cancer to the lymph node. SLCO1B3, a solute carrier organic anion transporter family member, is upregulated in colorectal cancer. The overexpression of SLCO1B3 changed p53-dependent pathways and conferred apoptotic resistance in colorectal cancer. SLCO1B3 protein expression was significantly correlated with proximal tumor location and the expression of mismatch repair genes, and SLCO1B3 was identified as a cell-surface marker differentially expressed in colon adenocarcinoma relative to its expression in the surrounding normal colon tissue. In this study, SLCO1B3 was coexpressed with CCAT1 and GAS5. Therefore, we presumed that both CCAT1 and GAS5 might be involved in the development of READ by regulating SLCO1B3.

According to KEGG pathway enrichment analysis of DEmRNAs and DElncRNAs coexpressed with DElncRNAs, the p53 signaling pathway, intestinal immune network for IgA production and colorectal cancer pathway were three READ-related pathways. MSH6 coexpressed with two DElncRNAs (LOC105374879 and CASC15) and BCL2 coexpressed with B3GALT5-AS1 were significantly enriched in the colorectal cancer signaling pathway. TNFRSF17 coexpressed with B3GALT5-AS1 was enriched in the intestinal immune network for IgA production. CCNB2 coexpressed with LOC105374879 was enriched in the p53 signaling pathway. MSH6 is a mismatch repair gene involved in colorectal cancers, and it was reported that most patients with colorectal
| Chr | lncRNA | Symbol        | Start − 100kb | End + 100kb | Chr | mRNA | Symbol | Start | End  |
|-----|--------|---------------|---------------|-------------|-----|------|--------|-------|------|
| chr8 | CCAT1  | 127107381     | 127319268     |             |      |      |        |       |      |
| chr17| LOC102723961 | 79715942 | 79923284   |             |      |      |        |       |      |
| chr17| LOC102723961 | 79715942 | 79923284 |             |      |      |        |       |      |
| chr11| LOC105369370 | 69266824 | 69472512 |             |      |      |        |       |      |
| chr6 | LOC105374879 | 1184930  | 1391486   |             |      |      |        |       |      |
| chr6 | LOC105374879 | 1184930  | 1391486 |             |      |      |        |       |      |
| chr13| MIR17HG | 91247819 | 91454575 |             |      |      |        |       |      |
| chr19| UCA1   | 15727044      | 15936732     |             |      |      |        |       |      |
| chr1  | GAS5   | 173763247     | 173967987     |             |      |      |        |       |      |
| chr11| SNHG1  | 62751987      | 62955888      |             |      |      |        |       |      |
| chr15| LINC00926 | 57200364 | 57407769 |             |      |      |        |       |      |
| chr21| B3GALT5-AS1 | 39497146 | 39712822 |             |      |      |        |       |      |
| chr17| LINC00482 | 81208223 | 81409248 | LOC100130370 | 81375496 | 81392947 |      |       |      |
| chr7 | SNHG15 | 44883027      | 45086660      |             |      |      |        |       |      |
| chr8 | LOC105375752 | 127040057 | 127269518 |             |      |      |        |       |      |
| chr8 | LOC105375752 | 127040057 | 127269518 |             |      |      |        |       |      |
| chr8 | PVT1   | 127694532     | 128201253     |             |      |      |        |       |      |
| chr17| SNHG16 | 76457763      | 76665348      |             |      |      |        |       |      |
| chr7 | LOC105375431 | 100842058 | 101069565 |             |      |      |        |       |      |
| chr7 | LOC105375431 | 100842058 | 101069565 |             |      |      |        |       |      |
| chr7 | LOC105375431 | 100842058 | 101069565 |             |      |      |        |       |      |
| chr6 | SNHG5  | 85577006      | 85778733      |             |      |      |        |       |      |
| chr19 | LOC400706 | 45957677 | 46177629 |             |      |      |        |       |      |
| chr1  | BLACAT1 | 205273251     | 20556068      |             |      |      |        |       |      |
| chr6 | CASC15 | 21566443      | 22294400      |             |      |      |        |       |      |
| chr4 | DANCOR | 52612149      | 52823436      |             |      |      |        |       |      |
| chr17 | MAFG-AS1 | 81827828 | 82030753 |             |      |      |        |       |      |
| chr17 | MAFG-AS1 | 81827828 | 82030753 | MYADML2 | 81939644 | 81947233 |      |       |      |
| chr17 | MAFG-AS1 | 81827828 | 82030753 | PYCR1  | 81932383 | 81937328 |      |       |      |
| chr17 | MAFG-AS1 | 81827828 | 82030753 | NOTUM  | 81952506 | 81961181 |      |       |      |
| chr12 | LOC105369827 | 70368087 | 70616501 | KCNMB4 | 70366219 | 70434292 |      |       |      |
| chr19 | LOC101927522 | 35305606 | 35534730 | FFAR2  | 35447964 | 35451767 |      |       |      |
| chr19 | LOC101927522 | 35305606 | 35534730 | CD22   | 35329165 | 35347361 |      |       |      |
| chr19 | LOC101927522 | 35305606 | 35534730 | DMKN   | 35497216 | 35513678 |      |       |      |
| chr19 | LOC101927522 | 35305606 | 35534730 | TMEM147 | 35533337 | 35547527 |      |       |      |
| chr1  | LOC105378625 | 31348257 | 31549586 | SERINC2 | 31409564 | 31434680 |      |       |      |
| chr16 | LOC105371100 | 16049564 | 16328616 | ABCCC6 | 16149564 | 16223616 |      |       |      |
| chr3  | LOC101928405 | 165050005 | 165258164 | SI     | 164978897 | 165083824 |      |       |      |
| chr17 | LOC105371919 | 79723794 | 79927704 | CBX2   | 79776253 | 79787650 |      |       |      |
| chr17 | LOC105371919 | 79723794 | 79927704 | CBX8   | 79794376 | 79797116 |      |       |      |
| chr1  | LOC105378687 | 43254683 | 43458673 | CDC20  | 43358954 | 43363203 |      |       |      |

(Continues)
cancer carrying an MSH6 mutation were diagnosed after the age of 50 and had distally localized tumors. TNFRSF17 may be a candidate gene associated with the pathogenesis of colon cancer, and the haplotypes of TNFRSF17 polymorphisms might be markers for colon cancer susceptibility.\(^2^8\) BCL2 is a well-known protein that prevents apoptosis in many kinds of tumors and is routinely assayed as a diagnostic marker in the clinical practice of pathology. Very recent studies found that BCL2 was downregulated in early-stage colon adenocarcinoma and that BCL2 was involved in the metastasis of colon adenocarcinoma to the lymph nodes.\(^2^9^,3^0\) In our study, BCL2 was reduced in READ, which indicated that BCL2 might regulate READ as well. Therefore, we hypothesized that LOC105374789, CASC15, and B3GALT5-AS1 might play pivotal roles in regulating the colorectal cancer signaling pathway, the intestinal immune network for IgA production and the p53 signaling pathway.

In summary, we identified 2113 DEmRNAs and 150 DElncRNAs in READ compared to their expression in normal tissues. The PPI network identified several hub proteins including CDK1, AURKB, CDC6, FOXQ1, NUF2, and TOP2A. DElncRNA-DEmRNA coexpression and DElncRNA-nearby DEmRNA interaction networks were constructed to identify hub IncRNAs, including CCAT1,
LOC105374879, GAS5, and B3GALT5-AS1. The colorectal cancer pathway, intestinal immune network for IgA production, and p53 signaling pathway were three significantly enriched pathways for DEMRNAs and DEMRNAs coexpressed with DElncRNAs. MSH6 coexpressed with two DElncRNAs (LOC105374879 and CASC15) and BCL2 coexpressed with B3GALT5-AS1 were significantly enriched in the colorectal cancer signaling pathway of. TNFRSF17 coexpressed with B3GALT5-AS1 was enriched in the intestinal immune network for IgA production. CCNB2 coexpressed with LOC105374879 was enriched in the p53 signaling pathway. Our results warrant further studies on these mRNAs and lncRNAs to improve our understanding of the mechanisms associated with the pathogenesis and progression of READ. However, there are limitations to our study. First, the sample size for RNA sequencing was small, and large numbers of READ samples are needed for further research. Second, DEMRNAs and DElncRNAs in READ were identified, but their biological functions were not studied. Therefore, in vivo and in vitro experiments are necessary to elucidate the biological roles of DEMRNAs and DElncRNAs in READ in future work.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflict of interest. No competing financial interests exist.

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DATA AVAILABILITY STATEMENT

The dataset supporting the conclusions of this article is included within the article.

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