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Research

Long non-coding RNA TMEM51-AS1 inhibits colorectal cancer progression

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Abstract
Colorectal cancer (CRC) is the third most common cause of death worldwide and has high mortality and a poor prognosis. Long non-coding RNAs (lncRNAs) are non-coding RNAs longer than 200 nucleotides that play roles in cancer through multiple mechanisms. TMEM51-AS1 is a newly discovered 40,650 bp lncRNA. Our results showed that TMEM51-AS1 expression was significantly downregulated in CRC tissues (fold change = 0.74, $P < 0.0001$). This finding was confirmed in 20 pairs of CRC carcinoma and paracancerous tissues (fold change = 0.5, $P < 0.001$). Additionally, TMEM51-AS1 expression was found to be significantly reduced in CRC cell lines compared to normal human intestinal epithelial cells ($P < 0.001$). Bioinformatic analysis revealed that TMEM51-AS1 expression was associated with immune escape, RNA methylation, and DNA damage and repair. TMEM51-AS1 may also activate energy metabolism pathways to participate in cancer development. Drug sensitivity analysis confirmed that several drugs are more effective in CRC patients with high expression of TMEM51-AS1. In conclusion, our study demonstrates that TMEM51-AS1 can suppress the progression of CRC.

Keywords LncRNA · TMEM51-AS1 · CRC · Expression · Downregulation · Progression

Abbreviations

chRCC	Chromophobe renal cell carcinoma
CRC	Colorectal cancer
FDR	False discovery rate
lncRNA	Long non-coding RNA
LSCC	Laryngeal squamous cell carcinoma
m1A	N1-methyladensoine
m5C	5-Methylcytosine
m6A	N6-methyladenosine

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NATs	Natural antisense transcripts
NES	Normalized enrichment score
TCGA	The Cancer Genome Atlas
TME	Tumor microenvironment
TAM	Tumor-associated macrophages

1 Introduction

Colorectal cancer (CRC) is a malignant tumor of the digestive system originating from the colorectal mucosal epithelium, with high morbidity and mortality rates [1]. Currently, CRC treatment typically involves surgical resection as the primary approach, supplemented by chemotherapy, radiotherapy, targeted therapy, and immunotherapy [2]. However, despite ongoing advancements in CRC treatments, a clinical cure remains uncommon, with most patients only able to extend their survival through these combination therapies [3, 4]. This phenomenon is primarily due to the development of treatment side effects, drug resistance, and the lack of predictive biomarkers for treatment efficacy [5–7]. Therefore, developing novel prognostic and therapeutic strategies for CRC, particularly targeting therapeutic approaches, is crucial to overcoming the limitations of existing therapies and improving clinical outcomes and patient prognosis [8].

Non-coding RNA (ncRNA) refers to RNA molecules that do not encode proteins. They play a key role in cancer development through gene regulation, shaping the tumor microenvironment, and acting as tumor-promoting or suppressing molecules [9, 10]. The expression patterns and functions of ncRNAs in the unique environment of cancer reveal their potential as diagnostic markers, prognostic indicators, and therapeutic targets [11]. With continuous research progress, treatments based on certain ncRNAs have gradually entered clinical application [12, 13].

TMEM51-AS1 is a 40,650 bp long non-coding RNA (lncRNA) located on human chromosome 1p36.21. According to the CNGBdb database, TMEM51-AS1 is expressed in 17 tissues including the brain, ovary, and prostate. A bioinformatics study of TCGA data found that TMEM51-AS1 expression was significantly positively correlated with increased overall survival of chromophobe renal cell carcinoma (chRCC) by comparing chRCC tissues and paracancerous tissues (59 vs 23). The study also established a ceRNA network of TMEM51-AS1 through correlation analysis [14]. Another bioinformatics study of TCGA data found that TMEM51-AS1 was highly expressed in laryngeal squamous cell carcinoma (LSCC) tissues and could be used as a potential biomarker for LSCC overall survival prognosis. This was determined by comparing 16 pairs of LSCC tissues and adjacent tissues. This study found that the regulation of TMEM51-AS1 is influenced by the methylation of its promoter CGI. When there is an increase in TMEM51-AS1 levels, it can function as a ceRNA for miR-106b, leading to a decrease in the expression of SNX21 and TRAPPC10 [15].

Overall, these advanced studies have strongly demonstrated the significant potential and value of TMEM51-AS1 as a novel cancer treatment target. However, current research on TMEM51-AS1 is limited in its coverage of various cancer types and its mechanisms of action. This study centers on TMEM51-AS1 as a critical regulatory molecule in colorectal cancer. We analyzed its differential expression patterns and explored its multifaceted regulatory roles within the tumor microenvironment. Our results reveal that TMEM51-AS1 is associated with multiple methylation events and contributes to tumor progression via pathways related to metabolic reprogramming and impaired DNA repair. Notably, TMEM51-AS1 is strongly correlated with cancer stem cell characteristics, suggesting a role in sustaining stemness and contributing to recurrence and treatment resistance. This provides a novel perspective for understanding the mechanisms of CRC progression. Furthermore, our findings indicate that TMEM51-AS1 is closely linked to the efficacy of chemotherapeutic agents and the regulation of immune checkpoint pathways. These associations highlight its potential as a therapeutic biomarker for both drug sensitivity and immunotherapy responsiveness. This study not only lays a solid foundation for further in-depth research but also provides new perspectives and strategies for the treatment of CRC.

2 Materials & methods

2.1 Sample selection and TMEM51-AS1 expression stratification in CRC analysis

We downloaded gene expression profiles and somatic mutation data for TCGA-COAD (521 tumor samples and 41 adjacent non-tumor samples) and TCGA-READ (177 tumor samples and 10 adjacent non-tumor samples) from the TCGA database. We also obtained data for normal intestinal tissue (308 samples) from the GTEx database. The GTF annotation file was downloaded from the Ensembl Genome Browser and gene IDs were converted into gene symbols. Genes with zero

expression (n = 20,061) were removed to extract an expression profile composed of 39,499 genes. After excluding samples lacking somatic mutation data, we successfully screened 614 TCGA colorectal cancer (CRC) samples. Subsequently, utilizing a median expression value of 0.889 (in transcripts/million, denoted as TPM) for TMEM51-AS1, we categorized the samples into two distinct groups: the TMEM51-AS1 high expression group and the low expression group. Specifically, CRC samples with TMEM51-AS1 expression values exceeding 0.889 were classified into the high expression group, comprising 307 patients. Conversely, samples with expression values below 0.889 were assigned to the low expression group, also consisting of 307 patients. This meticulous grouping approach provides robust data support for subsequent in-depth research endeavors.

2.2 Human CRC tissues and cell lines

In this study, we enrolled 20 CRC patients from Shaoxing People's Hospital with an average age of approximately 64 years (range: 37–83 years). Half of the patients were in T4 and N0 stages and 90% were in M0 stage. We found no significant difference in the expression ratio of TMEM51-AS1 between cancer tissues and adjacent non-tumor tissues when comparing patients with different tumor locations, TMN stages, stages or genders. All patients met the criteria of being diagnosed with CRC and having no other complications (Table 1). The study protocol received approval from the Ethics Committee of Shaoxing People's Hospital (2020-K-Y-254-01) and we confirm that all methods were performed in accordance with the relevant guidelines and regulations. These samples were stored at − 80 °C until required for use. This study utilized two types of human CRC cells: HCT-15 and LOVO cells, as well as immortalized human normal colonic epithelial NCM-460 cells (iCell Bioscience Inc., Shanghai, China). After being tested for mycoplasma contamination, all cell lines were cultured in 1640 medium supplemented with 10% bovine serum (Nanjing BioChannel Biotechnology Co., Ltd). The cells were maintained at 37℃ in a humidified atmosphere containing 5% CO_2.

2.3 TMEM51-AS1 correlation with RNA methylation modification

RNA methylation modification is an emerging player in post-transcriptional gene expression regulation. It has multiple roles in global regulation and fine-tuning of gene expression [16]. This modification affects gene expression by regulating RNA metabolism, splicing, stability, and translation. It plays an important role in regulating various biological functions and is associated with tumor development and progression [17]. As shown in Table S1, genes related to RNA methylation modification include 23 m6A modification genes [18], 12 m5C modification genes [19], and 10 m1A modification genes [20]. Our study explored the correlation between TMEM51-AS1 and RNA methylation modification by comparing the expression levels of RNA modification-related genes in high- and low-expression groups.

2.4 KEGG and GO Enrichment Analysis of TMEM51-AS1 in CRC Progression

To identify signaling pathways significantly associated with TMEM51-AS1 and investigate the molecular mechanisms underlying CRC progression, we conducted KEGG pathway and GO function enrichment analysis using GSEA software (version 4.2.3). Signature gene sets were collected from the Molecular Signature Database v7.1. The enrichment score indicates the extent to which a gene set in a pathway is upregulated or downregulated in a set of samples. Gene sets with an absolute normalized enrichment score greater than 1 and a false discovery rate (FDR) less than 0.25 were considered significantly enriched [21].

2.5 Association analysis of TMEM51-AS1 expression with somatic mutations and DNA repair genes in CRC

Somatic mutations play a crucial role in transforming normal cells into cancer cells by increasing cellular competitiveness and promoting cell proliferation [22]. We used the maftools package [23] to analyze somatic mutation data from CRC patients. This allowed us to identify genes with significantly different mutation rates between groups with high and low expression of TMEM51-AS1 and to determine which mutated genes were significantly associated with TMEM51-AS1. The DNA damage and repair system is responsible for repairing DNA damage caused by both endogenous and exogenous factors. It helps to repair gene mutations and maintain the genome stability of human cells [24]. We identified 210 DNA repair-related genes from the human DNA repair gene website (Table S2) and evaluated their relationship with TMEM51-AS1.

Table 1 Statistical analysis of clinical information from 20 CRC patients

Variables	Number	Expression ratio*	P value
Gender			
Male	14	0.59(0.4–0.85)	0.51
Female	6	0.65(0.28–0.92)	
Age			
> 64	10	0.68(0.39–0.92)	0.11
< 64	10	0.54(0.28–0.85)	
Tumor location			
Left colon cancer	12	0.60(0.28–0.85)	0.8
Right colon cancer	8	0.62(0.44–0.85)	
T			
T2	1	0.92	0.82
T3	8	0.58(0.40–0.84)	
T4	11	0.60(0.28–0.85)	
M			
M0	18	0.58(0.28–0.92)	0.23
M1	2	0.85	
Stage			
I	1	0.92	0.08
II	9	0.64(0.4–0.85)	
III	7	0.51(0.28–0.65)	
IV	3	0.85	
N			
N0	11	0.66(0.39–0.92)	0.4
N1	5	0.54(0.47–0.64)	
N2	4	0.56(0.28–0.85)	

*Expression ratio of TMEM51-AS1 between cancer tissues and adjacent non-tumor tissues

2.6 Correlation between TMEM51-AS1 and immune escape in CRC

The relationship between TMEM51-AS1 and immune checkpoint loci was evaluated using Pearson correlation analysis. Chemokines, a subfamily of cytokines, play a crucial role in immune cell trafficking and lymphoid tissue development [25]. They are involved in various cancer developmental processes such as angiogenesis, metastasis, cancer cell proliferation and stemness [26]. As key factors in tumor development, chemokines can be targets for immunotherapy [26]. We identified 46 chemokines from the literature [27] and investigated their correlation with TMEM51-AS1 by comparing their expression levels in high and low expression groups. The TIDE algorithm was used to predict the efficacy of immune checkpoint blockade therapy (anti-PD-1/anti-CTLA-4) [28]. The TIDE score represents the efficacy of immune drugs (anti-PD1, anti-CTLA4) on patients with CRC, IFNG can drive the maturation of innate immune cells [29]. Dysfunction represents T cell dysfunction and exclusion represents T cell-mediated rejection.

2.7 Exploring the relationship between TMEM51-AS1 and tumor stemness

Cancer stem cells (CSCs) are a type of tumor cell with the potential to differentiate into multiple lineages. They are often considered the initiating cells of cancer and have been shown to play a significant role in tumor growth, invasion, metastasis, and recurrence [30]. CSCs typically exhibit higher drug resistance and tolerance compared to normal tumor cells. CSC signaling can also modify the tumor microenvironment by directly modulating tumor-infiltrating lymphocytes for cancer development [31]. The stemness index is used to evaluate the similarity between tumor cells and stem cells and is associated with active biological processes in stem cells and a higher degree of tumor dedifferentiation. To investigate the relationship between TMEM51-AS1 and tumor stemness, we identified 26 stemness gene sets from the literature (Table S3) and used the ssGSEA algorithm to quantify the enrichment score of stemness genes between two groups. We

also used stem cell populations from the PCBC database and their differentiated ectoderm, mesoderm, and endoderm progenitor cell information as an initial dataset to train and derive the stemness index mRNAsi using the OCLR algorithm.

2.8 Assessing chemotherapeutic drug sensitivity in CRC patients based on TMEM51-AS1 expression levels

We used the pRRophic package to calculate the half-inhibitory concentration (IC50) of chemotherapeutic drugs for each CRC patient. This allowed us to assess each patient's sensitivity to chemotherapeutic drugs. We then compared the drug sensitivity differences between the high TMEM51-AS1 expression group and the low TMEM51-AS1 expression group for commonly used chemotherapeutic drugs.

2.9 Correlation between TMEM51-AS1 and energy metabolism pathways

Alterations in energy metabolism pathways are crucial to support cancer cell growth, division, and migration. We investigated the potential correlation between TMEM51-AS1 and these pathways. First, we identified 590 genes related to energy metabolism from the GeneCards database. We then compared the expression of these energy metabolism-related genes between high and low TMEM51-AS1 expression groups.

2.10 Statistical analysis

All statistical analyses were performed using R software (version 4.10). The ggplot2 package was utilized for graph visualization [32], while the maftools package was employed to analyze somatic mutation data. The mafCompare function was used to screen for genes with differences in mutation rates between high and low expression. GSEA software (Broad Institute, Massachusetts, USA) was employed to study the signaling pathway related to TMEM51-AS1. The Wilcoxon test was used to compare expression differences of RNA methylation modification-related genes between TMEM51-AS1 high and low expression groups. Additionally, the Wilcoxon test was used to compare the expression difference of TMEM51-AS1 between cancer and normal tissues. Statistical significance was defined as $p < 0.05$.

3 Results

3.1 TMEM51-AS1 is downregulated in CRC

We combined the COAD and READ samples from the TCGA database and removed samples without somatic mutation data. This resulted in a total of 614 CRC samples and 359 paracancerous samples. Wilcoxon test analysis revealed that TMEM51-AS1 expression in CRC tissues was significantly lower than in normal tissues (fold change = 0.74, $P < 0.0001$) (Fig. 1A). Additionally, we used qRT-PCR experiments to measure TMEM51-AS1 expression in 20 pairs of CRC cancer and paracancerous tissues (Fig. 1B). Our results showed that TMEM51-AS1 was significantly downregulated in CRC tissues (fold change = 0.6, $P < 0.001$). We also observed an decrease in TMEM51-AS1 expression in CRC cell lines compared to human normal epithelial cells (NCM460) (HCT-15: 0.6 times; LOVO: 0.5 times; $P < 0.001$) (Fig. 1C).

3.2 TMEM51-AS1 positively correlates with RNA methylation

RNA methylation can be catalyzed by writer enzymes [33] and reversed by eraser enzymes through demethylation [34]. Reader enzymes recognize and bind to modified RNA [35]. Our research found that writer-related genes such as ZC3H13, METTL3, METTL14, RBM15, RBM15B, VIRMA, TRMT6, TRMT61B, DNMT1, DNMT3 A, DNMT3B, NSUN2, NSUN3, and NSUN4 were more highly expressed in the high-expression group. Additionally, YTHDC1, YTHDC2, YTHDF1, YTHDF2, YTHDF3, HNRNPA2B1, IGF2BP2, ALYREF, and other reader-related genes had higher expression levels in the high-expression group (Fig. 2A-C). Therefore, TMEM51-AS1 may promote RNA methylation modification in CRC by increasing the expression of writer and reader-related genes and decreasing the expression of eraser genes.

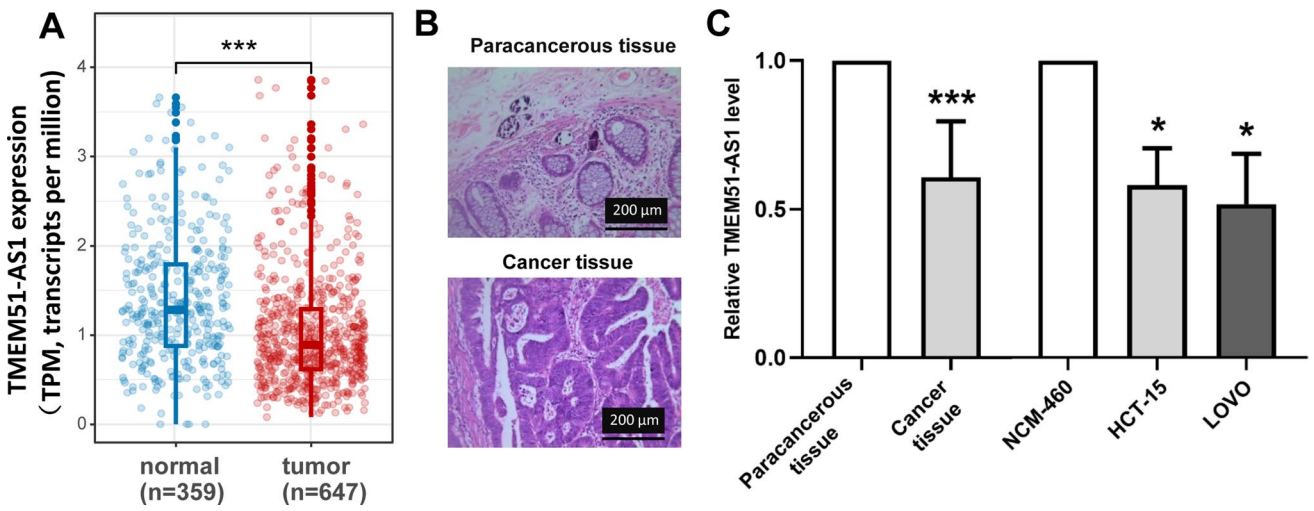


Fig. 1 TMEM51-AS1 is downregulated in CRC tissues. **A**. Compared with normal intestinal tissues, TMEM51-AS1 was underexpressed in CRC tissues. *** indicates P < 0.001. **B**. Pathological sections of CRC and paracancerous tissue were stained with HE. In the cancer tissue, tumor cells were arranged in irregular glandular and nest-like structures with large nuclei and visible mitotic figures. **C**. Paracancerous tissue showed mucosa interstitial edema, normal gland distribution, and locally visible calcified schistosome egg nodules. TMEM51-AS1 is also significantly upregulated in CRC HCT-15 and LOVO cells relative to human normal epithelial intestinal cells (NCM-460)

3.3 TMEM51-AS1-related pathways and biological functions

To investigate the potential mechanisms by which TMEM51-AS1 participates in cancerogenesis, we displayed the top three KEGG pathways with significant enrichment results based on Net enrichment score (NES) and P value. As shown in Fig. 3A, the biological functions associated with the high TMEM51-AS1 expression group include deubiquitinase activity, guanyl nucleotide exchange factor activity, and PDZ domain binding. The main biological functions associated with the low TMEM51-AS1 expression group include NADH dehydrogenase complex assembly, proton motive force-driven ATP synthesis, and respirasome. Figure 3B shows that the biological pathways associated with the high TMEM51-AS1 expression group include adherens junction, lysine degradation, and phosphatidylinositol signaling system pathways. The main biological pathways associated with the low TMEM51-AS1 expression group include allograft rejection, oxidative phosphorylation, and ribosome pathways.

3.4 TMEM51-AS1 is associated with TP53 somatic mutations and DNA repair

Somatic mutations can deactivate tumor suppressor genes and cause mutations in proto-oncogenes, leading to the development of tumors [36]. Our research examined the differences in somatically mutated genes among various subgroups and discovered that the TP53 mutation rate was significantly higher in the TMEM51-AS1 high expression group compared to the low expression group (60% vs 41%, P < 0.001) (Fig. 3C). TP53 plays a vital role in how cells respond to different stresses and ensures genome integrity. Defective TP53 genes can result in cancer development [37]. TMEM51-AS1 may increase tumor malignancy by promoting TP53 gene mutation. According to the results shown in Fig. 3C, the mutation rate of 11 genes was significantly lower in the TMEM51-AS1 high expression group compared to the TMEM51-AS1 low expression group (CDH5: 1.3% vs 7.5%; KIRREL3: 0.6% vs 5.9%; NOTCH2: 1.3% vs 7.2%; MYO10: 1.3% vs 7.8%; CTC1: 0.3% vs 4.6%; CYP4 F12: 0.3% vs 4.2%; PKN3: 0.3% vs 4.2%; ALDH18 A1: 0% vs 3.3%; RALBP1: 0% vs 3.3%; SPECC1: 1.9% vs 7.5%; KCNQ2: 1.6% vs 6.8%; all P < 0.001). Notably, these genes play critical roles in tumor progression by modulating key biological processes, including cell proliferation, invasion, angiogenesis, metabolic reprogramming, and chromosomal stability [38–47]. Dysregulation or functional aberrations in these genes may contribute to tumorigenesis and tumor maintenance, thereby positioning them as potential molecular targets for the development of novel therapeutic strategies in cancer treatment.

TMEM51-AS1 may reduce tumor malignancy by inhibiting proto-oncogene mutations. Figure 3D shows that the expression levels of 35 DNA repair-related genes were higher in the high TMEM51-AS1 expression group. This suggests

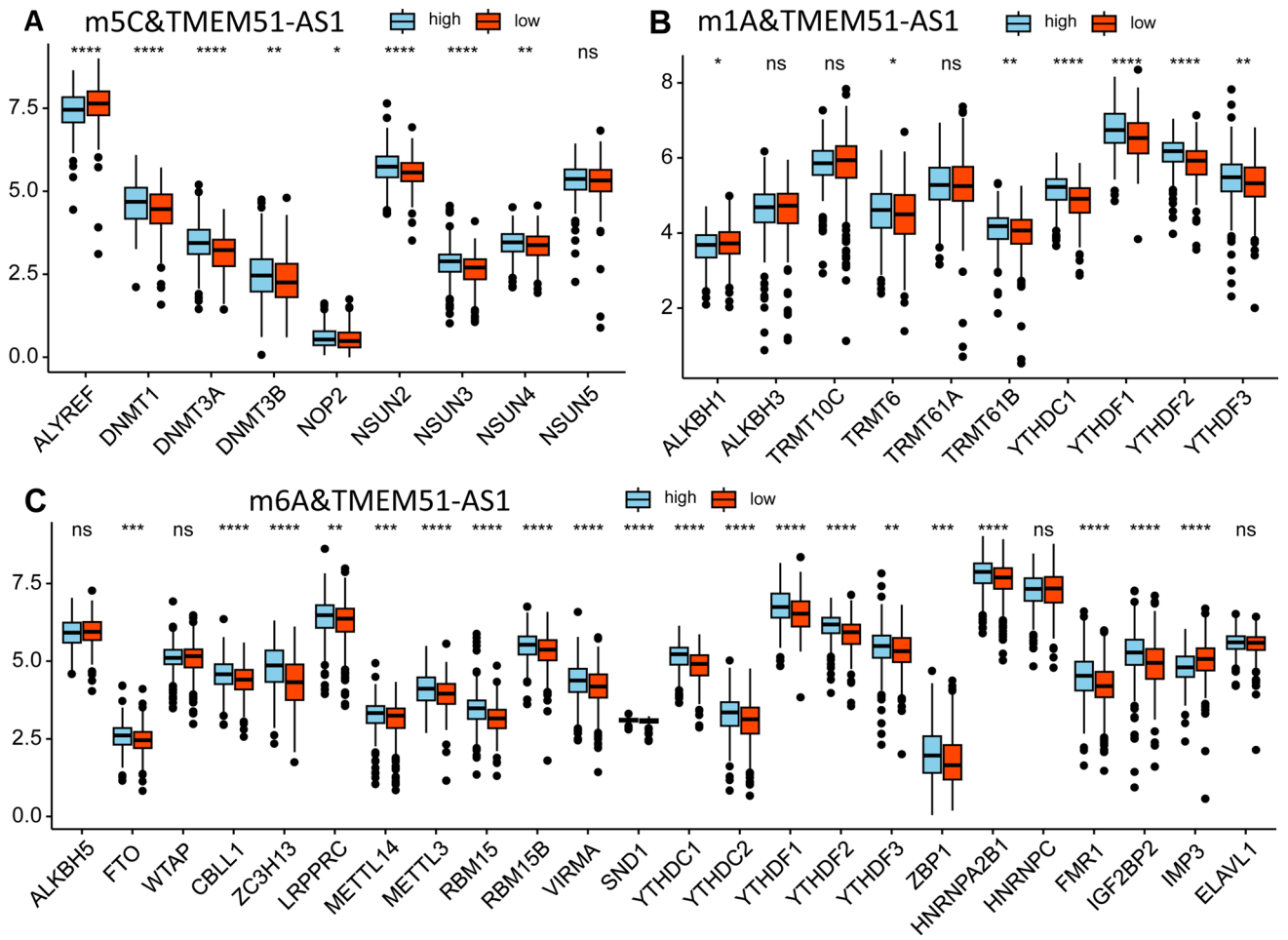


Fig. 2 TMEM51-AS1 positively correlates with RNA methylation modification. TMEM51-AS1 is positively correlated with m1 A (**A**), m6 A (**B**), and m5 C (**C**) modification-related genes. The statistical significance of these correlations is indicated by P-values less than 0.0001 (****) and 0.05 (*), with no significant difference indicated by "ns"

that the DNA repair system may be inhibited in patients with low TMEM51-AS1 expression. Therefore, TMEM51-AS1 may reduce tumor malignancy by enhancing DNA repair pathways.

3.5 TMEM51-AS1 inhibits immune escape of CRC cells

To investigate the relationship between immune escape of CRC cells and TMEM51-AS1, we used correlation analysis to assess the association of TMEM51-AS1 with stimulatory and inhibitory immune checkpoints. As shown in Fig. 4A, TMEM51-AS1 was inversely associated with multiple inhibitory immune checkpoint genes, including CD244, CD366, IDO1, LAG3, LGALS9, PDCD1, PD-L2, and TGFB. TMEM51-AS1 was positively associated with several stimulatory immune checkpoint genes, including ADORA2 A, CD160, KDR and IL6R, KLRK1, TNFRSF25, and TNFSF15. These results suggest that patients with low TMEM51-AS1 expression may be more prone to immune tolerance.

As shown in Fig. 4B, we found that 20 chemokines were more highly expressed in the TMEM51-AS1 low expression group compared to the TMEM51-AS1 high expression group. Monocytes are recruited to the tumor microenvironment (TME) and differentiate into tumor-associated macrophages (TAMs). Chemokines such as CCL2 [48], CCL5 [49], and CCL8 [50] are responsible for recruiting TAMs into the TME, where they promote tumor progression and suppress immune pathways. CCL18 is produced and secreted by TAMs and promotes tumor cell proliferation, migration, and invasion through receptors PITPNM3 and CCR8. It also recruits naive $CD4^+$ T cells into the TME and promotes their transformation into Treg cells [51]. Treg cells suppress anti-tumor immune responses and contribute to cancer immune escape [52]. Chemokines such as CCL1, CCL17, and CCL22 recruit Treg cells into the TME [53]. Chemokines such as CXCL9, CXCL10, and CXCL11

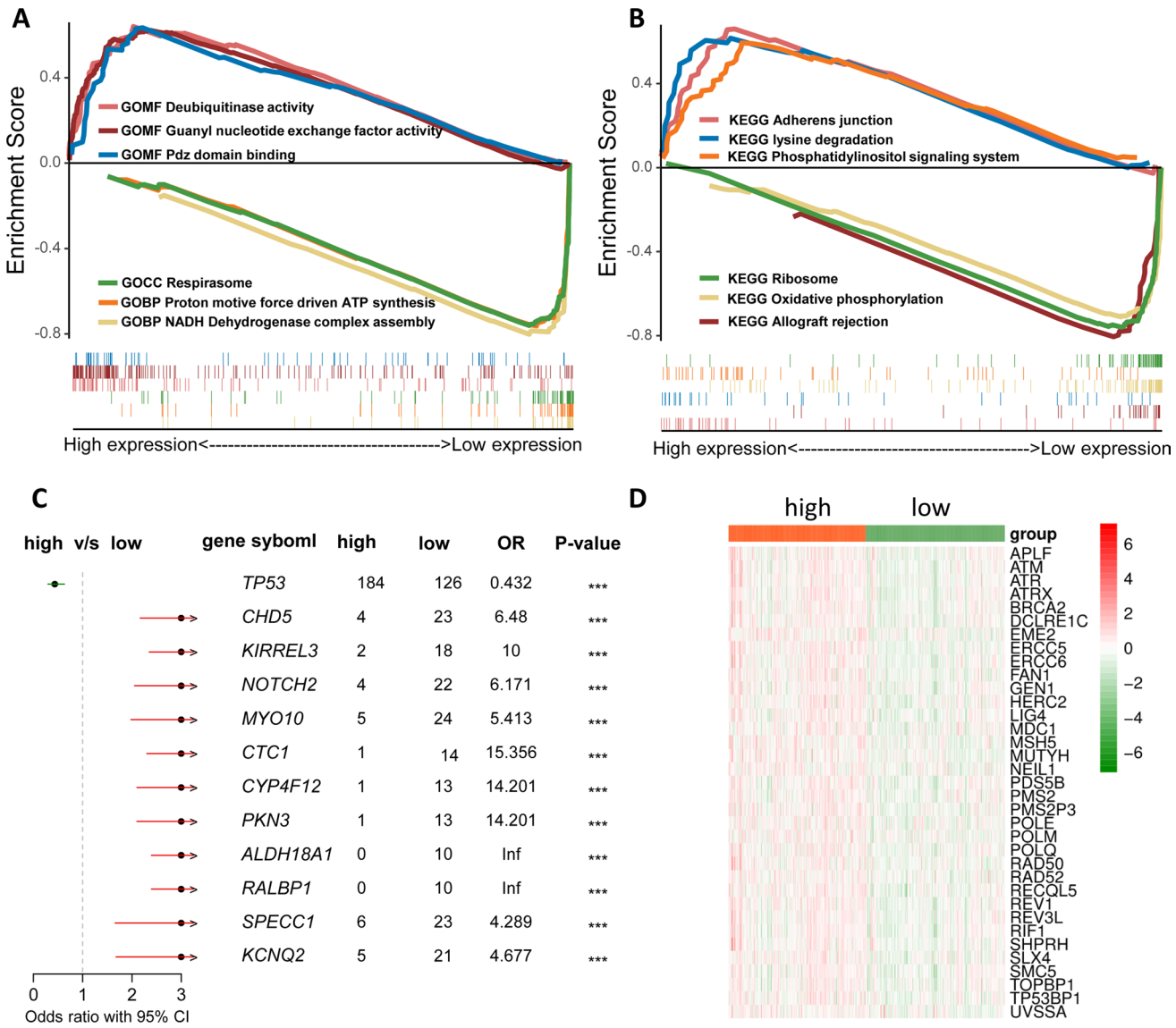


Fig. 3 TMEM51-AS1 is associated with DNA damage and repair. The main enriched GO molecular functions (**A**) and KEGG pathways (**B**) in the high and low expression groups of TMEM51-AS1. **C**. TMEM51-AS1 is also related to somatic mutation, with the OR representing the ratio and a P-value less than 0.001 indicating statistical significance. **D**. DNA repair genes associated with TMEM51-AS1

promote the proliferation and metastasis of liver cancer and colorectal cancer tumors [54]. Therefore, TMEM51-AS1 may enhance patients' immune response ability by inhibiting the expression of chemokines.

TIDE analysis showed that patients with low TMEM51-AS1 expression had higher TIDE scores, IFNG gene expression levels and T cell dysfunction scores compared to patients with high TMEM51-AS1 expression (Fig. 4C). A high TIDE score indicates a higher chance of immune escape. This suggests that patients with low TMEM51-AS1 expression may be more likely to experience immune escape while those with high TMEM51-AS1 expression may be more likely to benefit from immunotherapy. In summary, TMEM51-AS1 may inhibit CRC immune escape.

3.6 Relationship between TMEM51-AS1 and tumor stemness and chemotherapeutic drug sensitivity

As shown in Fig. 5A, the stemness score was higher in the TMEM51-AS1 low expression group and TMEM51-AS1 was significantly negatively correlated with tumor stemness index (mRNAsi) ($P < 0.001$) (Fig. 5B). This indicates that patients with low TMEM51-AS1 expression had a higher stemness index. We also observed that patients with high TMEM51-AS1

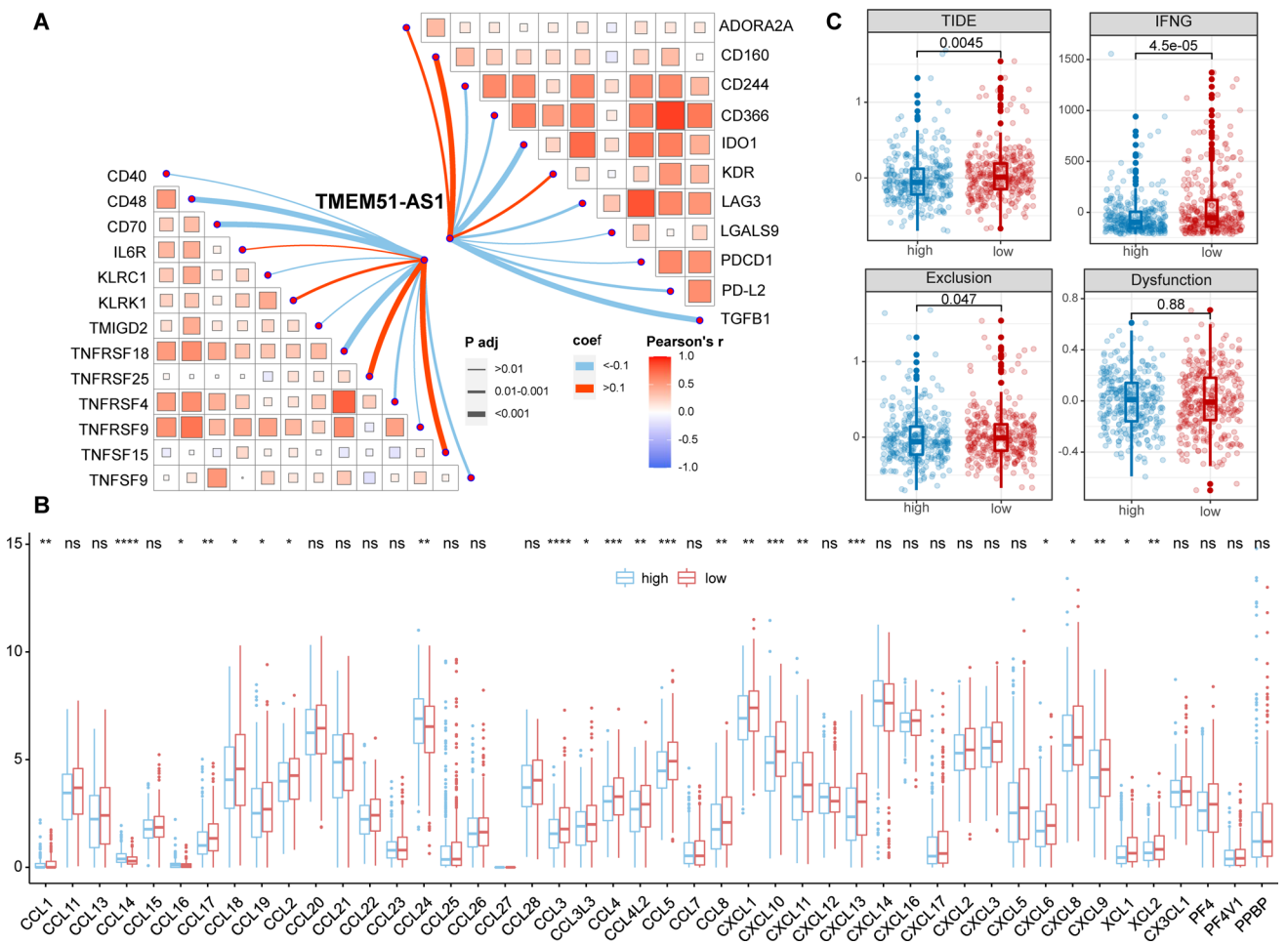


Fig. 4 TMEM51-AS1 inhibits immune escape in CRC cells. Correlation of TMEM51-AS1 with immune checkpoint loci (left: stimulatory loci, right: inhibitory loci). **B**. TMEM51-AS1 is significantly correlated with chemokines **C**. There is a significant difference in the response to immunotherapy between the high and low expression groups of TMEM51-AS1. The statistical significance of these correlations is indicated by P-values less than 0.0001 (****), 0.001 (***) and 0.01(**), with no significant difference indicated by "ns"

expression were more sensitive to chemotherapeutic drugs such as Axitinib, Gefitinib, Nilotinib, PD173074, PLX-4720, RO-3306, Vorinostat, and ZM447439 (Fig. 5C).

3.7 Relationship between TMEM51-AS1 and energy metabolism pathways

We conducted Wilcoxon detection analysis and identified 22 differentially expressed energy metabolism-related genes between the high and low TMEM51-AS1 expression groups. Among these, 17 genes were significantly negatively correlated with TMEM51-AS1 expression (Fig. 5D). Key regulatory genes such as ECHS1, GSTO1, TP1, VDAC1, and UBB were included in this group. Based on these findings, we speculate that TMEM51-AS1 may contribute to cancer progression by activating energy metabolism pathways.

4 Discussion

In this study, we analyzed the TCGA and GTEx databases and found that TMEM51-AS1 was significantly underexpressed in CRC tissues. Our experimental results further confirmed that TMEM51-AS1 expression was significantly lower in CRC tissues compared to normal intestinal tissues. We also found that TMEM51-AS1 expression was significantly lower in CRC cells compared to human normal intestinal epithelial cells. Through bioinformatics analysis, we obtained the following results: TMEM51-AS1 was positively correlated with RNA methylation modification and was mainly involved in energy

Fig. 5 Association of TMEM51-AS1 with tumor stemness and chemotherapeutic drug sensitivity. **A**. TMEM51-AS1 expression was correlated with a lower tumor stemness score. **B**. TMEM51-AS1 is negatively correlated with tumor stemness index mRNAsi. **C**. TMEM51-AS1 expression correlates with lower IC50 of multiple anticancer drugs. **D**. TMEM51-AS1 expression was correlated with energy metabolism pathway

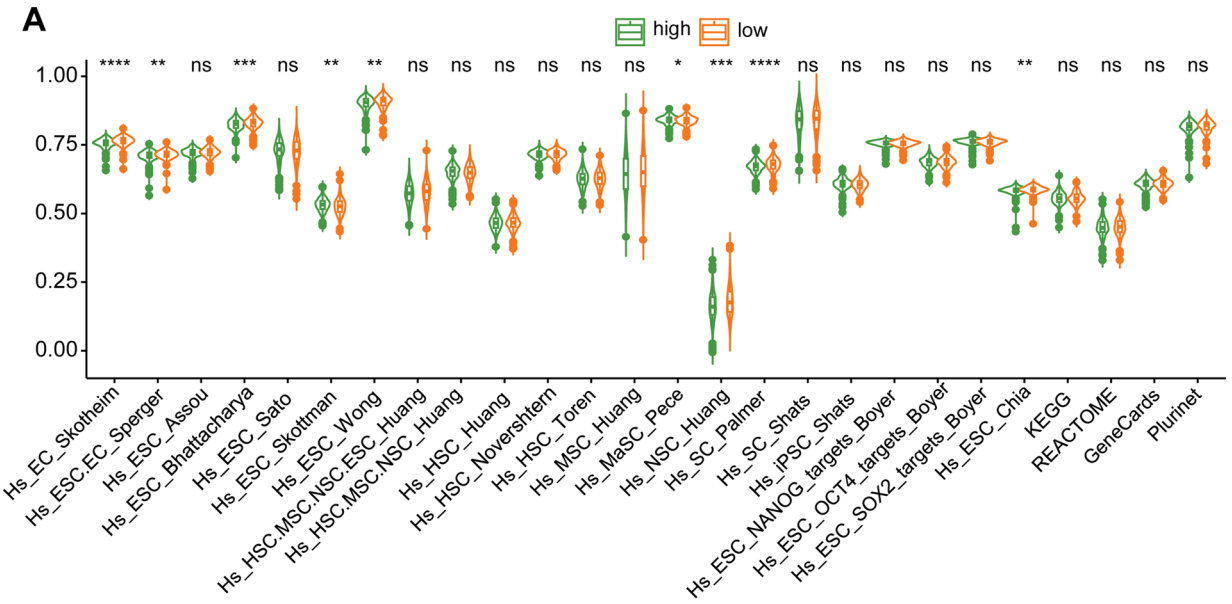
metabolism and signal transduction-related pathways. TMEM51-AS1 can reduce proto-oncogene mutations by enhancing DNA repair ability. Additionally, TMEM51-AS1 can inhibit immune escape of CRC cells, reduce tumor stemness, and increase sensitivity to anticancer drugs.

The expression of immune checkpoints affects the effectiveness of immunotherapy [55]. It has been reported that lncRNA POU3F3 promotes the migration and invasion of nasopharyngeal carcinoma cells by upregulating the expression of immune checkpoint TGFB1 [56]. In this study, we found that TMEM51-AS1 was inversely associated with inhibitory immune checkpoints. Chemokines can aggravate tumor cell malignancy [57]. Additionally, lncRNA-135528 suppresses tumor progression by upregulating CXCL10 through the JAK/STAT pathway [58]. RAMP2-AS1 inhibits the malignant phenotype of breast cancer by recruiting DNMT11 and DNMT1B to suppress CXCL3 expression [59]. We found that TMEM51-AS1 was negatively associated with chemokines. Therefore, high expression of TMEM51-AS1 in CRC may inhibit the expression of inhibitory immune checkpoints and chemokines, thereby inhibiting tumor progression.

Epigenetic modifications can trigger the silencing or overexpression of lncRNAs in cancer [60]. N1-methyladenosine (m1A) is a common and reversible post-transcriptional RNA modification that influences the processing, structure, and function of target RNA. It is positively linked to cancer development [61]. 5-Methylcytosine (m5C) is a reversible epigenetic modification that plays a crucial role in regulating RNA stability, protein synthesis, and transcription [62]. N6-methyladenosine (m6A) is an abundant and conserved internal transcriptional modification that has significant effects on RNA production and metabolism. It is involved in the development of various diseases, including cancer [33]. Research has shown that the m6A methylation modifier gene METTL3 can increase the expression of LINC01559, regulating the miR-106b-5p/PTEN axis to inhibit CRC progression [63]. Our paper found that the expression of m6A, m1A, and m5C reader- and writer-related genes was higher in the TMEM51-AS1 high expression group. At the same time, the expression of the m1A eraser gene ALKBH1 was lower in the TMEM51-AS1 high expression group. Notably, the m6A eraser gene FTO was highly expressed in the TMEM51-AS1 high expression group, and its specific mechanism of action requires further study. Down-regulation of RNA methylation modification-related gene METTL14 in liver cancer can affect the expression of cell cycle-related proteins and inhibit cell cycle progression [64]. Our study found that TMEM51-AS1 expression was associated with high levels of RNA methylation modification.

Deubiquitinase is a multistep enzymatic process involved in various cellular biological activities [65]. Guanyl nucleotide exchange factors promote cell proliferation and migration [66]. PDZ domain proteins may play key roles in cytoskeletal organization and in the organization of macromolecular complexes involved in intercellular signaling, transport, and cell wall formation [67]. The products of lysine degradation are involved in mitochondrial metabolism [68]. The phosphatidylinositol signaling system is a signal transduction system that can initiate subsequent signaling cascade reactions when signaling molecules such as hormones and neurotransmitters bind to its receptors [69]. Therefore, TMEM51-AS1 may be positively associated with signaling-related pathways. The NADH dehydrogenase complex catalyzes the oxidation of NADH to NAD^+ and participates in energy metabolism [70]. ATP synthase couples transmembrane proton transport to ATP synthesis from ADP and inorganic phosphate driven by a proton motive force [71]. The respirasome, an important part of the oxidative phosphorylation system, transfers electrons from electron donors to oxygen and generates a proton concentration gradient on the mitochondrial membrane through coupled proton translocation [72]. Oxidative phosphorylation is often upregulated in tumor cells to support the energetic demands required for tumor growth and proliferation [73]. Hyperactive ribosome biogenesis promotes cell growth and proliferation and enhances protein synthesis [74]. Therefore, TMEM51-AS1 may be negatively associated with pathways related to energy metabolism.

While most lncRNAs have been shown to promote cancer stemness traits, some lncRNAs also hinder cancer and cancer stem cell (CSC) traits [75]. For example, lncRNA DILC inhibits the expansion of hepatic CSCs by directly binding to the IL-6 promoter and inhibiting IL-3/STAT6 signaling [76]. LINC00467 has been found to promote Axitinib resistance through the miR-509-3p/PDGFRA axis [77]. LncRNA OSER1-AS1 interacts with the miR-612/FOXM1 axis to regulate Gefitinib resistance in lung adenocarcinoma [78]. Previous studies have demonstrated that lncRNA H19 enhances the stemness of CRC cells and promotes oxaliplatin resistance in CRC cells by activating the Wnt/β-catenin pathway [79]. LncRNA TUG1 accelerates CRC stem cell identity and oxaliplatin resistance [80]. Our study found that patients with high TMEM51-AS1 expression had higher sensitivity to chemotherapy drugs such as Axitinib, Gefitinib, Nilotinib, PD173074, PLX-4720, RO-3306, Vorinostat, and ZM447439 and lower tumor stemness. Therefore, we speculate that TMEM51-AS1 may enhance the sensitivity of cancer cells to chemotherapeutic drugs by reducing tumor stemness.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Hs_NSC_Huang
Hs_SC_Palmer
Hs_SC_Shats
Hs_iPSC_Shats
Hs_ESC_NANOG_targets_Boyer
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Hs_ESC_SOX2_targets_Boyer
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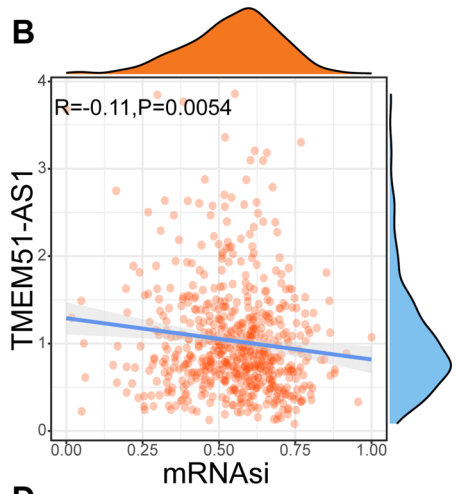
REACTOME
GeneCards
Plurinet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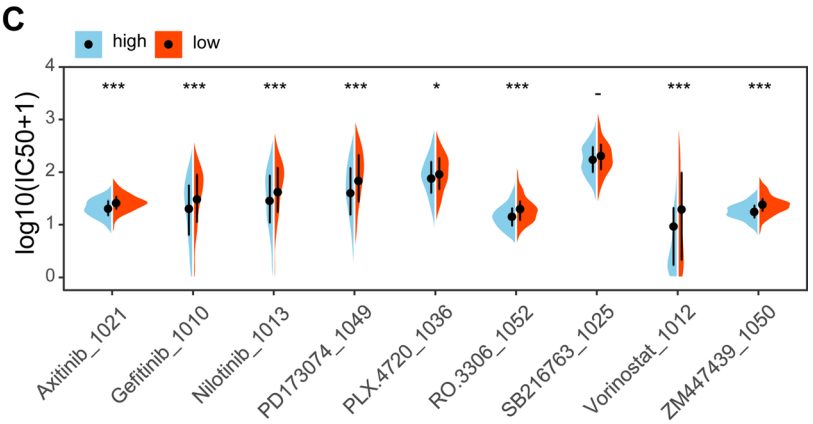
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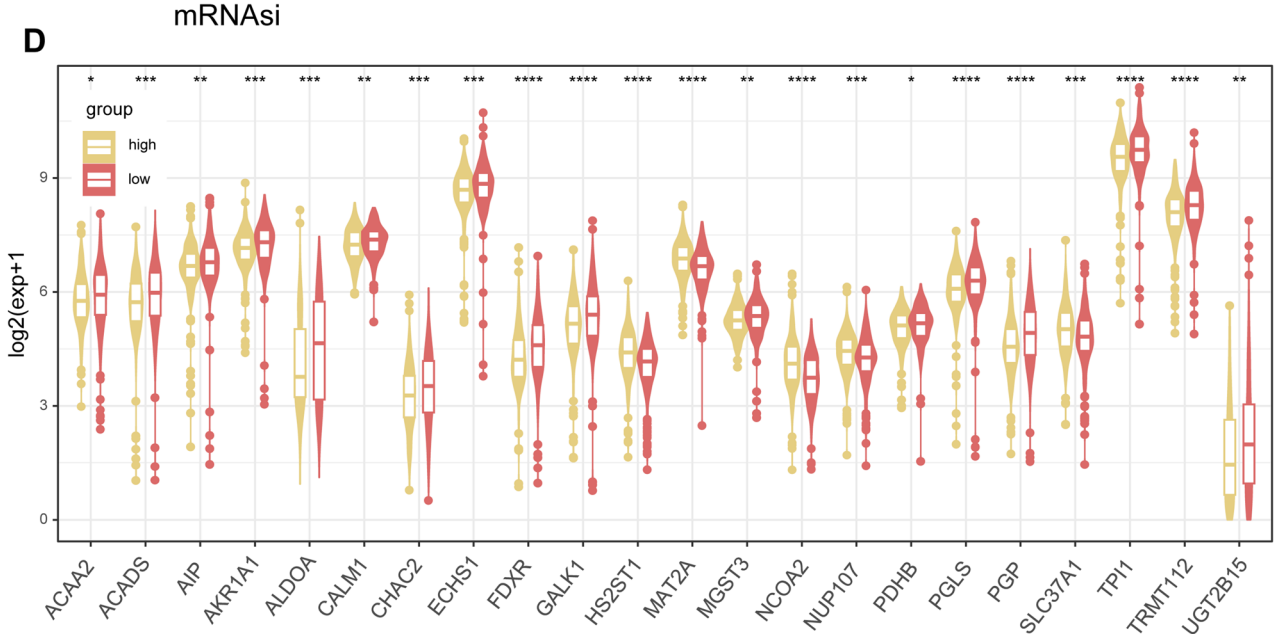
Vorinostat_1012
ZM447439_1050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GALK1
HS2ST1
MAT2A
MGST3
NCOA2
NUP107
PDHB
PGLS
PGP
SLC37A1
TPI1
TRMT112
UGT2B15

Despite the progress made in understanding the role of TMEM51-AS1 in CRC, there are still several limitations to the current research. Firstly, this study had a small sample size and its findings need to be confirmed with larger samples and in other populations. Secondly, only HCT-15 and LOVO cell lines were used in the experiment and further research should include more CRC cell lines. Additionally, animal models are required to validate the results under in vivo conditions. Lastly, the molecular mechanism of TMEM51-AS1 in CRC is not yet fully understood and more research is needed to provide a theoretical basis for targeted therapy through TMEM51-AS1 in CRC. Broadly, strategies for restoring or reactivating gene expression are typically employed to counteract gene silencing caused by aberrant methylation or histone modifications. In contrast, gene upregulation strategies aim to enhance transcriptional or translational efficiency beyond physiological levels through various technical approaches [81, 82]. Numerous studies have demonstrated that lncRNAs participate in genome organization, cellular architecture, and gene expression through diverse mechanisms, including epigenetic regulation—such as the formation of nucleic acid–protein complexes—as well as modulation of RNA, DNA, or protein molecules implicated in cancer progression. LncRNAs often display disease-specific, tissue-specific, and cell-type-specific expression patterns [83]. In this context, downregulation of TMEM51-AS1 may facilitate immune escape by promoting T cell exhaustion and modulating immune checkpoint expression [84]. It may also contribute to chemotherapy resistance by regulating apoptotic signaling pathways or enhancing the activity of drug efflux pumps [85]. Additionally, TMEM51-AS1 appears to promote tumor cell migration and invasion through its influence on extracellular matrix remodeling [86]. Despite these associations, the precise regulatory mechanisms of TMEM51-AS1 remain to be elucidated. Further in vitro and in vivo studies are necessary to validate its direct molecular targets, which will be essential for guiding the development of effective, targeted therapeutic strategies. Meanwhile, the emergence of comprehensive cancer omics platforms and advanced gene interference technologies is significantly accelerating the discovery and functional annotation of lncRNAs involved in tumor biology [83].

No studies have reported the role of TMEM51-AS1 in CRC so far. Our study suggests that TMEM51-AS1 may function as a tumor suppressor gene to suppress CRC progression. This was confirmed by experiments showing low expression of TMEM51-AS1 in CRC tissues and cells. TMEM51-AS1 is associated with RNA methylation and plays a role in energy metabolism and signal transduction. It can enhance DNA repair ability to reduce proto-oncogene mutations. TMEM51-AS1 can also prevent immune escape of CRC cells, decrease tumor stemness, and increase the effectiveness of anticancer drugs. These findings provide new insights into the role of lncRNAs in CRC progression and suggest a potential new therapeutic target for CRC.

5 Websites

TCGA database: https://www.cancer.gov/about-nci/organization/ccg/research/structural-genomics/tcga
CNGBdb: https://db.cngb.org/search/gene/200197/
Ensembl genome browser: http://www.ensembl.org
Human DNA repair genes: https://www.mdanderson.org/documents/Labs/Wood-Laboratory/human-dna-repair-genes.html
GTEx: https://www.gtexportal.org/home/

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Author contributions DW, QX, XS, YM, JM, JL, WZ, XZ collected and analyzed the literature, drafted the figures, and wrote the manuscript. QD collected the colorectal and paracancerous tissues. SD and HL conceived the idea and gave the final approval of the submitted version. All authors have read and agreed to the published version of the manuscript.

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Data availability All relevant data are within the paper and its Supporting Information files.

Declarations

Ethics approval and consent to participate This study was approved by the Ethics Committee of Shaoxing People's Hospital (No. 2020-K-Y-254-01). We confirm that the informed consent was obtained from all the participants and/or their legal guardian(s) in the study.

Competing interests The authors declare no competing interests.

References

1. Krul MF, Elferink MAG, Kok NFM, Dekker E, Lansdorp-Vogelaar I, Meijer GA, et al. Initial impact of national CRC screening on incidence and advanced colorectal cancer. Clin Gastroenterol Hepatol. 2023;21(797–807): e3.
2. Biller LH, Schrag D. Diagnosis and treatment of metastatic colorectal cancer: a review. JAMA. 2021;325:669–85.
3. Singh P, Waghambare P, Khan TA, Omri A. Colorectal cancer management: strategies in drug delivery. Expert Opin Drug Deliv. 2022;19:653–70.
4. Ciardiello F, Ciardiello D, Martini G, Napolitano S, Tabernero J, Cervantes A. Clinical management of metastatic colorectal cancer in the era of precision medicine. CA Cancer J Clin. 2022;72:372–401.
5. Yang L, Yang J, Kleppe A, Danielsen HE, Kerr DJ. Personalizing adjuvant therapy for patients with colorectal cancer. Nat Rev Clin Oncol. 2024;21:67–79.
6. Zhao H, Ming T, Tang S, Ren S, Yang H, Liu M, et al. Wnt signaling in colorectal cancer: pathogenic role and therapeutic target. Mol Cancer. 2022;21:144.
7. Ganesh K. Optimizing immunotherapy for colorectal cancer. Nat Rev Gastroenterol Hepatol. 2022;19:93–4.
8. Sveen A, Kopetz S, Lothe RA. Biomarker-guided therapy for colorectal cancer: strength in complexity. Nat Rev Clin Oncol. 2020;17:11–32.
9. Yan H, Bu P. Non-coding RNA in cancer. Essays Biochem. 2021;65:625–39.
10. Slack FJ, Chinnaiyan AM. The Role of Non-coding RNAs in oncology. Cell. 2019;179:1033–55.
11. Anastasiadou E, Jacob LS, Slack FJ. Non-coding RNA networks in cancer. Nat Rev Cancer. 2018;18:5–18.
12. Rupaimoole R, Slack FJ. MicroRNA therapeutics: towards a new era for the management of cancer and other diseases. Nat Rev Drug Discov. 2017;16:203–22.
13. Mishra R, Patel H, Alanazi S, Kilroy MK, Garrett JT. PI3K inhibitors in cancer: clinical implications and adverse effects. Int J Mol Sci. 2021. https://doi.org/10.3390/ijms22073464.
14. He HT, Xu M, Kuang Y, Han XY, Wang MQ, Yang Q. Biomarker and competing endogenous RNA potential of tumor-specific long noncoding RNA in chromophobe renal cell carcinoma. Onco Targets Ther. 2016;9:6399–406.
15. Hui L, Wang J, Zhang J, Long J. lncRNA TMEM51-AS1 and RUSC1-AS1 function as ceRNAs for induction of laryngeal squamous cell carcinoma and prediction of prognosis. PeerJ. 2019;7: e7456.
16. Helm M, Motorin Y. Detecting RNA modifications in the epitranscriptome: predict and validate. Nat Rev Genet. 2017;18:275–91.
17. Song P, Tayier S, Cai Z, Jia G. RNA methylation in mammalian development and cancer. Cell Biol Toxicol. 2021;37:811–31.
18. Zhao Q, Zhao Y, Hu W, Zhang Y, Wu X, Lu J, et al. m(6)A RNA modification modulates PI3K/Akt/mTOR signal pathway in Gastrointestinal Cancer. Theranostics. 2020;10:9528–43.
19. Hu J, Othmane B, Yu A, Li H, Cai Z, Chen X, et al. 5mC regulator-mediated molecular subtypes depict the hallmarks of the tumor microenvironment and guide precision medicine in bladder cancer. BMC Med. 2021;19:289.
20. Zhao M, Shen S, Xue C. A Novel m1A-score model correlated with the immune microenvironment predicts prognosis in hepatocellular carcinoma. Front Immunol. 2022;13: 805967.
21. Domingues P, Gonzalez-Tablas M, Otero A, Pascual D, Miranda D, Ruiz L, et al. Tumor infiltrating immune cells in gliomas and meningiomas. Brain Behav Immun. 2016;53:1–15.
22. Frank SA, Nowak MA. Problems of somatic mutation and cancer. BioEssays. 2004;26:291–9.
23. Mayakonda A, Lin DC, Assenov Y, Plass C, Koeffler HP. Maftools: efficient and comprehensive analysis of somatic variants in cancer. Genome Res. 2018;28:1747–56.
24. Zhang L, Meng F. Zhong D [DNA Damage Repair System and Antineoplastic Agents in Lung Cancer]. Zhongguo Fei Ai Za Zhi. 2022;25:434–42.
25. Kohli K, Pillarisetty VG, Kim TS. Key chemokines direct migration of immune cells in solid tumors. Cancer Gene Ther. 2022;29:10–21.
26. Bule P, Aguiar SI, Aires-Da-Silva F, Dias JNR. Chemokine-directed tumor microenvironment modulation in cancer immunotherapy. Int J Mol Sci. 2021. https://doi.org/10.3390/ijms22189804.
27. Dangaj D, Bruand M, Grimm AJ, Ronet C, Barras D, Duttagupta PA, et al. Cooperation between constitutive and inducible chemokines enables t cell engraftment and immune attack in solid tumors. Cancer Cell. 2019;35(885–900): e10.
28. Jiang P, Gu S, Pan D, Fu J, Sahu A, Hu X, et al. Signatures of T cell dysfunction and exclusion predict cancer immunotherapy response. Nat Med. 2018;24:1550–8.
29. Benci JL, Johnson LR, Choa R, Xu Y, Qiu J, Zhou Z, et al. Opposing functions of interferon coordinate adaptive and innate immune responses to cancer immune checkpoint blockade. Cell. 2019;178(933–48): e14.
30. Sun JH, Luo Q, Liu LL, Song GB. Liver cancer stem cell markers: Progression and therapeutic implications. World J Gastroenterol. 2016;22:3547–57.
31. Lytle NK, Barber AG, Reya T. Stem cell fate in cancer growth, progression and therapy resistance. Nat Rev Cancer. 2018;18:669–80.
32. Ito K, Murphy D. Application of ggplot2 to Pharmacometric Graphics. CPT Pharmacometrics Syst Pharmacol. 2013;2: e79.

33. Deng LJ, Deng WQ, Fan SR, Chen MF, Qi M, Lyu WY, et al. m6A modification: recent advances, anticancer targeted drug discovery and beyond. Mol Cancer. 2022;21:52.
34. Chen YS, Yang WL, Zhao YL, Yang YG. Dynamic transcriptomic m(5) C and its regulatory role in RNA processing. Wiley Interdiscip Rev RNA. 2021;12: e1639.
35. Wiener D, Schwartz S. The epitranscriptome beyond m(6)A. Nat Rev Genet. 2021;22:119–31.
36. Wang E. Understanding genomic alterations in cancer genomes using an integrative network approach. Cancer Lett. 2013;340:261–9.
37. Aubrey BJ, Strasser A, Kelly GL. Tumor-suppressor functions of the TP53 pathway. Cold Spring Harb Perspect Med. 2016. https://doi.org/10.1101/cshperspect.a026062.
38. Geng P, Qin W, Xu G. Proline metabolism in cancer. Amino Acids. 2021;53:1769–77.
39. Mayca Pozo F, Geng X, Tamagno I, Jackson MW, Heimsath EG, Hammer JA, et al. MYO10 drives genomic instability and inflammation in cancer. Sci Adv. 2021;7:6908.
40. Wu Z, Owens C, Chandra N, Popovic K, Conaway M, Theodorescu D. RalBP1 is necessary for metastasis of human cancer cell lines. Neoplasia. 2010;12:1003–12.
41. Priimak AA, Bentsianov AD, Red'kin AN. Electric stimulation of phrenic nerves in pulmonary phthisiosurgery. Grudn Khir. 1986;3:56–9.
42. Wang Y, Zhou X, Han P, Lu Y, Zhong X, Yang Y, et al. Inverse correlation of miR-27a-3p and CDH5 expression serves as a diagnostic biomarker of proliferation and metastasis of clear cell renal carcinoma. Pathol Res Pract. 2021;220: 153393.
43. Ciaccio C, Leonardi E, Polli R, Murgia A, D'Arrigo S, Granocchio E, et al. A missense de novo variant in the CASK-interactor KIRREL3 gene leading to neurodevelopmental disorder with mild cerebellar hypoplasia. Neuropediatrics. 2021;52:484–8.
44. Yang B, Zhang X, Zhou H, Zhang X, Yang W, Lu J, et al. Preliminary study on the role and mechanism of KIRREL3 in the development of esophageal squamous cell carcinoma. Pathol Res Pract. 2022;237: 154025.
45. Jarrar YB, Lee SJ. Molecular functionality of cytochrome P450 4 (CYP4) genetic polymorphisms and their clinical implications. Int J Mol Sci. 2019. https://doi.org/10.3390/ijms20174274.
46. Zhang Y, Zou J, Chen R. An M0 macrophage-related prognostic model for hepatocellular carcinoma. BMC Cancer. 2022;22:791.
47. Gu P, Min JN, Wang Y, Huang C, Peng T, Chai W, Chang S. CTC1 deletion results in defective telomere replication, leading to catastrophic telomere loss and stem cell exhaustion. EMBO J. 2012;31:2309–21.
48. Xu M, Wang Y, Xia R, Wei Y, Wei X. Role of the CCL2-CCR2 signalling axis in cancer: mechanisms and therapeutic targeting. Cell Prolif. 2021;54: e13115.
49. Walens A, DiMarco AV, Lupo R, Kroger BR, Damrauer JS, Alvarez JV. CCL5 promotes breast cancer recurrence through macrophage recruitment in residual tumors. Elife. 2019. https://doi.org/10.7554/eLife.43653.
50. Zhang X, Chen L, Dang WQ, Cao MF, Xiao JF, Lv SQ, et al. CCL8 secreted by tumor-associated macrophages promotes invasion and stemness of glioblastoma cells via ERK1/2 signaling. Lab Invest. 2020;100:619–29.
51. Korbecki J, Olbromski M, Dziegiel P. CCL18 in the Progression of Cancer. Int J Mol Sci. 2020. https://doi.org/10.3390/ijms21217955.
52. Attias M, Al-Aubodah T, Piccirillo CA. Mechanisms of human FoxP3(+) T(reg) cell development and function in health and disease. Clin Exp Immunol. 2019;197:36–51.
53. Ohue Y, Nishikawa H. Regulatory T (Treg) cells in cancer: can Treg cells be a new therapeutic target? Cancer Sci. 2019;110:2080–9.
54. Cambien B, Karimdjee BF, Richard-Fiardo P, Bziouech H, Barthel R, Millet MA, et al. Organ-specific inhibition of metastatic colon carcinoma by CXCR3 antagonism. Br J Cancer. 2009;100:1755–64.
55. Chan TA, Yarchoan M, Jaffee E, Swanton C, Quezada SA, Stenzinger A, Peters S. Development of tumor mutation burden as an immunotherapy biomarker: utility for the oncology clinic. Ann Oncol. 2019;30:44–56.
56. Li W, Wu X, She W. LncRNA POU3F3 promotes cancer cell migration and invasion in nasopharyngeal carcinoma by up-regulating TGF-beta1. 2019. Biosci Rep. https://doi.org/10.1042/BSR20181632.
57. Li S, Hu X, Yu S, Yi P, Chen R, Huang Z, et al. Hepatic stellate cell-released CXCL1 aggravates HCC malignant behaviors through the MIR4435-2HG/miR-506-3p/TGFB1 axis. Cancer Sci. 2023;114:504–20.
58. Wang P, Peng X, Zhang J, Wang Z, Meng J, Cen B, et al. LncRNA-135528 inhibits tumor progression by up-regulating CXCL10 through the JAK/STAT pathway. Apoptosis. 2018;23:651–66.
59. Li L, Gan YP, Peng H. RAMP2-AS1 inhibits CXCL11 expression to suppress malignant phenotype of breast cancer by recruiting DNMT1 and DNMT3B. Exp Cell Res. 2022;416: 113139.
60. Dong Z, Zhang A, Liu S, Lu F, Guo Y, Zhang G, et al. Aberrant methylation-mediated silencing of lncRNA MEG3 functions as a ceRNA in esophageal Cancer. Mol Cancer Res. 2017;15:800–10.
61. Li J, Zhang H, Wang H. N(1)-methyladenosine modification in cancer biology: current status and future perspectives. Comput Struct Biotechnol J. 2022;20:6578–85.
62. Song H, Zhang J, Liu B, Xu J, Cai B, Yang H, et al. Biological roles of RNA m(5)C modification and its implications in Cancer immunotherapy. Biomark Res. 2022;10:15.
63. Shi K, Yang S, Chen C, Shao B, Guo Y, Wu X, et al. RNA methylation-mediated LINC01559 suppresses colorectal cancer progression by regulating the miR-106b-5p/PTEN axis. Int J Biol Sci. 2022;18:3048–65.
64. Ma JZ, Yang F, Zhou CC, Liu F, Yuan JH, Wang F, et al. METTL14 suppresses the metastatic potential of hepatocellular carcinoma by modulating N(6) -methyladenosine-dependent primary MicroRNA processing. Hepatology. 2017;65:529–43.
65. Sun T, Liu Z, Yang Q. The role of ubiquitination and deubiquitination in cancer metabolism. Mol Cancer. 2020;19:146.
66. Hamel B, Monaghan-Benson E, Rojas RJ, Temple BR, Marston DJ, Burridge K, Sondek J. SmgGDS is a guanine nucleotide exchange factor that specifically activates RhoA and RhoC. J Biol Chem. 2011;286:12141–8.
67. Gardiner J, Overall R, Marc J. PDZ domain proteins: "dark matter" of the plant proteome? Mol Plant. 2011;4:933–7.
68. Nemeria NS, Nagy B, Sanchez R, Zhang X, Leandro J, Ambrus A, et al. Functional versatility of the human 2-oxoadipate dehydrogenase in the L-lysine degradation pathway toward its non-cognate substrate 2-oxopimelic acid. Int J Mol Sci. 2022. https://doi.org/10.3390/ijms23158213.
69. Bizzarri M, Fuso A, Dinicola S, Cucina A, Bevilacqua A. Pharmacodynamics and pharmacokinetics of inositol(s) in health and disease. Expert Opin Drug Metab Toxicol. 2016;12:1181–96.

70. Marquez J, Kratchmarova I, Akimov V, Unda F, Ibarretxe G, Clerigue AS, et al. NADH dehydrogenase complex I is overexpressed in incipient metastatic murine colon cancer cells. Oncol Rep. 2019;41:742–52.
71. Feniouk BA, Suzuki T, Yoshida M. Regulatory interplay between proton motive force, ADP, phosphate, and subunit epsilon in bacterial ATP synthase. J Biol Chem. 2007;282:764–72.
72. Wu M, Gu J, Zong S, Guo R, Liu T, Yang M. Research journey of respirasome. Protein Cell. 2020;11:318–38.
73. Boreel DF, Span PN, Heskamp S, Adema GJ, Bussink J. Targeting oxidative phosphorylation to increase the efficacy of radio- and immune-combination therapy. Clin Cancer Res. 2021;27:2970–8.
74. Elhamamsy AR, Metge BJ, Alsheikh HA, Shevde LA, Samant RS. Ribosome biogenesis: a central player in cancer metastasis and therapeutic resistance. Cancer Res. 2022;82:2344–53.
75. Jahangiri L, Ishola T, Pucci P, Trigg RM, Pereira J, Williams JA, et al. The Role of autophagy and lncRNAs in the maintenance of cancer stem cells. Cancers. 2021. https://doi.org/10.3390/cancers13061239.
76. Wang X, Sun W, Shen W, Xia M, Chen C, Xiang D, et al. Long non-coding RNA DILC regulates liver cancer stem cells via IL-6/STAT3 axis. J Hepatol. 2016;64:1283–94.
77. Li W, He Y, Chen W, Man W, Fu Q, Tan H, et al. Knockdown of LINC00467 contributed to Axitinib sensitivity in hepatocellular carcinoma through miR-509-3p/PDGFRA axis. Gene Ther. 2021;28:634–45.
78. Shi T, Sun W, Shi YL, Wang Q, Yan ZX, Zhang M. LncRNA OSER1-AS1 interacts with miR-612/FOXM1 axis to modulate gefitinib resistance of lung adenocarcinoma. Am J Transl Res. 2021;13:1365–76.
79. Ren J, Ding L, Zhang D, Shi G, Xu Q, Shen S, et al. Carcinoma-associated fibroblasts promote the stemness and chemoresistance of colorectal cancer by transferring exosomal lncRNA H19. Theranostics. 2018;8:3932–48.
80. Sun J, Zhou H, Bao X, Wu Y, Jia H, Zhao H, Liu G. lncRNA TUG1 facilitates colorectal cancer stem cell characteristics and chemoresistance by enhancing GATA6 protein stability. Stem Cells Int. 2021;2021:1075481.
81. Nuñez JK, Chen J, Pommier GC, Cogan JZ, Replogle JM, Adriaens C, et al. Genome-wide programmable transcriptional memory by CRISPR-based epigenome editing. Cell. 2021;184:2503-19.e17.
82. Khorkova O, Stahl J, Joji A, Volmar CH, Wahlestedt C. Amplifying gene expression with RNA-targeted therapeutics. Nat Rev Drug Discov. 2023;22:539–61.
83. Coan M, Haefliger S, Ounzain S, Johnson R. Targeting and engineering long non-coding RNAs for cancer therapy. Nat Rev Genet. 2024;25:578–95.
84. Kumar D, Gurrapu S, Wang Y, Bae SY, Pandey PR, Chen H, et al. LncRNA Malat1 suppresses pyroptosis and T cell-mediated killing of incipient metastatic cells. Nat Cancer. 2024;5:262–82.
85. Zhu C, Wang X, Wang Y, Wang K. Functions and underlying mechanisms of lncRNA HOTAIR in cancer chemotherapy resistance. Cell Death Discov. 2022;8:383.
86. Winkler J, Abisoye-Ogunniyan A, Metcalf KJ, Werb Z. Concepts of extracellular matrix remodelling in tumour progression and metastasis. Nat Commun. 2020;11:5120.