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***Basic Study***

**Potential role of long noncoding RNA RP5-881L22.5 as a novel biomarker and therapeutic target of colorectal cancer**

Zong H *et al*. Potential role of lncRNA RP5-881L22.5

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**Author contributions:** Zong H designed the project, wrote the paper and acquired the data; Zou JQ and Huang JP analyzed and interpreted the data; Huang ST modified the language of the article.

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**Abstract**

BACKGROUND

The incidence of colorectal cancer in humans is high, and it is in the top five for cancer-related morbidity and mortality. It is one of the main threats to human health. The function of long noncoding RNAs in tumor occurrence and development has gradually gained attention in recent years. In increasing numbers of studies, researchers have demonstrated that it plays an important role in the pathogenesis of colorectal cancer.

AIM

To find out if long noncoding RNA RP5-881L22.5 played a role in the pathogenesis of colorectal cancer in relation to the tumor microenvironment.

METHODS

We analyzed the transcriptome data and clinical data in The Cancer Genome Atlas-colon adenocarcinoma. The CIRBERSORT algorithm was applied to evaluate these tumor-infiltrating immune cells in The Cancer Genome Atlas-colon adenocarcinoma cancer tissue samples. Using the “estimate” package in R, we assessed the tumor immune microenvironment. The expression level of RP5-881L22.5 in tumor tissue and adjacent normal tissue samples from 4 pairs of colorectal cancer patients was determined by quantitative reverse transcription PCR. Colorectal cancer cells were tested for invasiveness using a transwell invasion assay after RP5-881L22.5 expression was knocked down.

RESULTS

The expression of lncRNA RP5-881L22.5 was related to the clinical characteristics of the tumors, and it was negatively related to the infiltration level of immune cells in the tumor microenvironment and the expression of T cell inhibitory receptors. A major function of its coexpressed mRNA was to regulate tumor immunity, such as the immune response. When quantitative reverse transcription PCR was performed on tumor tissues from 4 pairs of colorectal cancer patients, the results showed that RP5-881L22.5 was highly expressed. Subsequently, knocking down the expression of RP5-881L22.5, the invasiveness of colorectal cancer cell lines was reduced, and the apoptosis rate was increased.

CONCLUSION

RP5-881L22.5 plays a crucial role in the microenvironment of tumors as well as in the pathogenesis of colorectal cancer. The relationship between RP5-881L22.5 and the tumor immune microenvironment deserves further study.

**Key Words:** Colorectal cancer; Long noncoding RNA RP5-881L22.5; Tumor immune microenvironment; Biomarker; Therapeutic target

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**Core Tip:** Long noncoding RNA RP5-881L22.5 is related to the clinical characteristics of tumors, and it is negatively related to the infiltration level of immune cells in the tumor microenvironment and the expression of T cell inhibitory receptors. RP5-881L22.5 may play an important role in the tumor immune microenvironment as well as in the pathogenesis of colorectal cancer. The relationship between RP5-881L22.5 and the tumor immune microenvironment deserves further study.

**INTRODUCTION**

Among all malignant tumors, colorectal cancer is the most common worldwide. In total, 1148515 new cases were diagnosed worldwide in 2020[1]. Although the diagnosis and treatment of colorectal cancer have been continuously improved in recent years, the 5-year survival rate of colorectal cancer patients has not changed significantly. Greater than 20% of patients have already experienced metastasis at diagnosis, and approximately 50% of patients experience metastases during treatment[2]. Therefore, more research on the pathogenesis, early diagnosis, early treatment and prognosis of colorectal cancer is needed.

With the progression of research, it is increasingly recognized that tumors are a systemic disease, and the tumor immune microenvironment is a key factor involved in the pathogenesis and mechanism of tumors[3,4]. Innate immunity and adaptive immunity run through the whole disease process[5,6]. In the early stage of tumorigenesis, natural killer (NK) cells can recognize specific ligands on the surface of tumor cells, even if there are only a few tumor cells[3]. On the other hand, the activation of macrophages and dendritic cells, especially T cells and B cells, in the tumor immune microenvironment produces a large amount of additional cytokines, which further promotes the activation of CD8+ cytotoxic T cells and promotes immune memory[7]. However, given the long-term presence of tumors in the body, tumor cells can resist or suppress anti-tumor immune responses, leading to immune evasion and promoting tumor progression, which also represents a challenge for immunotherapy[8].

Long noncoding RNAs (lncRNAs) have a length over 200 nucleotides and are involved in many normal human physiological functions and pathogenic processes of disease[9,10]. In addition, extensive studies have found that lncRNAs have a pivotal function in the formation of the tumor immune microenvironment[11]. Therefore, we designed this study to explore whether lncRNAs can be used as molecular markers for a more accurate prediction of colorectal cancer prognosis and whether the molecular mechanisms of lncRNAs are the reasons for the differences in colorectal cancer prognosis as well as their impact on the tumor immune microenvironment.

**MATERIALS AND METHODS**

***Data preparation***

We downloaded the transcriptome sequencing data and clinical data from the Colon Adenocarcinoma (COAD) project in The Cancer Genome Atlas (TCGA) from the UCSC Xena (https://xenabrowser.net/) website[12,13]. The data included HTseq-FPKM data [log2 (FPKM+1)] (https://gdc-hub.s3.us-east-1.amazonaws.com/download/TCGA-COAD.htseq\_fpkm.tsv.gz), survival data (https://gdc-hub.s3.us-east-1.amazonaws.com/download/TCGA-COAD.survival.tsv) and clinical data (Phenotype including pathological staging and other data) (https://gdc-hub.s3.us-east-1.amazonaws.com/download/TCGA-COAD.GDC\_phenotype.tsv.gz). The dataset contains 512 colon cancer tissues and adjacent normal tissues, of which 448 colon cancer tissues have complete survival data and pathological stage data (tumor stage was determined according to the UICC and AJCC TNM classification system).

***Gene annotation and dataset construction***

According to the human reference genome GRch38 file (release 22) provided on the GENCODE website (https://www.gencodegenes.org), we converted gene IDs from Ensembl to symbols. Then, according to the annotation files provided by GENCODE, we extracted the mRNA and lncRNA expression datasets from the TCGA-COAD sequencing data[14].

***Differential expression analysis of mRNA and lncRNA***

Genes with extremely low expression levels [log2 (FKPM+1) < 0.5] were removed, and the differential expression of HTseq-FPKM data was analyzed with “limma” (R language, V4.1.1)[15]. We compared mRNA and lncRNA expression differences between 471 COAD tumor specimens and 41 adjacent normal tissue specimens. The lncRNA RP5-881L22.5 was selected as the research object, and a comparison of RP5-881L22.5 expression across different stages of COAD was conducted. Kaplan-Meier survival curves were drawn for patients grouped by different expression levels, and the survival differences of all patients grouped by expression levels as well as the effect of the expression differences of RP5-881L22.5 in different stages on survival were compared.

***Online analysis at the bioinformatics website***

The expression of RP5-881L22.5 in 33 cancers in TCGA was acquired from the GEPIA2 (http://gepia2.cancer-pku.cn/). The differential expression of RP5-881L22.5 between colon cancer tissue (TCGA-COAD) and normal colon tissue (data involved the TCGA-COAD project and Genotype-Tissue Expression (GTEx) database) were analyzed[16,17].

***Assessment of immune infiltration***

We downloaded the benchmark database file (LM22.txt) of 22 tumor-infiltrating immune cells (CD8+ T cells, CD4 naive T cells, regulatory reg cells, naïve B cells, memory B cells, plasma cells, CD4 memory resting T cells, CD4 memory activated T cells, follicular helper T cells, gamma delta T cells, resting NK cells, activated NK cells, monocytes, macrophages M0/M1/M2, resting dendritic cells, activated dendritic cells, resting mast cells, activated mast cells, eosinophils and neutrophils). The CIRBERSORT algorithm was applied to evaluate these tumor-infiltrating immune cells in TCGA-COAD cancer tissue samples[18]. Using the “estimate” package in the R language, we evaluated the tumor immune microenvironment[19]. All TCGA-COAD cancer tissue samples were divided into high expression and low expression groups according to the median expression of RP5-881L22.5, and the differences in immune components and immune cell infiltration between the two groups were compared. In addition, the expression levels of the coinhibitory receptors LAG3, CTLA4, HAVCR2, TIGIT and CD244 were compared between the two groups[20].

***Functional enrichment analysis***

Pearson correlation analysis was performed using the “cor.test” function of the basic R package. Among the differentially expressed mRNAs (logFC > 1 or logFC < -1, and adj.P.val < 0.05), the mRNAs coexpressed with RP5-881L22.5 were screened out. Gene set enrichment analysis of coexpressed mRNAs was performed using the “clusterProfiler” package in R language[21].

***Clinical specimens***

Colorectal cancer tissue specimens were obtained from the radical surgery specimens of colorectal cancer patients admitted to the Gastrointestinal Surgery Department of the Second Affiliated Hospital of Southern University of Science and Technology (Shenzhen Third People's Hospital) in August 2021. All patients were diagnosed by pathological diagnosis of colonoscopy biopsy: (1) Patients with acute infection, such as intestinal obstruction or intestinal perforation, were excluded; (2) Patients with HIV infection, autoimmune diseases, inflammatory bowel disease and other immune system-related diseases were excluded; (3) Preoperative neoadjuvant treatment patients were excluded; and (4) Patients with severe heart and lung insufficiency who could not tolerate surgery were excluded. Finally, eight tissue samples were obtained, which were colorectal cancer (3 cases of colon cancer, 1 case of rectal cancer), and four tumor tissues and four adjacent normal intestinal tissues were saved and transferred to a -80 °C freezer for storage. The experiment was ethically approved by the Ethics Committee of Shenzhen Third People’s Hospital (Shenzhen, China; license no. HE2022177).

***Cell culture and siRNA transfection***

The colorectal cancer cell line DLD-1 was obtained from ATCC (Manassas, VA, United States). 1640 medium supplemented with 10% fetal bovine serum, 1% penicillin-streptomycin and 3% glucose (HyClone, Logan, UT, United States) was used to incubate DLD-1 cells. Control siRNA and RP5-881L22.5 siRNA were purchased from RiboBio Co., Ltd. (GuangZhou, China). Lipofectamine 2000 Transfection Reagent (Invitrogen, CA, United States) was used to transfect siRNA into DLD-1 cells.

***RNA extraction and quantitative real-time PCR***

TRIzol reagent (ELK Biotechnology, Wuhan, China) was used to isolate total RNA from colorectal cancer tissues. On 1% agarose gels, degradation and contamination of RNA were monitored after isolation. Synthesis of first-strand cDNA was performed using an M-MLV Reverse Transcriptase kit (ELK Biotechnology, Wuhan, China). Quantitative real-time PCR was completed on a StepOne™ Real-Time PCR machine (Life Technologies, CA, United States) with 3 replicate per sample using QuFast SYBR Green PCR Master Mix kit (ELK Biotechnology, Wuhan, China). GAPDH was used as an endogenous control gene. The reactions were incubated at 95 °C for 1 min, followed by 40 cycles at 95 °C for 15 s, 58 °C for 20 s and 72 °C for 45 s. Calculation of relative expression levels was performed using the 2−ΔΔCT value relative quantification. The primer sequences were as follows: RP5-881L22.5 (ENSG00000226812) Sense: 5’- TATTGAGCACCTACTATGTACCAGG -3’ and Antisense: 5’- GTTAGAGCTCAGTCTCTCACAGCTC -3’; and GAPDH Sense: 5’- CATCATCCCTGCCTCTACTGG -3’ and Antisense: 5’- GTGGGTGTCGCTGTTGAAGTC -3’.

***Transwell invasion assay***

To prepare the transwell chamber for use, 50 μL of Matrigel (Corning, United States) and medium were diluted 1:3 and added to the chamber. Cell suspension (1 mL) was centrifuged at 1500 rpm for 5 min after being diluted to 105 cells/mL. The cell suspension was pipetted into a transwell chamber with 1 mL of serum-free medium. In a 24-well plate, 500 μL of complete medium containing 10% fetal bovine serum was added, and the chamber was inserted. The plate was incubated at 37 °C in a CO2 (5% content) incubator for 24 h (adjusted according to the experiment). A 0.5% crystal violet solution (1:4) was diluted into a 0.1% crystal violet staining solution with PBS solution. The medium was washed with PBS, and the glue and cells in the upper chamber were wiped off with a cotton swab, fixed with paraformaldehyde for 20 min, washed twice with PBS, stained with crystal violet for 10 min, and washed to remove the crystal violet on the surface. The side devoid of cell seeding was photographed under an inverted microscope (OLYMPUS).

***Apoptosis assay***

Precooled PBS was added at 4 °C, and an appropriate amount of binding buffer was diluted for use. After washing with PBS once, the cells in the 6-well plate were digested with 400 μL of 0.25% trypsin. The digestion was terminated by adding complete medium once the cells had become round and some had fallen off. We collected samples in 1.5 mL EP tubes and centrifuged them at 300 × g for 5 min, discarding the supernatant. One milliliter of PBS was added to resuspend the cells, they were centrifuged at 300 × g for 5 min, and the supernatant was discarded. A 200 μL solution of Binding Buffer was used to resuspend the pellet. Following this, 5 μL of Annexin V-FITC (Sungene Biotech, Tianjin, China) were added, mixed well and incubated for 10 min in the dark. Next, 5 μL propidium iodide was added, mixed well and incubated in the dark for 5 min. On-board inspection was performed within 1 h.

***Statistical analysis***

Comparing survival rates between groups was carried out using Kaplan-Meier survival curves and log-rank tests. The immune scores, the infiltration of immune cells and the expression levels of each gene were compared between groups using Wilcoxon tests. Differences were considered statistically significant when *P* < 0.05. All statistical procedures and graphs were completed using R programmer language (V4.1.1).

**RESULTS**

***Differential expression analysis of mRNAs and lncRNAs***

According to the GENCODE annotation file, we identified a total of 19712 mRNAs and 15878 lncRNAs in TCGA-COAD database. Genes with very low expression levels [log2 (FKPM+1) < 0.5] were deleted, and differential expression analysis was performed on the remaining 13285 mRNAs and 1850 lncRNAs. There were 1612 differentially expressed mRNAs and 122 differentially expressed lncRNAs (logFC > 1 or < -1 and adjusted *P* value < 0.05)(Figure 1A and B).

***RP5-881L22.5 expression in TCGA pan-cancer analysis***

By querying the expression of RP5-881L22.5 in all tumor tissues from the TCGA database on the GEPIA2 website, we found that among all 33 malignant tumors, only COAD and READ had significant differential expression (log2FC > 1.0, *q* value < 0.01)(Figure 1C) between cancer and normal tissues. Additionally, we found similar differences in gastric and esophageal cancers, both of which are malignant tumors of the digestive tract (Figure 1D).

***Association between RP5-881L22.5 expression and clinical features***

Based on the median expression of RP5-881L22.5 in 448 colon cancer tissue, we divided all samples into two groups with 224 samples in each. The 62 specimens of patients in stage IV were divided into two groups according to the median expression of rp5-881L22.5 with 31 samples in each group. Eighty specimens of patients in the N2 stage were divided into two groups according to the median expression of rp5-881L22.5 with 40 samples in each group. A positive correlation was found between RP5-881L22.5 expression and TNM stage (UICC and AJCC TNM staging system) as well as N stage and M stage, although no association was found with T stage (Figure 2A-D). However, after dividing all COAD samples into high and low groups according to RP5-881L22.5 expression levels, no difference in survival was noted between the two groups. For stage IV patients and patients with lymph node metastasis stage N2, high expression was associated with better survival. No differences in survival were noted among the other stages (Figure 2E-G).

***Association between RP5-881L22.5 expression and the tumor immune microenvironment***

To explore whether the expression of RP5-881L22.5 is related to the tumor immune microenvironment, we divided all samples from TCGA-COAD into high and low groups according to the median expression of RP5-881L22.5 and compared the immune microenvironment differences. According to our findings, the immune score and tumor stroma score in the high expression group were lower than those in the low expression group, and the differences were significant(Figure 3A-C). Comparing the infiltration of 22 types of immune cells in the high and low groups, four types of immune cells showed significant differences, including CD8 T cells, M0 macrophages, activated NK cells and neutrophils (*P* < 0.05) (Figure 3D). T cell exhaustion is one of the reasons for the formation of a tumor suppressive immune microenvironment, and the coinhibitory receptors PDCD1 (PD-1), LAG3, CTLA4, HAVCR2, TIGIT, CD244, *etc*, related to T cell exhaustion were also significantly different between the two groups. The expression levels of these genes in the RP5-881L22.5 high expression group were lower, and statistically significant differences were found (Figure 3E-J).

Afterward, coexpression analysis was performed on the transcriptome data from TCGA-COAD, and the genes coexpressed with RP5-881L22.5 among the differentially expressed mRNAs were selected. Through gene set enrichment analysis, it was found that the genes coexpressed with RP5-881L22.5 among the differentially expressed mRNAs were mainly involved in immune biological processes or pathways such as the immune response (Figure 4).

***Quantitative reverse transcription PCR and cell function assay results using clinical specimens***

Four pairs of tissue samples from colorectal cancer patients were assessed by quantitative reverse transcription PCR. We found that RP5-881L22.5 was generally highly expressed in tumor tissues compared with adjacent normal tissues (Figure 5A), which was consistent with the aforementioned bioinformatics analysis results. The apoptosis assay showed that the apoptosis rate of colorectal cancer cells increased after RP5-881L22.5 knockdown (Figure 5B-D). The transwell invasion assay revealed reduced invasiveness (Figure 5E). These results indicated that RP5-881L22.5 is a significant contributor to the occurrence and development of colorectal cancer.

**DISCUSSION**

LncRNAs play important functions in many regulatory mechanisms, such as transcriptional silencing, transcriptional activation, chromosome modification and intranuclear transport and play important roles in the occurrence and development of cancer[22,23]. Liu *et al*[24] found that three lncRNAs, LINC00114, LINC00261 and HOTAIR, can accurately judge the prognosis of colorectal cancer. Zhang *et al*[25] found that lncRNA-NEAT1 interacts with DDX5 to activate Wnt/β-catenin signaling, thereby promoting the progression of colorectal cancer. Ni *et al*[26] found that lncRNA-GAS5 controls colorectal cancer progression by regulating the phosphorylation and interpretation of YAP. This process is also negatively regulated by the m6A methylation reader protein YTHDF3.

We observed significant differences in lncRNA expression between colon cancer tumor tissues and normal colon tissues based on the analysis of TCGA dataset. This expression difference was also observed in rectal cancer, gastric cancer and esophageal cancer. Moreover, its expression was correlated with clinical features, such as the pathological stage of patients, and exhibited a significant correlation with survival in specific populations. These findings illustrate that RP5-881L22.5 may be a unique molecular marker in digestive tract cancer and may be involved in some important processes in the pathogenic process of digestive tract cancer. Subsequent clinical specimen detection and cell function experiments also verified this conclusion. All of these findings indicate that this lncRNA is likely to be related to the pathogenic process of colorectal cancer.

In addition to tumor cell characteristics, the microenvironment of the tumor also plays a substantial role in cancer[27]. Many lncRNAs show strong cell type-specific expression phenomena. Most mRNAs are expressed in the vast majority of cell types, whereas more than half of the cells contain only a few lncRNAs[28]. Cells with malignant characteristics had 9% of lncRNAs, lymphocytes had 11%, myeloid cells had 6%, and epithelial cells had 5%[29]. In particular, immune-specific lncRNAs recruit proteins to specific genomic loci to regulate target gene expression epigenetically and transcriptionally in most cases, thereby controlling the activity and differentiation of immune cells. For example, H19, ROCKI, lnc13 and HOXA-AS2 regulate target genes in immune cells by exerting protein recruitment functions or controlling chromatin accessibility[30-33]. At the same time, the tumor immune microenvironment can also be regulated by lncRNAs by targeting macrophages, dendritic cells, regulatory T cells and CD8+ T lymphocytes through a competing endogenous RNA mechanism, such as the SNHG1/miR-448/IDO regulator network[34], the SNHG16/miR-16-5p/SMAD5 regulator network[35], the FENDR/miR-423-5p/GADD45B regulator network[36], the SBF2-AS1miR-122-5p/XIAP regulator network[37] and the NEAT1/miR-155/Tim-3 regulator network[38].

Of course, in addition to their role in immune cells, lncRNAs also modulate the presentation of antigen or PD-L1 in tumor cells[39,40]. Li *et al*[41] identified a lncRNA, lncRNA inducing major histocompatibility complex I and immunogenicity of tumor, in humans and mice. They proposed the lncRNA inducing major histocompatibility complex I and immunogenicity of tumor-GBP-HSF1 axis as a therapeutic target for immunotherapy to modulate major histocompatibility complex I expression based on experimental validation *in* *vivo* and *in* *vitro*. In conclusion, increasing evidence indicates that the key mechanisms by which lncRNAs regulate tumor immunity involve various aspects, such as antigen presentation and T cell exhaustion.

In our study, RP5-881L22.5 expression in colorectal cancer was also strongly related to the tumor immune microenvironment. There was a negative correlation between its expression and the presence of CD8+ T lymphocytes and activated NK cells in the tumor microenvironment, and it was negatively correlated with the expression of various coinhibitory receptors on the surface of T lymphocytes. In addition, RP5-881L22.5 coexpressed genes were more involved in the pathways of tumor immune microenvironment formation, such as immune response and immune signal transmission.

In particular, TCGA data analysis revealed that RP5-881L22.5 expression was related to some clinical features of colorectal cancer patients but not to the T stage of the tumor, and no difference in survival was noted between the high expression group and the low expression group. Furthermore, a subset of advanced stage tumors showed better survival despite high marker expression. The expression of inhibitory receptors in the RP5-881L22.5 high expression group decreased. Therefore, T cells showed relatively stronger antitumor immune activity in the high expression group. This finding may explain why advanced tumors with higher RP5-881L22.5 expression showed a better survival rate. Further research on immunotherapy of advanced tumors using RP5-881L22.5 as a marker should be explored.

**CONCLUSION**

RP5-881L22.5 expression levels were significantly different between colorectal cancer tissues and nontumor tissues , and RP5-881L22.5 expression in tumor samples was considerably higher. RP5-881L22.5 expression levels were significantly correlated with clinicopathological stage and could predict prognosis for colorectal cancer. Moreover, RP5-881L22.5 showed an obvious immune correlation in colorectal cancer and might be a key molecule in the formation of the immunosuppressive microenvironment, which deserves further research. The relationship between RP5-881L22.5 and the tumor immune microenvironment, as well as the different prognostic differences it represents in tumors with different clinical characteristics, needs to further investigated in clinical tissue samples.

**ARTICLE HIGHLIGHTS**

***Research background***

Colorectal cancer is one of the most common malignant tumors in the world. Long noncoding RNAs (lncRNAs) are involved in many normal human physiological functions and pathogenic processes of diseases.

***Research motivation***

LncRNAs may be used as molecular markers for more accurate prediction of colorectal cancer prognosis. The molecular mechanisms of lncRNAs involved can impact colorectal cancer prognosis and impact the tumor immune microenvironment. Further research into these questions is needed.

***Research objectives***

To explore the differential expression analysis of mRNAs and lncRNAs in the colorectal cancer and to determine the association between RP5-881L22.5 expression and the tumor immune microenvironment.

***Research methods***

We analyzed the immune cell microenvironment through the bioinformatics combined with clinical data and cell experiments to verify the results.

***Research results***

RP5-881 L22.5 expression led to a difference in prognosis, which warrants further research on immunotherapy of advanced tumors with RP5-881 L22.5 as a marker.

***Research conclusions***

RP5-881 L22.5 plays an important role in the pathogenesis of colorectal cancer.

***Research perspectives***

RP5-881 L22.5 may be an important research target for the treatment of colorectal cancer.

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**Footnotes**

**Institutional review board statement:** The study was reviewed and approved by the Shenzhen Third People's Hospital (Approval No. G2022117).

**Informed consent statement:** Patients were not required to give informed consent to the study.

**Conflict-of-interest statement:** All authors declare that there is no conflict of interest in this work.

**Data sharing statement:** All data are publicly available for sharing use.

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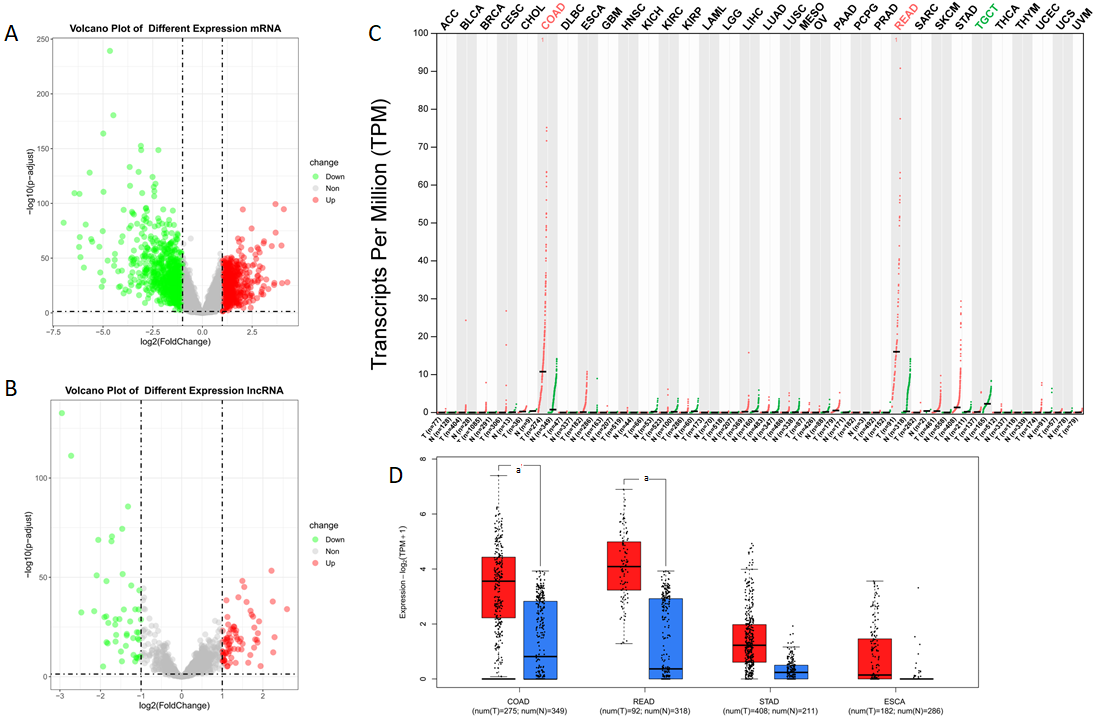
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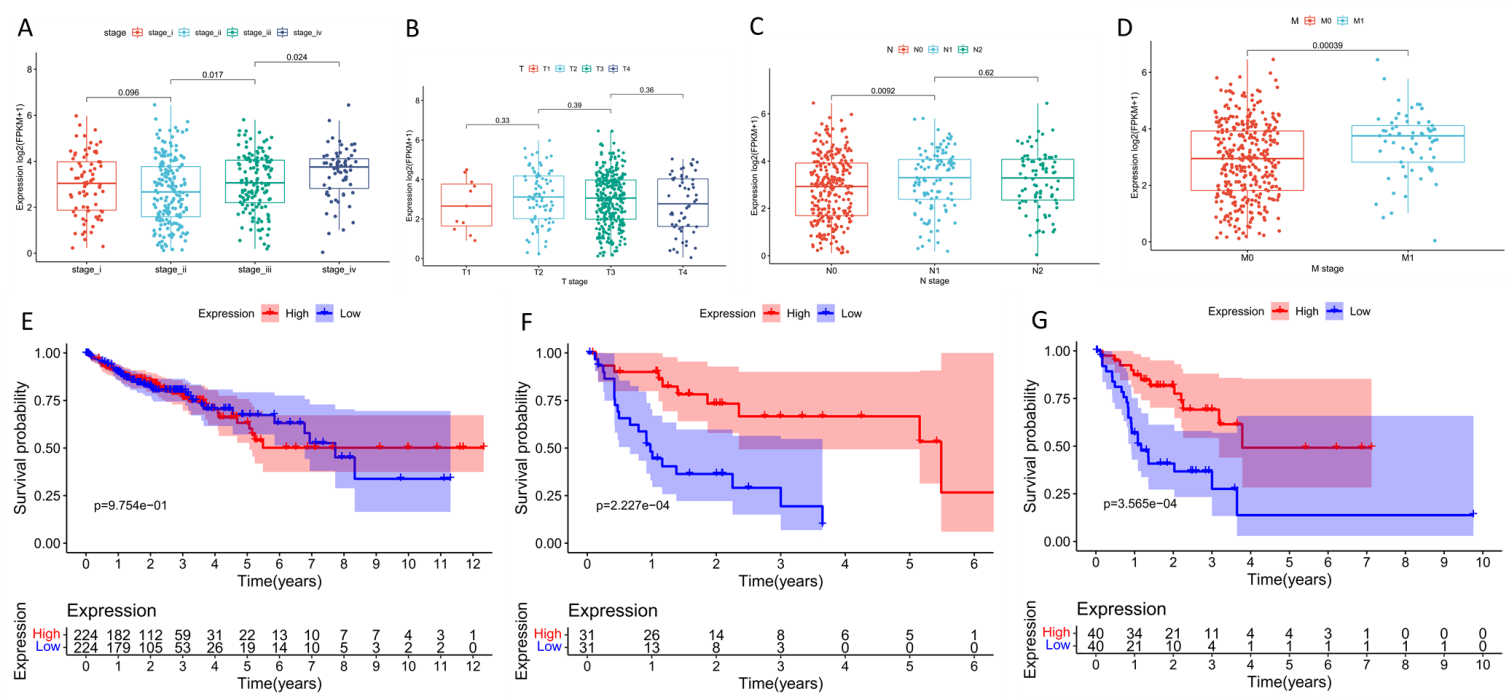
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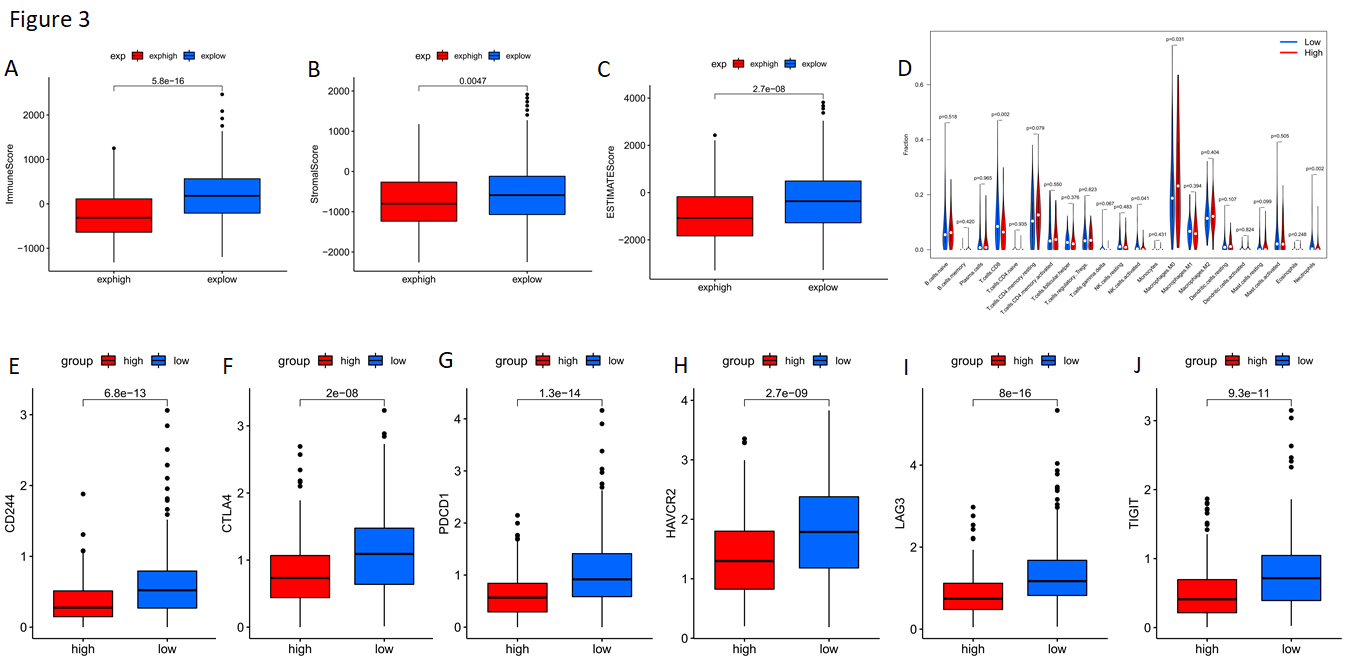
**Figure Legends**



**Figure 1 Volcano plot of the differentially expressed genes in The Cancer Genome Atlas-colon adenocarcinoma (LogFC > 1 or < -1 and adjust *P* value < 0.05 were defined as significant difference) and results from GEPIA2 website query.** A: Volcano plot of mRNA differential expression; B: Volcano plot of long noncoding (lnc) RNA differential expression. Red points referred to upregulated differentially expressed genes; green points referred to downregulated differentially expressed genes; grey points referred to non-differentially expressed genes; C: RP5-881L22.5 expression in the 33 cancers of The Cancer Genome Atlas. Log2FC > 1.0 or < -1.0 and *q* value < 0.05 were defined as a significant difference. Red points referred to tumor tissues; green points referred to normal tissues; D: Expression level of RP5-881L22.5 among colon adenocarcinoma, rectum adenocarcinoma, stomach adenocarcinoma and esophageal carcinoma (The Cancer Genome Atlas). Red referred to tumor tissues; blue referred to normal tissues (include data of GTEx); “a” referred to log2FC > 1.0 and *q* value < 0.01. ACC: Adrenocortical carcinoma; BLCA: Bladder urothelial carcinoma; BRCA: Breast invasive carcinoma; CESC: Cervical squamous cell carcinoma and endocervical adenocarcinoma; CHOL: Cholangiocarcinoma; COAD: Colon adenocarcinoma; DLBC: Lymphoid Neoplasm Diffuse Large B-cell Lymphoma; ESCA: Esophageal carcinoma; GBM: Glioblastoma multiforme; HNSC: Head and neck squamous cell carcinoma; KICH: Kidney chromophobe; KIRC: Clear cell renal cell carcinoma; KIRP: Kidney renal papillary cell carcinoma; LAML: Acute myeloid leukemia; LGG: Brain lower grade glioma; LIHC: Liver hepatocellular carcinoma; LUAD: Lung adenocarcinoma; LUSC: Lung squamous cell carcinoma; MESO: Mesothelioma; N: Normal; OV: Ovarian serous cystadenocarcinoma; PAAD: Pancreatic adenocarcinoma; PCPG: Pheochromocytoma and Paraganglioma; PRAD: Prostate adenocarcinoma; READ: Rectum adenocarcinoma; SARC: Sarcoma; SKCM: Skin Cutaneous Melanoma; STAD: Stomach adenocarcinoma; T: Tumor; TGCT: Testicular Germ Cell Tumors; THCA: Thyroid carcinoma; THYM: Thymoma; UCEC: Uterine corpus endometrial carcinoma; UCS: Uterine carcinosarcoma; UVM: Uveal melanoma.

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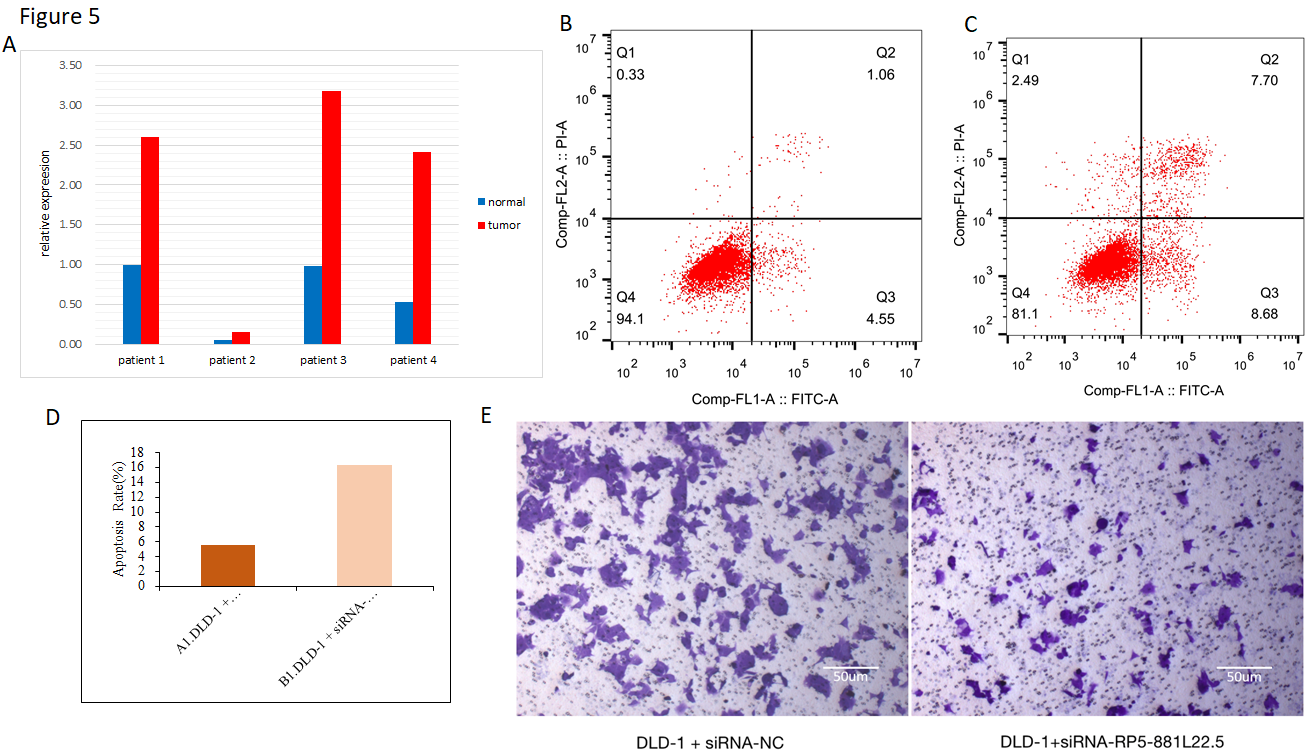
**Figure 2 Association between the expression level of RP5-881L22.5 and clinical characteristics.** A: Association between expression and TNM stage; B: Association between expression and T stage; C: Association between expression and N stage; D: The association between expression and M stage; E: Kaplan-Meier survival curve of the population at all stages; F: Stage IV; G: N2 stage. Red referred to the higher expression group, and blue referred to the lower expression group (grouped by median expression of RP5-881L22.5).



**Figure 3 Distribution of tumor microenvironment scores, 22 types of immune cells and expression of T lymphocyte coinhibitory receptor genes between different groups.** A: ImmuneScore, *P* =5.8 × 10-16; B: StromalScore, *P* =0.0047; C: ESTIMATEScore, *P* =2.7 × 10-8; D: The 22 immune cell types were represented in columns, and the proportions of immune cells were represented in rows; E: Expression of CD244, *P* =6.8 × 10-13; F: Expression of CTLA4, *P* =2.0 × 10-8; G: Expression of PDCD1, *P* =1.3 × 10-14; H: Expression of HAVCR2, *P* =2.7 × 10-9; I: Expression of LAG3, *P* =8.0 × 10-16; J: Expression of TIGIT, *P* =9.3 × 10-11. Red referred to the high expression group, and blue referred to the low expression group (grouped by median of RP5-881L22.5 expression). NK: Natural killer.



**Figure 4 Gene set enrichment analysis for the differentially expressed mRNAs.**



**Figure 5 Results of quantitative reverse transcription PCR for clinical specimens and cellular experiments.** A: Expression level of RP5-881L22.5 in tumor tissues and normal tissues among 4 patients’ surgical samples. Red referred to tumor tissues, and blue referred to adjacent normal tissues; B: Representative image of the siRNA + NC by flow cytometry; C: Representative image of the siRNA+RP5-881L22.5 by flow cytometry; D: The apoptosis ratio difference between siRNA + negative control (NC) group and siRNA + RP5-881L22.5; E: Effect of knockdown of RP5-881L22.5 on the transwell invasion assay of colorectal cancer cells. Scale bars: 50 μm. The magnification is 100 times the original size. Left: Representative image of the siRNA + NC; Right: Representative image of the siRNA + RP5-881L22.5.