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Overexpression of Long Non-Coding RNA, LINC01748, Predicts Extracellular Matrix Remodeling in Colorectal Cancer Through Let-7b-5p/CTHRC1 Axis

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Colorectal cancer (CRC) is a leading cause of cancer-related deaths. Long non-coding RNAs are emerging as key regulators in cancer by modulating functions of microRNAs thereby affecting expression of genes involved in the pathogenesis of cancers. Since the CTHRC1 gene (Collagen Triple-Helix-Repeat Containing-1) encodes an extracellular matrix protein involved in collagen regulation and cell migration, this study investigated the role of LINC01748 in CRC and its interaction with the Let-7b-5p/CTHRC1 axis. RNA sequencing data from The Cancer Genome Atlas database was analyzed to identify differentially expressed lncRNAs, miRNAs, and mRNAs in CRC. Interactions between LINC01748, miRNAs, and mRNAs were predicted, a protein-protein interaction network was constructed, and functional enrichment analysis was performed to reveal the biological activity of target genes. Additionally, the expression of LINC01748, Let-7b-5p, and CTHRC1 in CRC tissues and adjacent non-cancerous tissues were determined using RT-qPCR and western blotting. Collagen density was also evaluated by histopathological examination. 107 differentially expressed lncRNAs, 313 miRNAs, and 1,479 mRNAs were identified in CRC samples. Bioinformatics analysis indicated that LINC01748 may function as a competing endogenous-RNA for Let-7b-5p, regulating CTHRC1 expression. RT-qPCR confirmed upregulation of LINC01748 and CTHRC1 and downregulation of Let-7b-5p in CRC. Significant positive correlation of LINC01748 with CTHRC1, and a negative correlation with Let-7b-5p were detected. Gene Ontology and KEGG pathway analysis suggested the involvement of LINC01748/Let-7b-5p/CTHRC1 axis in ECM organization and collagen content. This study revealed the role of LINC01748 in the Let-7b-5p/CTHRC1 axis, highlighting its clinical significance in CRC.

Key Words: *Colorectal Neoplasms; CTHRC1 Protein, Human; RNA, Long Noncoding; MicroRNAs; Extracellular Matrix*

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INTRODUCTION

Colorectal cancer (CRC) is the third most common cancer and the second leading cause of cancer-related deaths worldwide. It accounts for approximately 10% of all cancer diagnoses and is responsible for 9% of cancer fatalities, which remains the leading cause of cancer mortality.[1] Multiple factors, including environmental, genetic, and epigenetic influences, may contribute to the development of CRC.[2] Despite significant advancements in chemotherapy, surgical techniques, and the availability of vari-

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ous targeted therapies and immunotherapies, the survival rate and prognosis for patients with CRC has remained suboptimal in recent years.[3] This persistent challenge can be attributed to an insufficient understanding of the mechanisms underlying tumorigenesis.[4] Consequently, it is essential to elucidate the molecular mechanisms of carcinogenesis to identify novel biomarkers, facilitate effective diagnoses, and establish viable therapeutic targets.

A large-scale genome sequencing has reported that only 1-2% of the human genome is related to protein-coding genes, and more than 90% of DNA is not translated into protein and is classified as non-coding RNAs.[5] Recent studies have shown that non-coding RNAs (ncRNAs) are regulatory molecules in cellular processes such as transcription, post-transcriptional changes, and chromatin remodeling.[6,7] As a result, nRNAs act as important regulators in the pathophysiology of diseases. In cancers, depending on their type, nRNAs have been identified as tumorigenic factors or tumor suppressors.[8,9] According to the competing endogenous-RNA (ceRNA) hypothesis, members of the ceRNA network, such as circular RNAs, Long non-coding RNAs (lncRNAs), mRNAs (mRNAs), and pseudogenes, interact competitively for shared microRNA response elements (MREs), thereby modulating one another's expression at the post-transcriptional level.[10] This molecular interaction can significantly influence a diverse array of biological processes, with many cancers arising from an imbalanced ceRNA network.[11] Recent studies suggested that numerous dysregulated RNAs in CRC may act as ceRNAs, thereby modulating various biological functions associated with tumorigenesis.[12] Consequently, the exploration of non-codingRNA dysregulation in the context of the ceRNA network could provide valuable insights into the mechanisms driving the development of CRC.

Long non-coding RNAs (lncRNAs), characterized by their length exceeding 200 nucleotides and lacking of coding capability, are significant regulatory molecules in cellular processes. By functioning as sponges for microRNAs (miRNAs), lncRNAs inhibit miRNA activity, thus modulating the expression of various target genes.[13] Increasing evidence illustrates that lncRNAs play crucial roles in biological functions such as cell growth, metabolism, migration, and angiogenesis.[14] Dysregulation of these lncRNAs has been linked to numerous diseases, particularly cancer, indicating their potential applications in diagnosis, prognosis, and targeted therapies.[15] For instance, the lncRNA-LINC2418 functions as a sponge for miR-34b-5p, thereby promoting CRC progression through the upregulation of BCL2-associated proteins.[16] In contrast, several studies have highlighted the tumor-suppressive roles of certain lncRNAs; specifically, lncRNA LINC01133 which exerts a tumor-suppressive effect in CRC by sequestering miR-186-5p.[17] Despite previous studies, the lncRNAs involved in CRC progression have not been sufficiently characterized. The potential roles and regulatory mechanisms of uncharacterized lncRNAs in CRC warrant further investigation, as they may represent emerging contributors to CRC carcinogenesis.

In this study we utilized RNA sequencing data from The Cancer Genome Atlas (TCGA) to identify novel lncRNAs associated with CRC, highlighting LINC01748 as a key regulatory molecule. Bioinformatics analyses, including miRNA sequencing and multiMiR, indicated that LINC01748 functions as a competing endogenous-RNA (ceRNA) for Let-7b-5p and CTHRC1. The CTHRC1 gene (collagen triple helix repeat containing 1) encodes a 30-kDa glycosylated extracellular matrix protein, and was initially identified in arteries subjected to balloon injury. This protein plays a crucial role in collagen regulation and vascular remodeling, promoting cell migration.[18] Evidence suggests that CTHRC1 induces smooth muscle cell dedifferentiation by suppressing transforming growth factor-beta (TGF-β). Additionally, CTHRC1 activates the Wnt/Planar Cell Polarity (PCP) signaling pathway, contributing to tissue remodeling and cellular responses relevant to vascular repair and pathological vascular remodeling.[19]

Based on these interactions, it is hypothesized that elevated LINC01748 expression in CRC reduces tumor-suppressor miRNAs like let-7b-5p, alleviating their repression of CTHRC1, thereby increasing its mRNA and protein levels. Therefore, this research experimentally evaluated the LINC01748/has-Let-7b-5p/CTHRC1 axis to confirm the predictions of our bioinformatics analysis and its potential role in CRC progression.

MATERIALS AND METHODS

1. Data preprocessing for RNA sequencing

In bioinformatics analysis, miRNA sequencing (miRNA-Seq), RNA sequencing (RNA-Seq), and clinical data relevant to colorectal cancer (CRC) were acquired from the GDC data portal of the TCGA database (https://portal.gdc.cancer.gov/), specifically under project ID: TCGA-COAD. The initial dataset featured 481 tumor samples and 41 adjacent tissue samples concerning lncRNAs and mRNAs, and 455 tumor samples with 8 adjacent tissue samples for miRNAs. Following the exclusion of duplicate and other non-relevant samples, the available number of RNA-Seq samples was adjusted to 458 tumor and 41 adjacent tissue samples, while miRNA-Seq was comprised of 444 tumor samples and 8 adjacent tissue samples.

2. Human tissue samples

In laboratory, we experiment 20 pairs of colorectal cancer (CRC) tissues and their relevant adjacent non-cancerous samples that were obtained from the Iran National Tumor Bank (Cancer Institute of Tehran University of Medical Sciences, Tehran-Iran). All tumor and corresponding adjacent tissues were validated by a pathologist as being colorectal cancer or non-cancerous tissues, respectively. Exclusion criteria encompassed patients with prior chemotherapy or radiotherapy, those exhibiting tumors in other regions, and patients with tumor recurrence. The non-cancerous samples were dissected at least 5 cm away from the

margin of their counterpart tumor tissues, promptly immersed in liquid nitrogen and stored at −70℃ until further analysis. The research adhered to the principles outlined in the Declaration of Helsinki [20]and was approved by the Ethics Committee of Hamadan University of Medical Sciences, Iran (Ethical code: IR.UMSHA.REC.1403.510) for experiments involving human samples.

Since all tumor tissue samples and marginal adjacent non-cancerous samples were purchased from the Iran National Tumor Bank (Cancer Institute of Tehran University of Medical Sciences, Tehran-Iran), the Cancer Institute collects informed consent forms from every human patient upon admission. Therefore, all informed consent forms are available to the cancer institute.

3. Analysis of COAD data to obtain differentially expressed genes

Normalization was first employed to standardize the COAD dataset. This process utilized the GDCRNAtools package[21] in the R programming language (version 4.3.2), applying the TMM[22] and Voom normalization techniques.[23] Following normalization, differentially expressed mRNAs (DEmRNAs), microRNAs (DEmiRNAs), and long non-coding RNAs (DElncRNAs) between primary tumor tissues and normal tissues were identified using the DESeq2 package–version 1.42.1.[24] The identification of DElncRNAs, DEmiRNAs, and DEmRNAs adhered to two criteria: a $|\log2\text{foldchange}| \geq 1.5$ and a false discovery rate (FDR) <0.01.

4. Prediction of interactions between lncRNAs and miRNAs (MREs)

The online DIANA-LncBase v3 platform (https://diana.e-ce.uth.gr/lncbasev3) was employed to explore interactions between lncRNAs and miRNAs. This resource is able to forecast potential miRNA response element (MRE) locations for any given lncRNA. Consequently, an intersection was established between differentially expressed miRNAs (DEmiRNAs) sourced from the TCGA database and the miRNAs predicted by DIANA-LncBase, facilitating the identification of target miRNA(s) associated with each lncRNA.

5. Prediction of the interaction between miRNAs and mRNAs

To obtain miRNA targets, we employed an R package known as multiMiR. This package features a web interface (http://multimir.org/) and integrates various miRNA and target databases that offer both predicted and experimentally confirmed miRNA-mRNA interactions. For this study, we utilized the TarBase (http://diana.imis.athena-innovation.gr/) and miRTarBase (http://mirtarbase.cuhk.edu.cn) databases available within the multiMiR package, both of which contain experimentally validated data.

6. Correlation analysis

The GDCRNATools package in R was utilized to calculate the correlations between differentially expressed lncRNAs (DElncRNAs) and their corresponding differentially expressed mRNAs (DEmRNAs). A significance threshold of p-values less than 0.05 was applied for correlation assessment.

7. Construction of the protein-protein interaction (PPI) network

A protein-protein interaction (PPI) network was constructed to include the mRNAs targeted by differentially expressed lncRNAs (DElncRNAs) using the STRING plugin[25] in Cytoscape software.[26] A total of 20 genes were evaluated based on criteria such as co-expression, protein homology, biological pathways, gene co-occurrence, gene neighborhood, and gene fusions.

8. Functional enrichment analysis

The clusterProfiler package[27] in R was utilized to conduct Gene Ontology (GO)[28] enrichment and Kyoto Encyclopedia of Genes and Genomes (KEGG)[29] analyses, highlighting the enriched pathways and functions for mRNAs associated with differentially expressed lncRNAs.

The clusterProfiler package was also used to conduct hypergeometric distribution tests, aiming to identify enriched differentially expressed mRNAs (DEmRNAs) in PPI network through two methods; enrichGO and enrichKEGG. Genes with p-values and q-values (the minimum acceptable false discovery rate) less than 0.05 were considered statistically significant in both GO and KEGG analyses.

9. RNA isolation and RT-qPCR assay

Total RNA was extracted from CRC tumors and adjacent non-cancerous tissues using the KIAZOL reagent (Kiazist Life Sciences, Hamadan-Iran) according to the manufacturer's instructions. Subsequently, cDNA synthesis was performed with a cDNA synthesis kit (Pars Tous, Mashhad-Iran). Reverse transcription of miRNA and U6 was carried out using the first Strand cDNA Synthesis Kit (Yekta Tajhiz, Tehran-Iran) employing the stem-loop method. RT-qPCR was conducted with RealQ Plus 2× Master Mix Green (Ampliqon, Denmark) in a Roche LightCycler 96 System (Roche Holding AG, Switzerland). U6 and ACTB (β-actin) served as internal controls for miRNA and mRNAs, respectively, and fold change in gene expression was calculated using the $2^{-\triangle\triangle Ct}$ slivak method.[30] The sequences of primers used in this study are listed in Table 1. It is worth noting that the primer designed for CTHRC1 target conserved regions shared among all four isoforms of the gene.

10. Western blotting

Total proteins were extracted from CRC and non-cancerous tissue samples using RIPA buffer (DNAbiotech®, Tehran-Iran) and a protease inhibitor from (Kiazist Life Sciences, Hamadan-Iran). The concentration of proteins was quantified using the bicinchoninic acid (BCA) assay.

TABLE 1. List of primers

Gene name	Accession number	Primer sequences	TM	Product size
LINC01748	NR_146508.1	F:5'-GCCCATCACATTATGCCCCAA-3' R: 5'-ACAGATGATCCCAAGTCCCAGA-3'	60	96 bp
Let-7b-5p	MIMAT0000063	F; 5'-GAATGCTGTGAGGTAGTAGG-3' R: 5'-GCGTATCCAGTGCAGGGT-3'	59	72 bp
CTHRC1	NM_138455.4, NM_001256099.2, XM_011516824.3, XM_054359715.1	F: 5'-CAGGTCGGGATGGATTCAAAG-3' R: 5'-TGAACACTGCTTGTAGTTGGG-3'	59	89 bp
ACTB	NM_001101.5	F: 5'-CATGTACGTTGCTATCCAGGC-3' R: 5'-CTCCTTAATGTCACGCACGAT-3'	60	250 bp
U6	NR_004394.1	F: 5'-GCAAGGATGACACGCAAATTC-3' R: 5'-GCGTATCCAGTGCAGGGT-3'	58	79 bp
Let-7b-5p RT	5'-GCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGCAACCAC-3'			
U6 RT	5'-GCGTATCCAGTGCAGGGTCCGTATTCGCACTGGATACGCAAAATATGGA-3			

RT primers were specifically designed to synthesize U6 and Let-7b-5p using the stem-loop method.

For western blot analysis an equal amount of protein from each sample were loaded onto SDS-PAGE and separated based on their molecular weights. Target proteins were then transferred onto a nitrocellulose membrane and a 5% non-fat dry milk was added to block non-specific bindings to the membrane. Primary antibodies targeting CTHRC1 (Santa Cruz, SC-293270) and β-actin (Santa Cruz, SC-517582) were added and samples incubated overnight at 4℃. The procedure was followed by a 1-hour incubation at room temperature with HRP-labeled secondary antibodies (m-IgGκ BP-HRP, Santa Cruz, SC-516102). The resultant protein bands were analyzed by densitometry employing a quantitative luminescence substrate for HRP. Beta-actin (β-actin) was used as a reference control protein for normalization.

11. Picrosirius red staining

To assess collagen density in selected tissue samples, we utilized the picrosirius red staining technique. Initially, 5-μm-thick sections were deparaffinized in 100% xylene and subsequently rehydrated through graded ethanol and water concentrations. The slides were then incubated in 0.1% Sirius Red solution (Sigma-Aldrich) dissolved in saturated picric acid for 60 minutes at room temperature. Following incubation, the excess stain was removed by rinsing with water, and the slides were treated with 0.1 N HCl for 2 minutes and then washed again. To facilitate dehydration, the sections were subjected to ascending ethanol concentrations and xylene before being mounted under coverslips. High-resolution images obtained from the microscope (LABMED Inc., USA) were analyzed using ImageJ software version 1.8.0 to quantitatively assess collagen content.

12. Statistical analysis

Data was analyzed and descriptive-analytical statistical methods were performed using SPSS version 26 and GraphPad Prism version 10.2.1. Qualitative data were described with percentage and frequency, and quantitative data were expressed as mean±SEM. The normality of the data was checked with the Shapiro-Wilk test. Normally distributed data were compared with t-test while the non-parametric Mann-Whitney test was used for data without normal distribution. Pearson and Spearman correlation analysis were conducted to examine the relationships among the expressions of mRNA, miRNA, and lncRNA. The Kaplan-Meier method was utilized to assess the overall survival rates in patients with colorectal cancer. To evaluate the independent prognostic value of CTHRC1 expression, univariate and multivariate Cox proportional hazards regression analyses were also performed, adjusting for key clinicopathological variables including age, gender, tumor stage, and histological grade.

RESULTS

1. Identification of differentially expressed lncRNAs, miRNAs and mRNAs

DESeq2 package for R environment was performed on patients from the TCGA-COAD database to identify DElncRNAs, DEmiRNAs, and DEmRNAs between tumor samples and margin control tissues. Volcano plot analysis revealed that 107 DElncRNAs (91 up- and 16 down-regulated), 313 DEmiRNAs (181 up- and 132 down-regulation) and 1,479 DEmRNAs (694 up- and 785 down-regulated) were identified (Fig. 1). DElncRNAs were initially analyzed based on literature review and filtered to find those that have not been experimentally studied in CRC. These potential new DElncRNAs were selected for further analysis ($|\log_2 FC| \geq 1.5$ and FDR<0.01).

2. Prediction of lncRNA-miRNA and miRNA-mRNA interactions

Following the novelty assessment of DElncRNAs and the identification of downstream miRNAs and mRNAs, our investigation centered on the long non-coding RNA LINC01748. Despite a lack of prior investigations into LINC01748 in colorectal cancer, it has been known to be

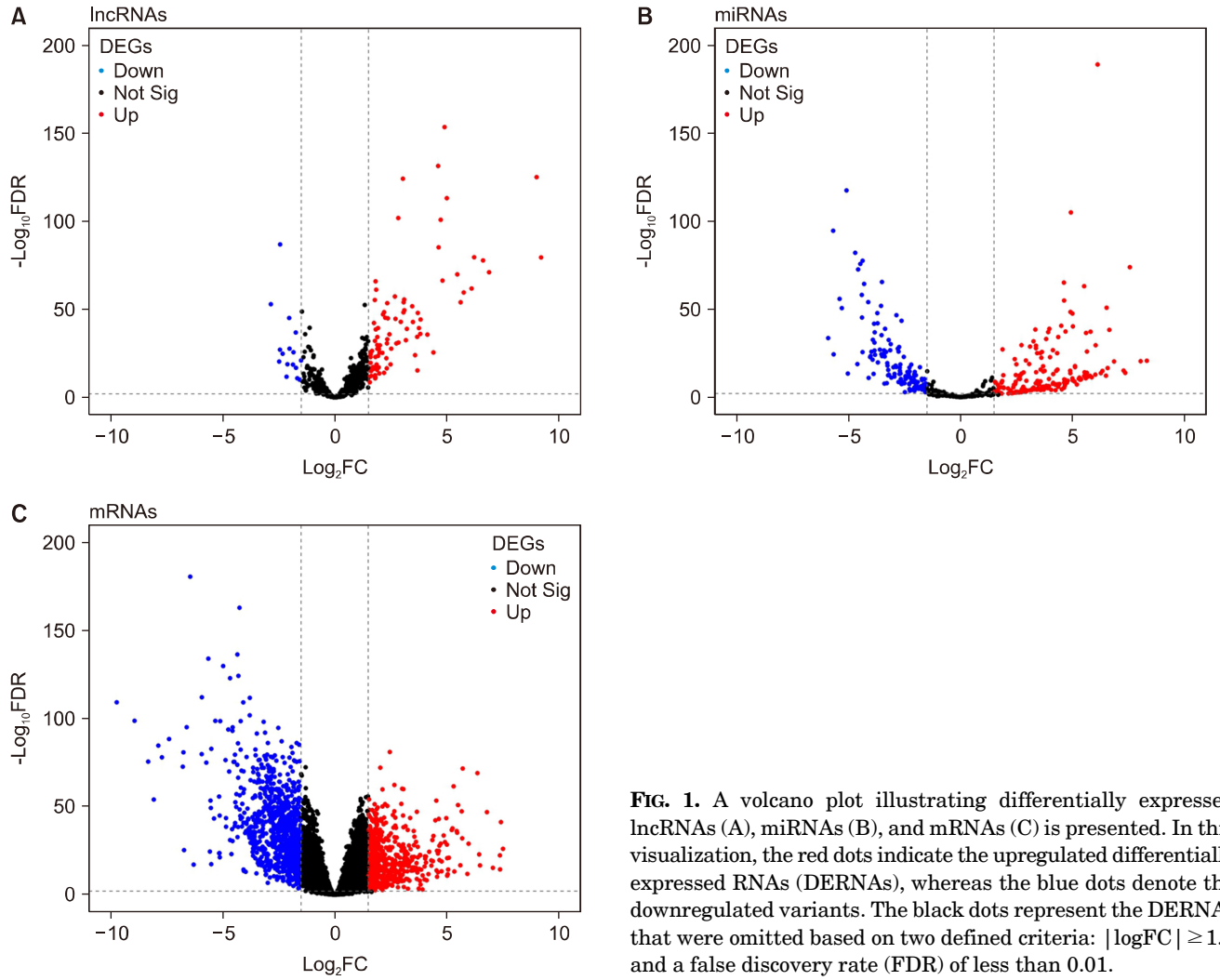

FIG. 1. A volcano plot illustrating differentially expressed lncRNAs (A), miRNAs (B), and mRNAs (C) is presented. In this visualization, the red dots indicate the upregulated differentially expressed RNAs (DERNAs), whereas the blue dots denote the downregulated variants. The black dots represent the DERNAs that were omitted based on two defined criteria: $|\log FC| \geq 1.5$ and a false discovery rate (FDR) of less than 0.01.

linked with significant implications in various other cancer types. According to the competing endogenous RNA (ceRNA) hypothesis, long non-coding RNAs can regulate target mRNA expression by competitively binding to their relevant microRNAs. To identify miRNAs that are associated with up-regulated DElncRNAs, we executed an intersection analysis between the miRNAs predicted by DIANA-LncBase v3 and the DEmiRNAs extracted from the TCGA-COAD dataset. In contrast, to predict miRNAs that correlate with down-regulated DElncRNAs, we intersected the predicted miRNAs from DIANA-LncBase v3 with the up-regulated DEmiRNAs obtained from TCGA. Based on these analyses, the ceRNA network associated with LINC01748 in colorectal cancer was constructed, identifying Let-7a-5p, Let-7b-5p, Let-7c-5p, Let-7e-5p, miR-15b-5p, miR-92a-3p, and miR-181a-5p as the primary miRNA targets of this lncRNA (Fig. 2A).

To select the most relevant miRNA for further study, we compared the differential expression metrics of these seven shared miRNAs in the TCGA-COAD dataset. Among them, Let-7b-5p exhibited the most pronounced down-regulation in tumor tissues, with the greatest magnitude of fold change ($\log_2 FC = -3.55$) and the smallest false discovery rate (FDR=1.88E-52). Based on these stringent statistical criteria, Let-7b-5p was prioritized for downstream analysis.

miRNAs interact with specific sequences within mRNA transcripts, culminating in either mRNA degradation or the inhibition of translation processes. To predict the targets of down-regulated miRNAs, an intersection analysis was performed, utilizing mRNAs sourced from TarBase, miRTarBase, and TCGA-derived up-regulated DEmRNAs via the multimir package in R. Ultimately, 42 mRNAs were identified as potential targets of Let-7b-5p (Fig. 2B). From this list, CTHRC1 was selected as the gene of interest for two key reasons: first, it displayed the highest upregulation ($\log_2 FC$) among the 42 candidates in our TCGA dataset; and second, and crucially, the interaction between Let-7b-5p and the 3'UTR of CTHRC1 has been previously confirmed by dual-luciferase reporter assays in other cancer contexts,[31,32] providing strong prior experimental support for this node within the axis. Therefore, through this stepwise bioinformatic filtering—prioritizing the most dysregulated miRNA and then the most dysregulated tar-

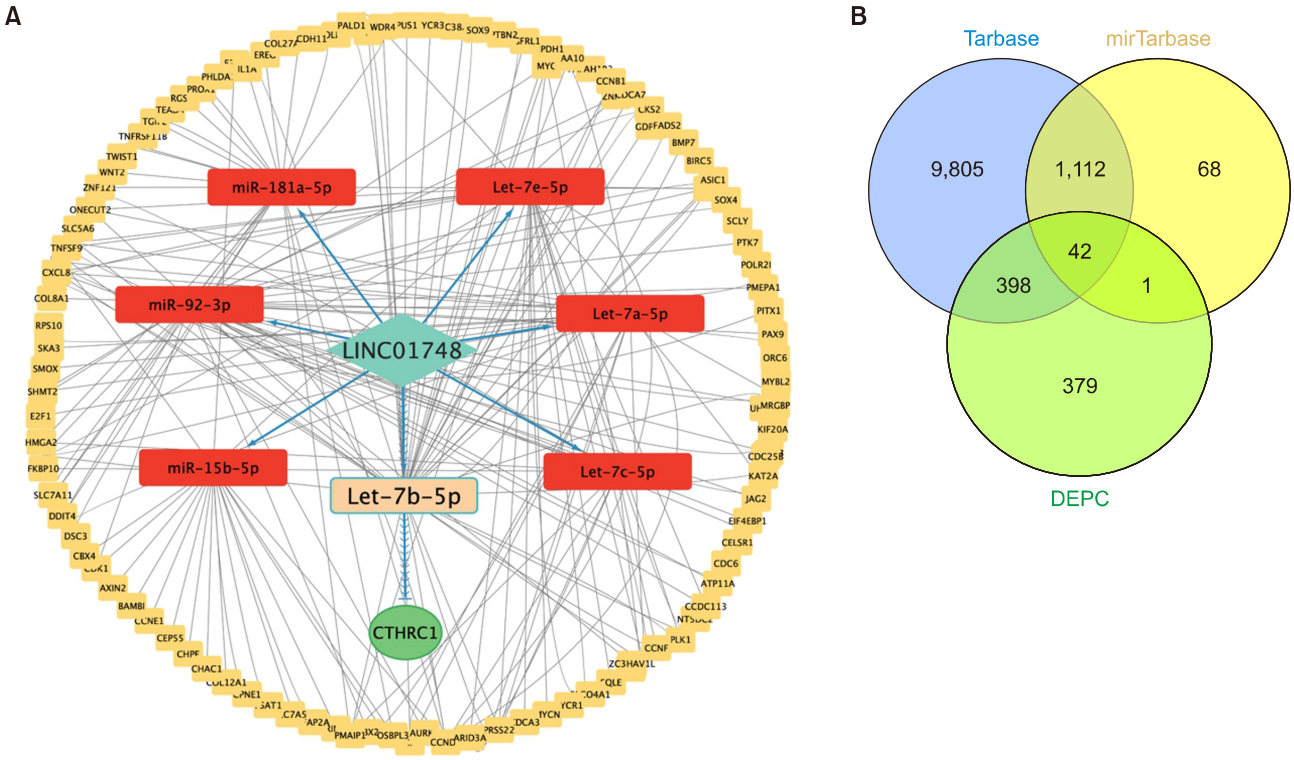

FIG. 2. LINC01748 may regulate its transcription via the let-7b-5p/CTHRC1 axis. (A) The ceRNA network diagram was generated based on the analyses conducted on LINC01748. (B) Integrating data from Tarbase, mirTarbase, and differentially expressed protein-coding genes created a Venn diagram illustrating the predicted target genes of let-7b-5p.

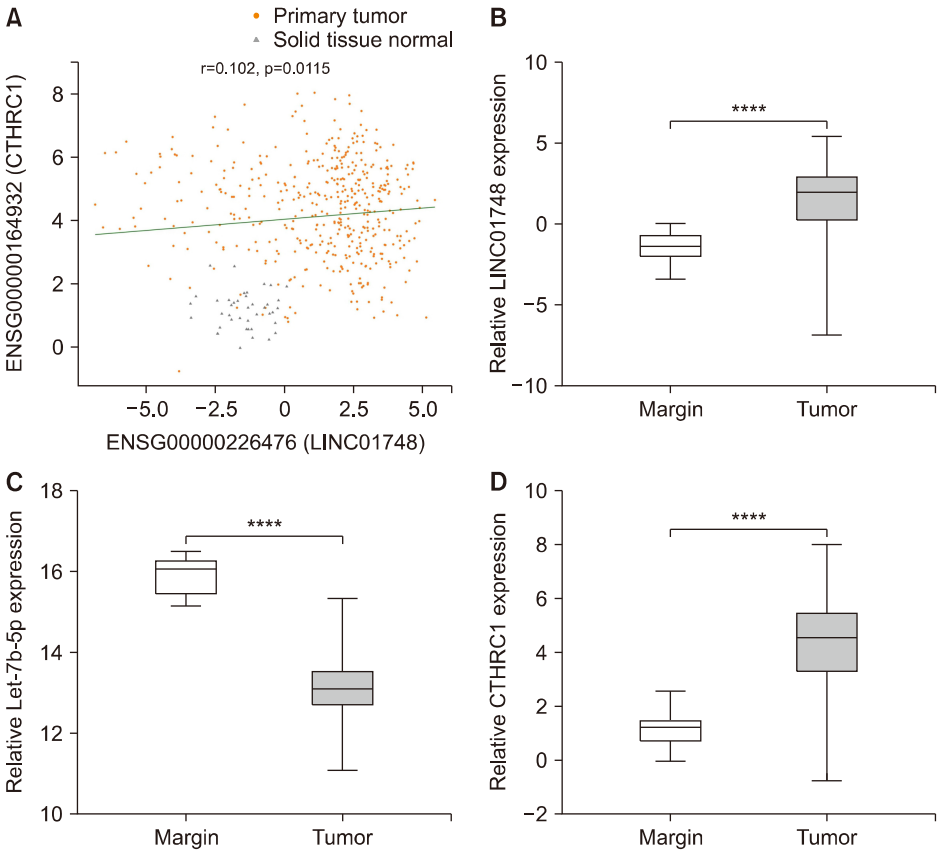

FIG. 3. Correlation and expression analysis of the LINC01748/Let-7b-5p/CTHRC1 axis accordant TCGA database data. (A) A Pearson correlation analysis was employed to investigate the correlation between the expression levels of LINC01748 and CTHRC1. (B) The expression of LINC01748, categorized as an oncogenic lncRNA, was compared between CRC tumor tissues (n= 458) and normal controls (n=41) (FDR= 3.56E-40). (C) The expression profile of Let-7b-5p, a microRNA functioning as a tumor suppressor, was analyzed across CRC tumor samples (n=444) in comparison to normal tissues (n=8) (FDR= 1.88E-52). (D) Moreover, the expression levels of CTHRC1 recognized as an oncogene, were assessed in both CRC tumor tissues and adjacent tissues (FDR= 1.57E-67). All results yielded statistically significant p-values and false discovery rates, confirming the relevance of the observed associations. ****p< 0.0001.

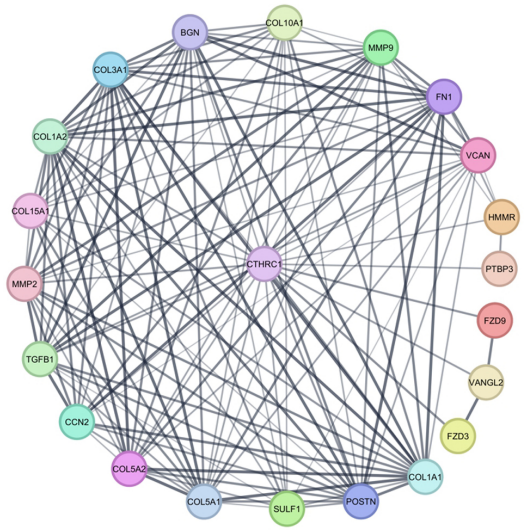

FIG. 4. Protein-protein interaction (PPI) network and functional enrichment of CTHRC1. The CTHRC1 protein-protein interaction network comprises 21 nodes and 119 edges. The edge thickness corresponds to the degree of value and strength of the data sources.

get gene with existing functional validation—we focused our experimental investigation into the LINC01748/Let-7b-5p/CTHRC1 regulatory axis.

3. Correlation analysis between LINC01748 and CTHRC1

The relationship between the expressions of LINC01748 and CTHRC1 was examined by Pearson correlation analysis using R software (Fig. 3A). Additionally, the expression levels of LINC01748, Let-7b-5p, and CTHRC1 derived from TCGA-COAD data were determined, as are presented in Fig. 3B-D. This analysis based on TCGA data indicated that LINC01748 and CTHRC1 exhibiting elevated expression levels in tumor tissue samples, whereas Let-7b-5p showed reduced expression in these samples compared to adjacent non-cancerous tissues.

4. Protein-protein interaction analysis of CTHRC1

The STRING Cytoscape program was used to generate a PPI network for CTHRC1 (Fig. 4). In the construction of the complex PPI network, the line thickness of each edge indicates the strength of data support, and each node represents all of the proteins produced by a single protein-coding gene locus. The network members (nodes) were connected through various interactions including protein homology, biological pathways, gene co-occurrence, gene co-expression, gene neighborhood, and gene fusion. The PPI network showed that CTHRC1 is associated with proteins that organizing extracellular matrix, and therefore confirmed the role of this protein in the progression of colorectal cancer (PPI enrichment p-value: <1.0e-16).

5. Functional enrichment analysis of PPI network

GO and KEGG enrichment analyses were performed to investigate the functional attributes of the CTHRC1 PPI network. GO analysis revealed that genes within the network are significantly associated with biological processes (BP) related to the organization of extracellular matrix, extracellular structure, and external encapsulating structure (Fig. 5A). At the cellular component (CC) level, the genes are enriched in locations including the collagen-containing extracellular matrix, endoplasmic reticulum lumen, and collagen trimer (Fig. 5B). In terms of molecular function (MF), the network genes are significantly enriched for activities such as extracellular matrix structural constituent, glycosaminoglycan binding, and conferring tensile strength to the extracellular matrix (Fig. 5C). KEGG pathway analysis indicated that the network genes participate in pathways including proteoglycans in cancer, cytoskeleton in muscle cells, and protein digestion and absorption (Fig. 5D). The relationships between individual proteins in the CTHRC1 PPI network and the enriched GO and KEGG terms were visualized using cnetplots generated with the ClusterProfiler package in R (Fig. 5E-H).

6. LINC01748, Let-7b-5p, and CTHRC1 expression in CRC tissues

The gene expression levels of the LINC01748/Let-7b-5p/CTHRC1 axis were quantified using a RT-qPCR technique. As shown in Fig. 6A, LINC01748 displayed significantly higher expression in tumor tissues than adjacent non-tumor tissues (2.34-fold, p=0.0003). Furthermore, analysis of Let-7b-5p expression (Fig. 6B) revealed a marked decrease (6.80 fold, p<0.0001) in cancerous tissues compared to adjacent non-cancerous tissues, contrasting with the results for LINC01748. Significant up-regulation was also observed in both CTHRC1 mRNA (2.56-fold, p=0.0003) and protein levels (1.453-fold, p=0.002) in CRC tissues compared with non-cancerous tissues, as illustrated in Fig. 6C, D. The Kaplan-Meier overall survival curve for patients with colorectal cancer shows that high expression of CTHRC1 is associated with reduced overall survival (Fig. 6E). Multivariate Cox proportional hazards analysis, adjusting for age, gender, tumor stage (I-II vs. III-IV), and histological grade, was performed to assess the independent prognostic value of CTHRC1 expression. Although the model indicated a trend toward increased hazard with high CTHRC1 expression (adjusted HR=1.51, 95% CI: 0.15-14.66, p=0.71), it did not reach statistical significance, likely due to the limited sample size of our cohort. Nevertheless, the consistent direction and magnitude of the hazard ratio across both models suggest a potential independent prognostic role for CTHRC1, warranting validation in larger, independent cohorts (Table 2). To assess the prognostic association of CTHRC1 in a larger population, we analyzed overall survival data from the TCGA-COAD cohort. Patients with high CTHRC1 expression (dichotomized by median) showed a trend toward poorer survival, though the result was not statistically significant in this dataset either (log-rank p=0.285, HR=1.23, 95% CI: 0.83-1.82) (Supplementary Fig. 1).

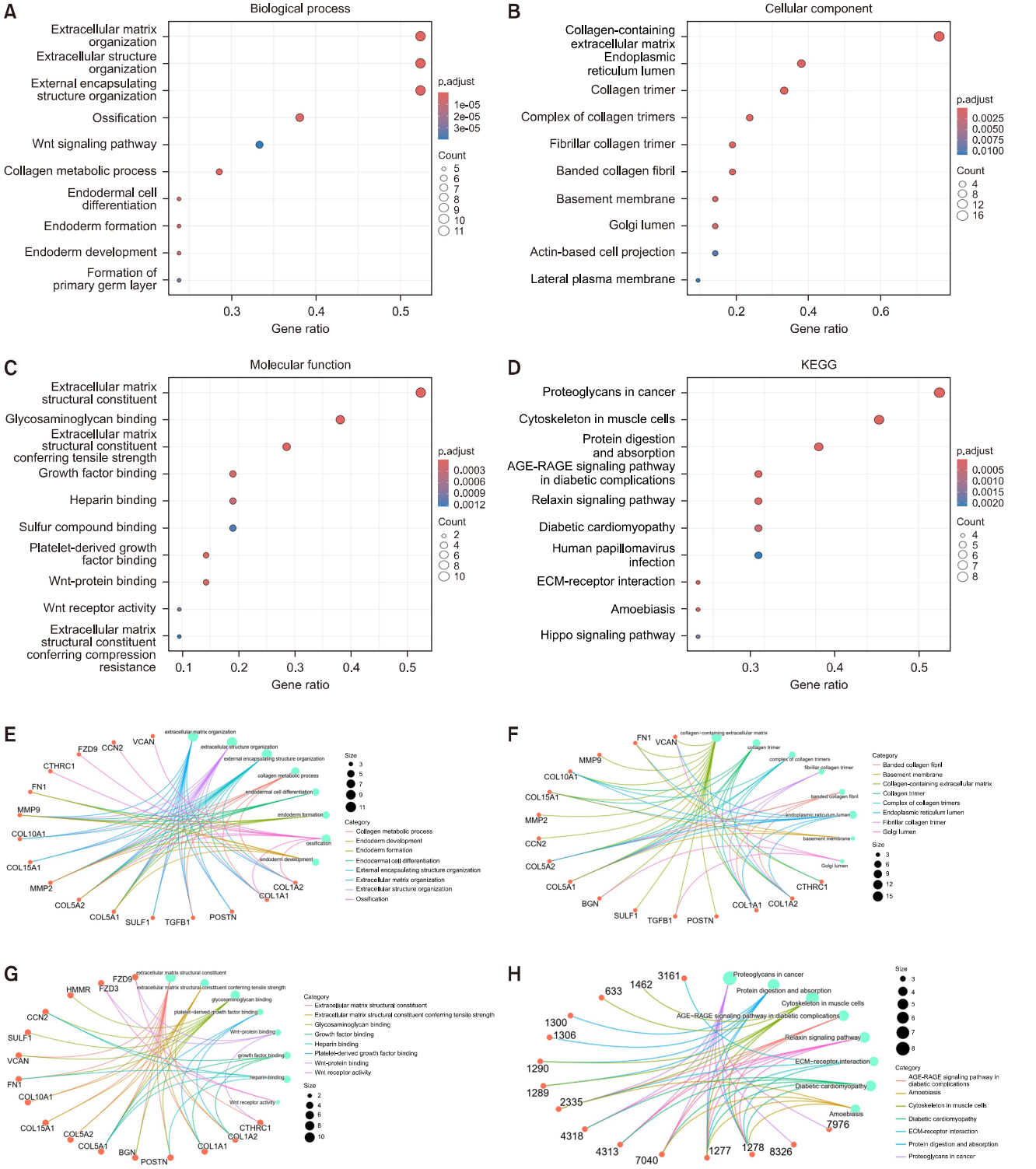

FIG. 5. Dot plots illustrate significantly enriched terms from Gene Ontology (GO) categories – (A) biological process (BP), (B) cellular component (CC), and (C) molecular function (MF) and (D) Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways. In each dot plot, terms are ranked by significance, with the most significant at the top. Cnetplots depict the relationships between individual proteins within the CTHRC1 PPI network and enriched GO terms for (E) BP, (F) CC, (G) MF, and (H) KEGG pathways.

7. Correlation analysis of expressed genes

The relationship between expression levels of the components in the LINC01748/Let-7b-5p/CTHRC1 axis was evaluated. Our analysis demonstrated a negative correlation between LINC01748 and Let-7b-5p (r= −0.59, p=0.005). Conversely, a significant positive correlation was found between LINC01748 and CTHRC1 mRNA levels (r=0.61, p=0.004), as shown in Fig. 7. An inverse correlation was al-

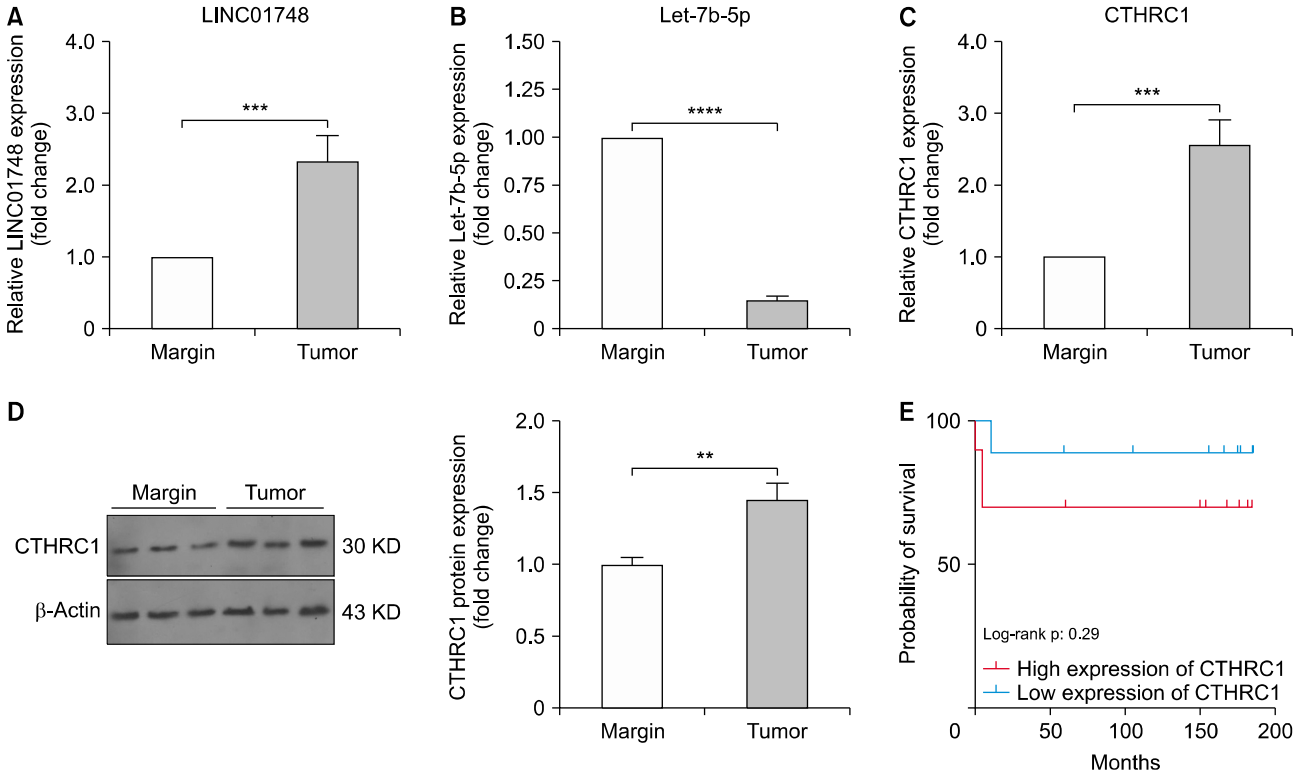

FIG. 6. Analysis of the expression levels of LINC01748/Let-7b-5p/ CTHRC1 axis components in CRC tissues. The relative expression of LINC01748 (A), Let-7b-5p (B), and CTHRC1 mRNA (C) in 20 CRC tissues vs tumor marginal tissues were evaluated using RT-qPCR. (D) The protein level of CTHRC1 was determined using western blotting. Cropped western blotting images are shown from only three marginal non-cancerous and three tumor samples as representative of all samples, but graph was plotted based on the mean protein levels of all tumor and marginal samples. (E) Kaplan-Meier overall survival curves stratified by expression level of CTHRC1 in CRC patient (corresponding graph is represented as mean±SEM following normalization against β-Actin). **p<0.01, ***p<0.001, and ****p<0.0001.

TABLE 2. Cox regression analysis

Characteristic	HR	95% CI	p-value
Age (years)			
<67			
≥67	1.577	0.15-15.63	0.69
Sex			
Female			
Male	0.489	0.04-5.86	0.57
Stage			
I-II			
III-IV	10.42	0.93, 117.03	0.057
Grade			
G1			
G2			
G3			
G4	1.11	0.38-3.22	0.84
CTHRC1			
Low expression			
High expression	1.51	0.15, 14.66	0.71

CI: confidence interval, HR: hazard ratio.

so observed between Let-7b-5p and CTHRC1 (r=−0.62, p=0.003).

8. Association of LINC01748, Let-7b-5p, and CTHRC1 gene expression levels with clinicopathological characteristics of CRC patients

This study consisted of 20 patients diagnosed with CRC, including 7 males and 13 females. Participants ranged in age from 30 to 85, with a mean age of 65.5±14.5 years (SD). Table 3 summarizes demographic characteristics and correlations between LINC01748, let-7b-5p, CTHRC1 expression, and clinicopathological features. LINCO1748 expression remained consistent across demographic and clinical variables, with no significant differences observed (p>0.05 for all comparisons). Let-7b-5p exhibited a borderline significant gender difference, with higher expression in males compared to females (0.18±0.03 vs. 0.13±0.03; p=0.053). Notably, CTHRC1 expression was significantly associated with aggressive tumor features, showing increased levels in poorly differentiated (G4) tumors relative to well-differentiated (G1-G2) tumors (5.13±0.81 vs. 1.64-2.43; p=0.011). Additionally, CTHRC1 expression was elevated in smaller tumors (T1-T2: 3.52±0.30) compared to larger tumors (T3-T4: 2.19±0.26; p=0.021). This explora-

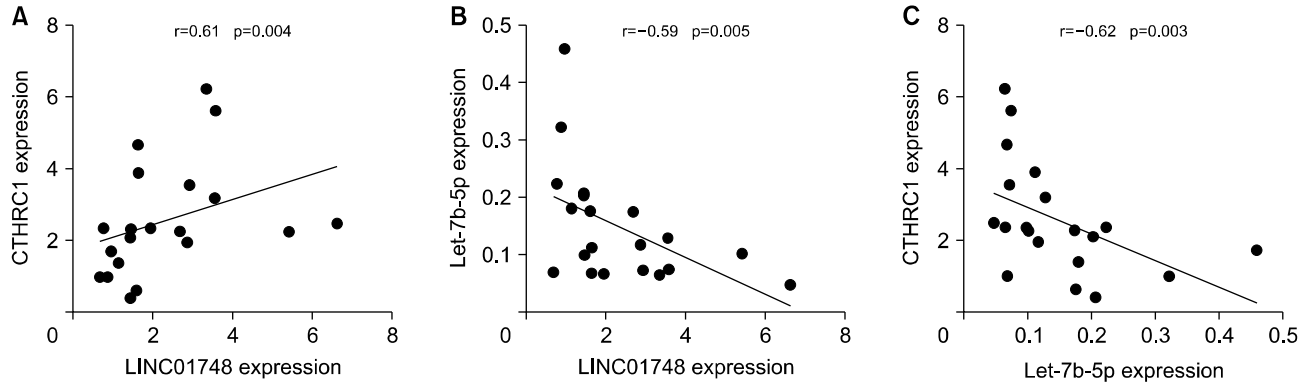

FIG. 7. Correlation analysis of the expression levels of LINC01748/Let-7b-5p/CTHRC1 axis components. Correlation analysis of (A) CTHRC1-LINC01748, (B) Let-7b-5p-LINC01748, and (C) CTHRC1-LINC01748 was performed based on the non-parametric Spearman test.

TABLE 3. Association of LINC01748, Let-7b-5p, and CTHRC1 expression with clinico-pathological parameters in CRC patients

Characteristics	Expression of LINC01748			Expression of let-7b-5p			Expression of CTHRC1		
	N (%)	Mean±SEM	p-value	N (%)	Mean±SEM	p-value	N (%)	Mean±SEM	p-value
Age (years)									
<67	10 (50)	1.97±0.26	0.309	10	0.12±0.02	0.315	10	2.39±0.51	0.653
≥67	10 (50)	2.70±0.65		10	0.17±0.41		10	2.72±0.50	
Gender									
Male	7 (35)	1.95±0.38	0.428	7	0.18±0.03	0.052	7	2.11±0.75	0.154
Female	13 (65)	2.55±0.50		13	0.13±0.03		13	2.80±0.37	
Clinical Stage									
I-II	16 (80)	2.49±0.43	0.478	16	0.14±0.03	0.571	16	2.55±0.41	0.925
III-IV	4 (20)	1.73±0.33		4	0.15±0.03		4	2.59±0.71	
Histological Grade									
G1/well	10 (50)	2.10±0.56	0.179	10	0.13±0.03	0.745	10	2.43±0.40	0.01*
G2/ moderate	7 (35)	2.27±0.60		7	0.14±0.02		7	1.64±0.25	
G4/undifferentiated	3 (15)	3.29±0.19		3	0.19±0.13		3	5.13±0.81	
Tumor Size									
T1-T2	2 (10)	3.47±0.11	0.101	2	0.06±0.005	0.101	2	5.92±0.30	0.02*
T3-T4	18 (90)	2.21±0.38		18	0.16±0.02		18	2.19±0.26	
Lymph Nodes									
N0	16 (80)	2.49±0.43	0.478	16	0.14±0.03	0.571	16	2.55±0.41	0.925
N1-N2	4 (20)	1.74±0.33		4	0.15±0.03		4	2.60±0.72	
Clinical Metastasis									
M0	19 (95)	2.38±0.37	0.664	19	0.15±0.02	0.433	19	2.45±0.35	0.175
M1	1 (5)	1.65		1	0.07		1	4.67	
Lymph invasion									
Yes	10 (50)	2.47±0.48	0.571	10	0.18±0.04	0.096	10	3.15±0.54	0.140
No	10 (50)	2.20±0.54		10	0.17±0.02		10	1.97±0.38	
Vascular invasion									
Yes	10	2.46±0.49	0.677	10	0.16±0.04	0.545	10	3.15±0.54	0.140
No	10	2.22±0.53		10	0.13±0.03		10	1.97±0.38	

*p<0.05.

tory analysis identified association between CTHRC1 expression and aggressive tumor features, suggesting a potential biological role in tumor progression that warrants further investigation in larger cohorts to assess its clinical relevance. Furthermore, the other markers demonstrated limited clinical correlations within this cohort. Further validation in larger patient cohorts is necessary to substantiate these associations.

9. Cancerous tissue exhibits higher collagen density

A comparative analysis of Picrosirius Red staining between non-cancerous colonic wall tissue and tumoral tis-

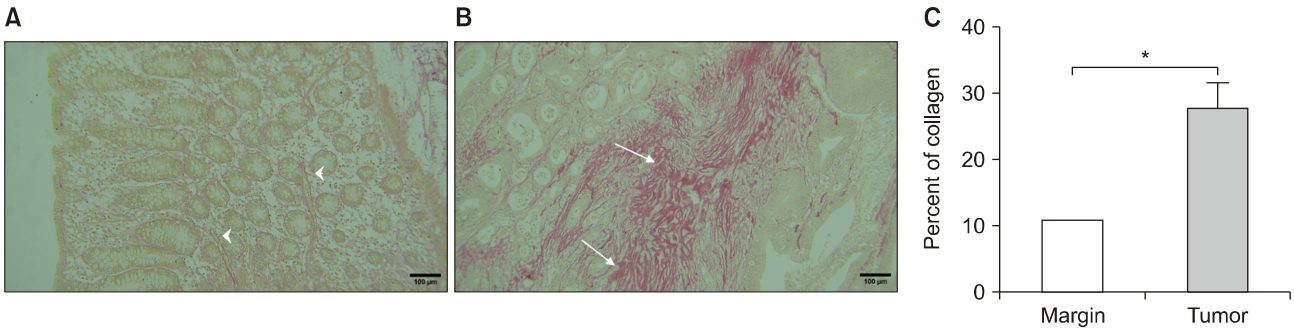

FIG. 8. Picrosirius red staining in CRC tissues (Bar= 100 μm). The adjacent normal tissue (A) exhibited only sporadic collagen bundles within the normal stroma (arrowheads) whereas, tumoral tissue (B) was characterized by substantial thick collagenous bundles interspersed among tumor nests (arrows). (C) The mean percent of collagen density of the tumor and marginal tissues. *p<0.05.

sue demonstrated significant differences in collagen organization. The adjacent normal tissue exhibited only sporadic collagen bundles within the normal stroma (Fig. 8; arrowhead) whereas, tumoral tissue was characterized by substantial thick collagenous bundles interspersed among tumor nests (Fig. 8; arrow), with pronounced desmoplastic reactions (increased thickness and density of collagen) observed around specific tumoral glands (Fig. 8).

DISCUSSION

In light of the emerging therapeutic strategies developed for CRC, it is concerning that the incidence and mortality rates associated with CRC have significantly escalated in recent years.[3] This trend emphasizes the urgent need for a comprehensive understanding of the molecular mechanisms driving tumorigenesis, which may aid in uncovering factors pertinent to the etiology, diagnosis, and effective therapeutic interventions for CRC.[8] The aberrant expression of non-codingRNAs is intricately associated with CRC incidence, progression, and the phenomenon of drug resistance.[33] The ceRNA mechanism provides insights into the regulatory interactions and networks that exist among various RNA molecules.[11] Emerging research indicates that numerous lncRNAs display dysregulated expression patterns in cancer.[9,14] As crucial constituents of ncRNAs, they may exert either oncogenic or tumor-suppressive effects.[34] In the present study, we investigated the bioinformatics analysis, expression patterns, and potential functions of the LINC01748/Let-7b-5p/CTHRC1 oncogenic axis in CRC (Fig. 9).

LINC01748, located at 1p32.1 and consisting of eight exons, 7 introns, and 3,229 nucleotide, was identified by Tan et al.[35] as being upregulated in tumor tissue approximately fourfold compared with adjacent non-cancerous, and correlated with migration, invasion, and cancer cell growth via regulation of miR-520a-5p/HMGA1. Bioinformatics analyses in the other cancer types, including hepatocellular carcinoma,[36] laryngeal squamous cell carcinoma,[37] and thymoma,[38] have also supported a tumorigenic role for this lncRNA through various mechanisms. In accordance with these studies, our results revealed a significant elevation of LINC01748 expression in CRC tissues compared with adjacent non-cancerous tissues.

Although different mechanisms for the effects of LINC01748 in cancers have been reported, the exact role of this lncRNA is still unclear. According to our bioinformatics analysis, let-7b-5p could be a potential target of LINC01748 in CRC patients. It has been reported that let-7b-5p inhibits HK2-mediated aerobic glycolysis and enhances breast tumor growth and metastasis both in vitro and in vivo.[39] Accordingly, Xue et al.[40] showed that increasing the expression of has-circ-0000264 by silencing let-7b-5p increased the expression of HMGA2 and ultimately increased the migration, invasion, and EMT of head and neck squamous carcinoma cells. Similarly, previous work by Xu et al showed that increased expression of let-7b-5p reduced lung adenocarcinoma growth by PI3K/AKT signaling pathway.[41] Inhibitory effect of Let-7b-5p on the proliferation, migration, and invasion of colon cancer cells has also repeatedly been observed in other studies.[42] In line with these findings, here we showed that let-7b-5p expression was significantly lower in colorectal tumor tissues than in adjacent tissues and strengthened our hypothesis that upregulation of LINC01748 is a causative factor for colorectal cancer cell growth probably likely through the downregulation of let-7b-5p.

Based on the bioinformatics predictions in the present study, the collagen triple helix repeat containing 1 (CTHRC1) gene could be targeted by let-7b-5p. The CTHRC1 gene is located on chromosome 8q22.3 and encodes an extracellular matrix protein. CTHRC1 increases collagen promoter activity by binding to ligands and contributes to vascular remodeling by limiting collagen matrix deposition and promoting cell migration.[18] The role of CTHRC1 in tumor progression is related to the regulation of several key signaling pathways, including TGF-β, Wnt, PKC-δ/ERK, PI3K/AKT/ERK, and MEK/ERK, which are also critical for cancer development and metastasis.[19] A study by Chen et al.[43] demonstrated that CTHRC1 expression is elevated in anaplastic thyroid carcinoma and upregulation of CTHRC1 resulted in a significant increase

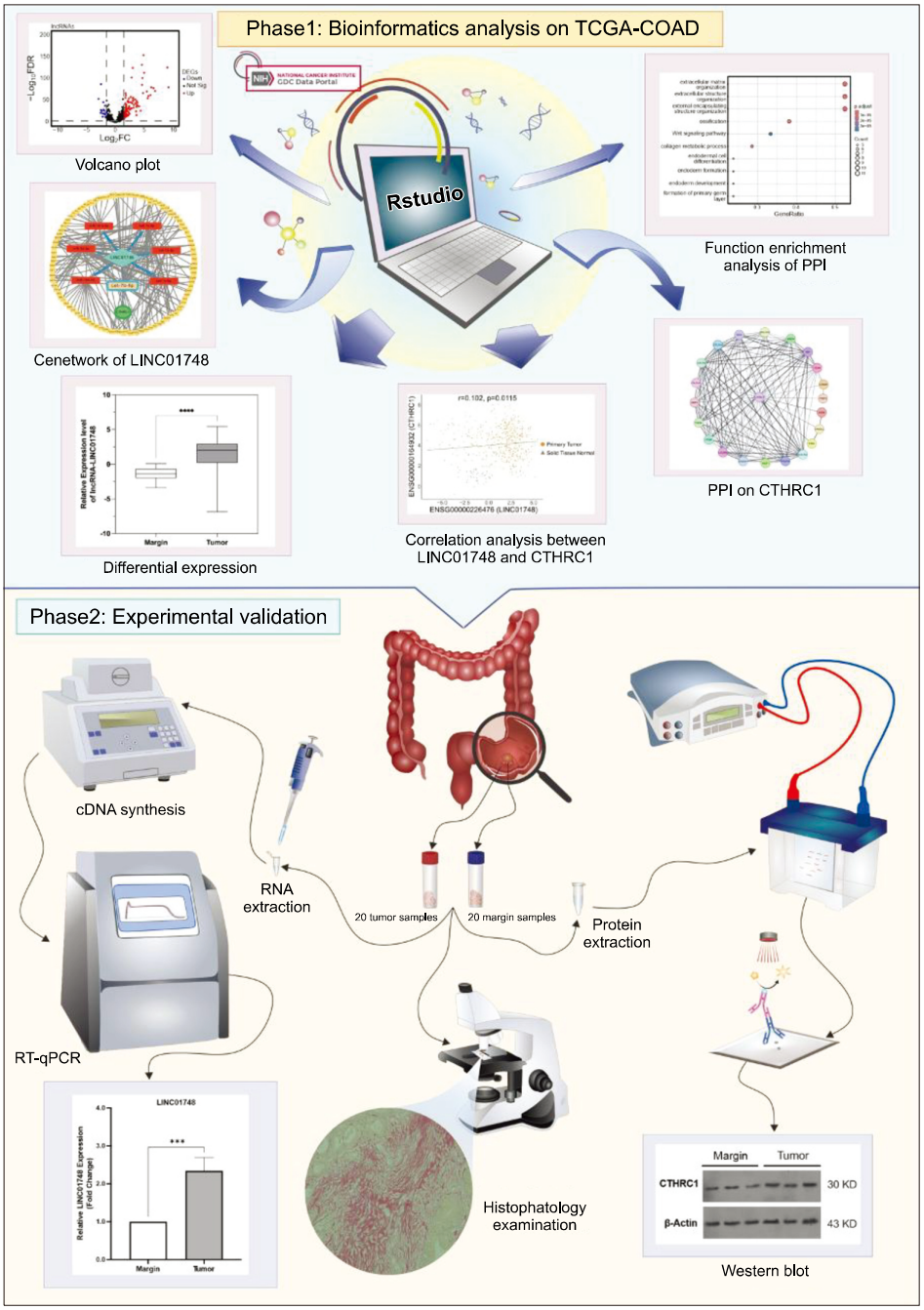

FIG. 9. Schematic diagram illustrating our bioinformatics analysis and experimental validation of the LINC01748/Let-7b-5p/CTHRC1 axis in the progression of CRC.

in the proliferation, invasion, and migration of tumor cells. Furthermore, a study on bladder carcinoma cell lines has revealed that high CTHRC1 expression is associated with enhanced migration, proliferation, and invasion of the cells.[44] In addition, heightened expression of CTHRC1 at the transcription and protein levels in colorectal cancer has been found associated with increased migration, invasion, and EMT of CRC cells, primarily due to the induction of the TGF-β pathway.[45] In accordance with previous observations, the current study showed that both the protein and mRNA levels of CTHRC1 were significantly elevated in CRC tissues. The enhancement in CTHRC1 expression was accompanied by an increased expression of LINC01748 and decreased levels of let-7b-5p in colorectal tissue samples compared with their corresponding adjacent non-cancerous tissues. Kaplan-Meier analysis showed no statistically significant difference in the overall survival of high expressers of CTHRC1 (log-rank p=0.29; HR=3.11, 95% CI: 0.43-22). Subsequent multivariate Cox regression, adjusting for major clinicopathological variables, yielded a similarly elevated hazard ratio, reinforcing the potential of CTHRC1 as an independent prognostic marker. The absence of statistical significance in our study is likely a consequence of the modest cohort size, which limited the statistical power. Future studies with larger patient populations are essential to confirm the independent prog-

nostic value of CTHRC1 in CRC.

Here, a functional enrichment analysis indicated that CTHRC1, a protein involved in the extracellular matrix, and its interacting proteins are significantly implicated in the organization of the extracellular matrix. This observation is in line with previous reports indicating that elevated expression of CTHRC1 promotes tumor development by modulating the tumor microenvironment and activating various signaling pathways.[46,47] More importantly, our picrosirius red staining revealed that collagen, a primary component of the ECM, was significantly elevated in CRC tissues compared to adjacent non-cancerous tissues.

This finding is also in accordance with several lines of evidence suggesting that increased production of ECM proteins, including collagen, plays a role in poor prognosis and even cancer recurrence.[48]

This study investigated the relationship between demographic and clinical variables and gene expression. The results indicated no significant differences in the expression of the three variables age, tumor stage, lymph node status, metastasis, and vascular invasion. Notably, CTHRC1 expression was significantly correlated with tumor size; consistent with findings in other cancers, larger tumors are generally associated with a higher risk of mortality. A study on epithelial ovarian cancer demonstrated that CTHRC1 expression was significantly related to tumor size.[49] Additionally, our demographic analysis revealed a significant association between CTHRC1 expression and tumor grade, aligning with prior research indicating that CTHRC1 expression correlates with histological grade in epithelial ovarian cancer.[50]

The interaction between LINC01748 and Let-7b-5p remains to be fully elucidated.

Similar to previously confirmed interactions between Let-7b-5p and the CTHRC1 gene expression in gastric cancer and glioma malignancy utilizing dual-luciferase reporter assays,[31,32] our investigation revealed a significant positive correlation between LINC01748 and CTHRC1, a strong negative correlation between LINC01748 and Let-7b-5p, and an inverse correlation between Let-7b-5p and CTHRC1 in colorectal cancer.

In summary, enhancement of LINC01748 expression significantly suppressed the inhibitory effect of Let-7b-5p on the expression of CTHRC1 in CRC tissues which led to the pathological features, including tumor grade and tumor size. This study suggests that the interaction between LINC01748 and the Let-7b-5p-CTHRC1 axis may enhance our understanding of the pathobiological significance of LINC01748 and its functional role in CRC pathogenesis, but also acknowledge the limitations inherent to bulk-tissue analyses. CTHRC1, an ECM-associated gene, is enriched in cancer-associated fibroblasts and other stromal components, suggesting that the observed positive correlation between LINC01748 (likely enriched in cancer cells) and CTHRC1 may partly reflect sample-specific tumor purity or stromal content rather than a direct, cell-autonomous ceRNA interaction. Future work using single-cell RNA sequencing or spatial transcriptomics is needed to localize LINC01748, Let-7b-5p, and CTHRC1 within tumor compartments and determine whether co-regulation occurs cell-autonomously in cancer cells or within stromal-epithelial interfaces. However, additional validation through more in vitro and in vivo functional studies are essential to definitively establish causality. Specifically, loss-of-function (e.g., siRNA/CRISPR against LINC01748) and gain-of-function (e.g., Let-7b-5p mimic) experiments in CRC cell lines will be crucial, along with the examination of a larger cohort of clinical samples, particularly blood samples from CRC patients as well as be necessary to further substantiate our findings. Furthermore, luciferase reporter assays are required to confirm the interactions between LINC01748 and Let-7b-5p, as well as the correlation between Let-7b-5p and CTHRC1 in the context of CRC.

In conclusion, this study presents the novel finding that LINC01748 expression and its interaction with the Let-7b-5p- CTHRC1 axis play a biologically significant role in colorectal cancer development. These results contribute to our understanding of the essential role of non-coding RNAs in oncogenesis and may guide the formulation of targeted therapies for CRC.

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CONFLICT OF INTEREST STATEMENT

None declared.

REFERENCES

1. Li Q, Geng S, Luo H, Wang W, Mo YQ, Luo Q, et al. Signaling pathways involved in colorectal cancer: pathogenesis and targeted therapy. Signal Transduct Target Ther 2024;9:266.
2. Marandi Y, Hashemzade S, Tayebinia H, Karimi J, Zamani A, Khodadadi I. NLRP3-inflammasome activation is associated with epithelial-mesenchymal transition and progression of colorectal cancer. Iran J Basic Med Sci 2021;24:483-92.
3. Hu SS, Fu L, Han SY, Li XL, Zhang LD. LINC01605 regulates proliferation, migration and invasion of colorectal cancer cells via targeting miR-3960/SOX11. Eur Rev Med Pharmacol Sci 2021;25: 1322-9.
4. Dienstmann R, Vermeulen L, Guinney J, Kopetz S, Tejpar S, Tabernero J. Consensus molecular subtypes and the evolution of precision medicine in colorectal cancer. Nat Rev Cancer 2017;17: 79-92.
5. Yamamura S, Imai-Sumida M, Tanaka Y, Dahiya R. Interaction and cross-talk between non-coding RNAs. Cell Mol Life Sci 2018;75:467-84.

6. Khalil AM, Guttman M, Huarte M, Garber M, Raj A, Rivea Morales D, et al. Many human large intergenic noncoding RNAs associate with chromatin-modifying complexes and affect gene expression. Proc Natl Acad Sci U S A 2009;106:11667-72.
7. Soni DK, Biswas R. Role of non-coding RNAs in post-transcriptional regulation of lung diseases. Front Genet 2021;12:767348.
8. Nemeth K, Bayraktar R, Ferracin M, Calin GA. Non-coding RNAs in disease: from mechanisms to therapeutics. Nat Rev Genet 2024;25:211-32.
9. Kalantary-Charvadeh A, Morovat S, Aslani S, Ziamajidi N, Emami Razavi A, Abbasalipourkabir R. The role of long non-coding RNA LINC00839 in oral squamous cell carcinoma based on bioinformatics and experimental research. Sci Rep 2024;14: 31817.
10. Salmena L, Poliseno L, Tay Y, Kats L, Pandolfi PP. A ceRNA hypothesis: the Rosetta Stone of a hidden RNA language? Cell 2011;146:353-8.
11. Qi X, Zhang DH, Wu N, Xiao JH, Wang X, Ma W. CeRNA in cancer: possible functions and clinical implications. J Med Genet 2015; 52:710-8.
12. Akhbari MH, Zafari Z, Sheykhhasan M. Competing endogenous RNAs (ceRNAs) in colorectal cancer: a review. Expert Rev Mol Med 2022;24:e27.
13. Nandwani A, Rathore S, Datta M. LncRNAs in cancer: regulatory and therapeutic implications. Cancer Lett 2021;501:162-71.
14. Chang L, Ding J, Pu J, Zhu J, Zhou X, Luo Q, et al. A novel lncRNA LOC101928222 promotes colorectal cancer angiogenesis by stabilizing HMGCS2 mRNA and increasing cholesterol synthesis. J Exp Clin Cancer Res 2024;43:185.
15. Lee YJ, Kim WR, Park EG, Lee DH, Kim JM, Shin HJ, et al. Exploring the key signaling pathways and ncRNAs in colorectal cancer. Int J Mol Sci 2024;25:4548.
16. Tian J, Cui P, Li Y, Yao X, Wu X, Wang Z, et al. LINC02418 promotes colon cancer progression by suppressing apoptosis via interaction with miR-34b-5p/BCL2 axis. Cancer Cell Int 2020;20: 460.
17. Yao Y, Zhang F, Liu F, Xia D. Propofol-induced LINC01133 inhibits the progression of colorectal cancer via miR-186-5p/NR3C2 axis. Environ Toxicol 2024;39:2265-84.
18. Pyagay P, Heroult M, Wang Q, Lehnert W, Belden J, Liaw L, et al. Collagen triple helix repeat containing 1, a novel secreted protein in injured and diseased arteries, inhibits collagen expression and promotes cell migration. Circ Res 2005;96:261-8.
19. Mei D, Zhu Y, Zhang L, Wei W. The role of CTHRC1 in regulation of multiple signaling and tumor progression and metastasis. Mediators Inflamm 2020;2020:9578701.
20. Wen B, Zhang G, Zhan C, Chen C, Yi H. The 2024 revision of the Declaration of Helsinki: a modern ethical framework for medical research. Postgrad Med J 2025;101:371-82.
21. Li R, Qu H, Wang S, Wei J, Zhang L, Ma R, et al. GDCRNATools: an R/Bioconductor package for integrative analysis of lncRNA, miRNA and mRNA data in GDC. Bioinformatics 2018;34:2515-7.
22. Robinson MD, Oshlack A. A scaling normalization method for differential expression analysis of RNA-seq data. Genome Biol 2010;11:R25.
23. Law CW, Chen Y, Shi W, Smyth GK. Voom: precision weights unlock linear model analysis tools for RNA-seq read counts. Genome Biol 2014;15:R29.
24. Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol 2014;15:550.
25. Szklarczyk D, Kirsch R, Koutrouli M, Nastou K, Mehryary F, Hachilif R, et al. The STRING database in 2023: protein-protein association networks and functional enrichment analyses for any sequenced genome of interest. Nucleic Acids Res 2023;51:D638-46.
26. Doncheva NT, Morris JH, Gorodkin J, Jensen LJ. Cytoscape StringApp: network analysis and visualization of proteomics data. J Proteome Res 2019;18:623-32.
27. Yu G, Wang LG, Han Y, He QY. ClusterProfiler: an R package for comparing biological themes among gene clusters. OMICS 2012; 16:284-7.
28. Ashburner M, Ball CA, Blake JA, Botstein D, Butler H, Cherry JM, et al. Gene ontology: tool for the unification of biology. The gene ontology consortium. Nat Genet 2000;25:25-9.
29. Kanehisa M, Furumichi M, Tanabe M, Sato Y, Morishima K. KEGG: new perspectives on genomes, pathways, diseases and drugs. Nucleic Acids Res 2017;45:D353-61.
30. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods 2001;25:402-8.
31. Yu J, Feng J, Zhi X, Tang J, Li Z, Xu Y, et al. Let-7b inhibits cell proliferation, migration, and invasion through targeting Cthrc1 in gastric cancer. Tumour Biol 2015;36:3221-9.
32. Wei C, Zhang X, Peng D, Zhang X, Guo H, Lu Y, et al. LncRNA HOXA11-AS promotes glioma malignant phenotypes and reduces its sensitivity to ROS via Tpl2-MEK1/2-ERK1/2 pathway. Cell Death Dis 2022;13:942.
33. Kishore C, Karunagaran D. Non-coding RNAs as emerging regulators and biomarkers in colorectal cancer. Mol Cell Biochem 2022;477:1817-28.
34. Lin Y, Zhao W, Lv Z, Xie H, Li Y, Zhang Z. The functions and mechanisms of long non-coding RNA in colorectal cancer. Front Oncol 2024;14:1419972.
35. Tan Y, Xu F, Xu L, Cui J. Long non-coding RNA LINC01748 exerts carcinogenic effects in non-small cell lung cancer cell lines by regulating the microRNA-520a-5p/HMGA1 axis. Int J Mol Med 2022;49:22.
36. Wang W, Zhang J, Wang Y, Xu Y, Zhang S. Non-coding ribonucleic acid-mediated CAMSAP1 upregulation leads to poor prognosis with suppressed immune infiltration in liver hepatocellular carcinoma. Front Genet 2022;13:916847.
37. Qian L, Ni T, Fei B, Sun H, Ni H. An immune-related lncRNA pairs signature to identify the prognosis and predict the immune landscape of laryngeal squamous cell carcinoma. BMC Cancer 2022; 22:545.
38. Liu W, Yang HS, Zheng SY, Weng JH, Luo HH, Lei YY, et al. Thymic epithelial tumors: examining the GTF2I mutation and developing a novel prognostic signature with LncRNA pairs to predict tumor recurrence. BMC Genomics 2022;23:656.
39. Li L, Zhang X, Lin Y, Ren X, Xie T, Lin J, et al. Let-7b-5p inhibits breast cancer cell growth and metastasis via repression of hexokinase 2-mediated aerobic glycolysis. Cell Death Discov 2023; 9:114.
40. Xue F, Feng H, Wang T, Feng G, Ni N, Wang R, et al. Hsa_circ_

0000264 promotes tumor progression via the hsa-let-7b-5p/HMGA2 axis in head and neck squamous cell carcinoma. Oral Dis 2023; 29:2677-88.
41. Xu Y, Chen X. MicroRNA (let-7b-5p)-targeted DARS2 regulates lung adenocarcinoma growth by PI3K/AKT signaling pathway. Oncol Res 2024;32:517-28.
42. Dai Y, Liu J, Li X, Deng J, Zeng C, Lu W, et al. Let-7b-5p inhibits colon cancer progression by prohibiting APC ubiquitination degradation and the Wnt pathway by targeting NKD1. Cancer Sci 2023;114:1882-97.
43. Chen Y, Jia L, Zhao K, Chen Z, Han Y, He X. CTHRC1 promotes anaplastic thyroid cancer progression by upregulating the proliferation, migration, and invasion of tumor cells. PeerJ 2023;11: e15458.
44. Li Y, Cheng X, Yan J, Jiang S. CTHRC1 facilitates bladder cancer cell proliferation and invasion through regulating the PI3K/Akt signaling pathway. Arch Med Sci 2022;18:183-94.
45. Ni S, Ren F, Xu M, Tan C, Weng W, Huang Z, et al. CTHRC1 overexpression predicts poor survival and enhances epithelial-mesenchymal transition in colorectal cancer. Cancer Med 2018;7: 5643-54.
46. Malvia S, Chintamani C, Sarin R, Dubey US, Saxena S, Bagadi SAR. Aberrant expression of COL14A1, CELRS3, and CTHRC1 in breast cancer cells. Exp Oncol 2023;45:28-43.
47. Zhao H, Chen Z, Fang Y, Su M, Xu Y, Wang Z, et al. Prediction of prognosis and recurrence of bladder cancer by ECM-related genes. J Immunol Res 2022;2022:1793005.
48. Ghasemi H, Mousavibahar SH, Hashemnia M, Karimi J, Khodadadi I, Tavilani H. Transitional cell carcinoma matrix stiffness regulates the osteopontin and YAP expression in recurrent patients. Mol Biol Rep 2021;48:4253-62.
49. Hou M, Cheng Z, Shen H, He S, Li Y, Pan Y, et al. High expression of CTHRC1 promotes EMT of epithelial ovarian cancer (EOC) and is associated with poor prognosis. Oncotarget 2015;6:35813-29.
50. Yang C, Liu T, Xia B, Gu L, Lou G. Correlation of collagen triple helix repeat containing 1 overexpression with lymph node and peritoneal metastasis in epithelial ovarian cancer. Int J Gynecol Cancer 2017;27:22-7.