LncRNA PCAT6 as a Predictor of Poor Colorectal Cancer Patient Prognosis: A TCGA Dataset Analysis

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Research Article

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Abstract

Background: The long non-coding RNA (lncRNA)) PCAT6 has been studied in many cancers, yet its relationship with colorectal cancer (CRC) remains poorly defined. Here, we conducted an analysis of The Cancer Genome Atlas (TCGA) database to better clarify the role of PCAT6 in this cancer type.

Materials and Methods: Wilcoxon rank-sum tests were utilized to assess relative levels of PCAT6 in CRC tumors and normal tissues, while logistic regression analyses were utilized to compare the relationships between PCAT6 levels and clinicopathological findings. Kaplan-Meier curves and Cox regression analyses were used to gauge correlations between PCAT6 and patient survival outcomes, while the biological roles of this lncRNA were investigated via a gene set enrichment analysis (GSEA) approach.

Results: PCAT6 levels were significantly correlated with CRC patient N stage (OR = 1.8 for N1&N2 vs. N0), lymphatic invasion (OR = 1.9 for Yes vs. No), M stage (OR = 2.1 for M1 vs. M0), CEA level (OR = 1.9 for >5 vs. ≤5), perineural invasion (OR = 1.9 for Yes vs. No), pathologic stage (OR = 1.9 for Stage IIIIV vs. Stage I/II), and neoplasm type (OR = 2.1 for rectal adenocarcinoma vs. colon adenocarcinoma) (all $P < 0.05$). CRC patients expressing higher PCAT6 levels exhibited poorer survival outcomes than those expressing low levels of this lncRNA ($P = 0.017$), and in univariate analyses, higher PCAT6 levels were linked to worse overall survival (OS) (HR = 1.540; 95% CI: 1.079-2.1997; $P = 0.017$), with this relationship also being preserved in a multivariate analysis (HR = 6.892; 95% CI: 1.713-27.727, $P = 0.007$). GSEA revealed high PCAT6 expression to be linked to differential DNA methylation enrichment, with high PCAT6 levels being associated with changes in base excision repair, cellular senescence, G2 M DNA damage checkpoint, chromatin-modifying enzyme, and gene silencing by RNA activity.

Conclusions: PCAT6 represents a promising prognostic biomarker of poor CRC patient survival outcomes, with DNA methylation and RNA-mediated gene silencing being potentially promising mechanistic pathways whereby this lncRNA may shape patient outcomes.

Introduction

Colorectal cancer (CRC) is a common form of gastrointestinal malignancy that accounts for roughly 9000,000 deaths throughout the world each year, making it the fourth leading cause of cancer-associated death. Rates of CRC are rising both due to the general aging of the global population and to a number of modifiable risk factors such as poor diet, obesity, smoking, and a lack of exercise [1]. CRC tumors arise when healthy cells of the colonic epithelium mutate and form benign adenomas that in turn progress to a malignant state [2]. Quantitative analyses suggest that early CRC-predisposing mutations occur in stem cells, while the acquisition of malignant and metastatic phenotypes can be a prolonged process that can take upwards of 10 years to complete [3]. As such, diagnosing CRC in its early stages is essential yet clinically challenging. Roughly 43% and 25% of CRC patients exhibit liver and liver/lung metastases, respectively, and for individuals with stage IV disease, the 5-year overall survival (OS) rate is under 10% [4, 5]. The primary treatment for CRC patients is surgical tumor resection supplemented by

chemotherapeutic and immunotherapeutic treatment. However, these treatments are of limited value for individuals with advanced disease. Several promising biomarkers have been tested as potential predictors of CRC onset or progression in clinical contexts [6], including carcinoembryonic antigen (CEA) and carbohydrate antigen 199 (CA199) [7], yet their utility remains a matter of some controversy. There is thus an urgent need to develop novel diagnostic and therapeutic biomarkers for this cancer type.

Prostate Cancer-Associated Transcript 6 (PCAT6; PCAN-R1, ncRNA-a2, KDM5B-AS1) is a recently described long non-coding RNA (lncRNA) associated with oncogenic processes [8]. PCAT6 is a 764 bp lncRNA encoded in an intergenic region on chromosome 1q32.1, where it flanks the histone demethylase JARID1B/KDAM 5B gene. In prostate tumor cells, this lncRNA was shown to induce colony formation and keratinocyte proliferation [9]. Its upregulation has also been reported in a variety of tumors including bladder, ovarian, lung, and gastric cancers, as well as in osteosarcoma, hepatocellular carcinoma (HCC), and glioblastoma [10]. PCAT6 can increase the proliferative, migratory, and angiogenic activity of triple-negative breast cancer (TNBC) cells *in vitro* and *in vivo*, and VEGF derived from M2 macrophages can stimulate PCAT6 upregulation to promote angiogenic activity in the context of TNBC [11]. PCAT6 upregulation in HCC has been associated with worse OS and disease-free survival, in addition to enhancing the proliferative activity and survival of HCC cells, whereas the knockdown of this lncRNA induced cell cycle arrest and apoptotic death in these same tumor cells [12]. In bladder cancer tissues, PCAT6 levels have been reported to be higher than in paracancerous healthy tissues, with bladder cancer patients exhibiting increased serum PCAT6 levels as compared to healthy controls suggesting that it may represent a valuable diagnostic biomarker for this cancer type [13]. However, there have been few studies to date assessing the relationship between PCAT6 and CRC.

As such, we sought to establish the relationship between PCAT6 and CRC, and to assess the prognostic relevance of this lncRNA based on an analysis of CRC patient data compiled within The Cancer Genome Atlas (TCGA). To that end, we compared PCAT6 expression in CRC tumors and normal tissue samples in the TCGA database, and examined correlations between PCAT6 levels and patient prognosis. Moreover, a gene set enrichment analysis (GSEA) technique was used to identify PCAT6-related biological processes with potential relevance to the progression of CRC.

In this study, we found PCAT6 upregulation to be evident in CRC patients, associated with worse CRC patient prognostic outcomes, and related to mechanistic functions including base excision repair, the G2/M DNA damage checkpoint, cellular senescence, chromatin-modifying enzymes, DNA methylation, and RNA-mediated gene silencing via a GSEA approach. As such, PCAT6 may represent a valuable biomarker for the diagnosis and prognostic assessment of individuals with CRC.

Material And Methods

TCGA data collection

Level 3 RNA-seq data in the HTSeq-FPKM format with corresponding clinical details were downloaded from the TCGA (https://portal.gdc.cancer.gov/) CRC COAD and READ projects, with any samples for which clinical information was missing being discarded, yielding 644 samples for subsequent analysis (**Table 1**). The detailed clinicopathologic information such as patient age, pathologic stage, neoplasm type (READ vs. COAD), height, weight, gender, race, history of colon polyps, colon polyps present, and lymphatic invasion status were assessed, as were CEA levels, residual tumor status, and TNM staging. All data were obtained from a publically accessible database, and no direct human or animal research was conducted by the authors of this study.

Gene Set Enrichment Analysis (GSEA)

GSEA approaches represent a computational means of establishing whether a given set of genes differ significantly between a given set of biological states [14]. GSEA analyses were performed based on patients in the TCGA COAD and READ datasets with low and high PCAT6 expression levels using the clusterPorfiler package [15]. Ordered gene lists were generated based on the strength of individual correlations with PCAT6 in this analysis, with GSEA being conducted to clarify differences in survival outcomes between patients with low and high PCAT6 expression levels. A preliminary GSEA version was utilized for data analysis [16]. Analyses were conducted with 10,000 genome permutations, with PCAT6 expression levels as a phenotypic label. Pathway enrichment was assessed according to nominal P-values and normalized enrichment score (NES) value.

ssGSEA-based immune cell analysis

A single-sample GSEA 9(ssGSEA) analysis was used to evaluate immune cell infiltration into CRC tumors with the R GSVA package [17]. This approach allowed for GSEA-based analyses of 24 different immune cell populations including natural killer (NK) cells, eosinophils, CD8+ T cells, B cells, Th17 cells, T cells, gamma delta T cells (Tgd), immature dendritic cells (iDCs), mast cells, eosinophils, Th1 cells, plasmacytoid DCs (pDCs), neutrophils, activated DCs (aDCs), DCs, T helper cells, NK CD56dim cells, T follicular helper (Tfh) cells, T effector memory (Tem) cells, macrophages, Th2 cells, central memory T (Tcm) cells, and cytotoxic cells. Based upon characteristic gene expression patterns for these cell types [18], their relative enrichment in each tumor sample was established. Spearman correlation analyses and Wilcoxon rank-sum tests were then used to assess correlations between PCAT6 expression and such immune cell infiltration in patients with different levels of PCAT6 expression.

Statistical Analysis

R (3.6.3) was used to analyze all data in the present study. Associations between PCAT6 expression levels and clinicopathological variables were assessed via Wilcoxon rank-sum tests and logistic regression analyses, while the associations between patient OS and specific clinicopathological variables were assessed through Kaplan-Meier and Cox regression analyses. The relationship between PCAT6 expression levels, other variables of interest, and patient survival was assessed via a multivariate Cox analysis. <edian values were used to stratify patients into subgroups with low and high levels of PCAT6 expression. $P < 0.05$ was the threshold of significance.

Results

Patients and Samples

In total, 644 patients were identified for inclusion in this study (343 male, 301 female, **Table 1**). Of these patients, 232 (39.9%) exhibited lymphatic invasion, 178 (32.1%) had a pre-treatment history of colon polyps, and 92 (30.7%) exhibited colonic polyps. Patients with Stage I, II, III, and IV disease accounted for 17.8% (n=111), 38.2% (n=238), 29.5% (n=184), and 14.4% (n=90) of the overall cohort, respectively, with 442 (74.2%) and 166 (25.8%) respective cases of colon adenocarcinoma and rectal adenocarcinoma. With respect to staging, 3.1% (n = 20), 17.3% (n = 111), 68% (n = 436), and 11.5% (n = 74) of patients had T1, T2, T3, and T4 disease; 57.5% (n =368), 23.9% (n = 153), and 18.6% (n = 119) had N0, N1, and N2 disease; and 84.2% (n =475) and 15.8% (n = 89) had M0 and M1 disease, respectively. In addition, 154 patients (37.1%) had pre-treatment CEA levels greater that five.

Assessment of PCAT6 expression and diagnostic utility in CRC

Using a Wilcoxon rank-sum test, PCAT6 expression was next compared between 644 CRC tumor samples and 51 normal tissue samples, revealing this lncRNA to be significantly upregulated in tumor tissue samples relative to healthy control tissues (P <0.001) (**Figure 1A**). When PCAT6 levels were compared between CRC patient tumors and matched paracancerous tissues, this lncRNA was similarly found to be downregulated in tumor tissues (P<0.001) (**Figure 1B**), suggesting that it may be linked to CRC development and/or progression. Receiver operating characteristic (ROC) curves were then generated to assess the diagnostic efficacy of PCAT6 based upon TCGA data, yielding an area under the ROC curve (AUC) value of 0.859 (**Figure 1C**), consistent with the ability of this lncRNA to reliably discriminate between tumors and healthy tissues.

The association between PCAT6 expression and CRC patient clinicopathological characteristics

Next, the relationship *between PCAT6* expression levels *and* clinical characteristics for 647 CRC patients in the TCGA database was assessed. As shown in **Figure 2,** high levels of PCAT6 expression were significantly correlated with tumor N stage (N0 vs. N1 vs. N2, p<0.01), M stage (M1 vs. M0, p<0.01), pathological stage (Stage I vs. Stage II vs. Stage III vs. Stage IV, p<0.05), lymphatic invasion (Yes vs. No, p<0.001), tumor type (READ vs. COAD, p<0.001), and CEA level (≤5 vs.>5, p<0.01). In univariate analyses, PCAT6 expression was associated with poor clinicopathological characteristics when using a median expression cutoff threshold of 2.5 (**Table 2**). Higher PCAT6 expression levels in CRC were associated with N stage (OR=1.81 for N1&N2 vs. N0, p<0.001), M stage (OR=2.07 for M1 vs. M0, p=0.002), CEA level (OR=1.88 for >5 vs. ≤5, p=0.002), lymphatic invasion (OR=1.95 for Yes vs. No, p<0.001), pathological

stage (OR=1.94 for stage III/IV vs. stage I/II, p<0.001), and tumor type (OR=2.14 for READ vs. COAD, p<0.001). These findings suggested that CRC patients exhibiting increased PCAT6 expression were more likely to harbor lymph node metastases and more advanced disease.

Examination of the relationship between PCAT6 and patient survival

Kaplan-Meier survival analyses revealed CRC patients with high PCAT6 levels to exhibit a worse prognosis than that of patients expressing lower levels of this lncRNA (P = 0.017) (**Figure 3A**), with similar results being obtained when assessing the progression-free interval (P = 0.001) (**Figure 3B**). In univariate analyses, high PCAT6 expression was associated with poorer OS (hazard ratio [HR]: 1.540; 95% confidence interval [CI]: 1.079-2.199; P = 0.017), and other variables associated with lower survival rates included age, CEA levels, lymphatic invasion, pathological stage, and TNM stage. In multivariate analyses, an independent association between PCAT6 expression and OS was detected (HR = 6.892; 95% CI: 1.713-27.727, P = 0.007), with the same being true for age and M stage (**Table 3**).

GSEA-based identification of signaling pathways associated with PCAT6

Next, a GSEA approach was used to compare patterns of gene expression between CRC tumors with low and high levels of PCAT6 expression, revealing significant differences in enrichment levels for MSigDB collections (c2.cp.v7.0.symbols.gmt) (FDR <0.05, normalized P < 0.05). NES values were then used to select the most enriched signaling pathways (**Figure 4 and Table 4**), revealing high levels of PCAT6 expression to be associated with the enrichment of DNA methylation, RNA-mediated gene silencing, cellular senescence, base excision repair, chromatin-modifying enzyme, and G2/M DNA damage checkpoint pathways.

Immune Cell Infiltration Analysis of PCAT6 in the CRC

Finally, we examined the relationship between PCAT6 expression and immune cell infiltration as quantified via an ssGSEA analysis in CRC samples through a Spearman correlation approach. This strategy revealed higher levels of PCAT6 expression to be positively correlated with pDC and NK cell infiltration (P<0.05, **Figure 5**).

Discussion

A growing body of research has shown PCAT6 to play key roles in many different cancers, with the upregulation of this lncRNA being associated with worse prognostic outcomes and clinical features, underscoring its promising utility as a prognostic biomarker [19]. For example, one study of osteosarcoma samples revealed higher PCAT6 expression in these tumors relative to paracancerous

normal bone tissue, with such upregulation being linked to worse patient survival and a more malignant phenotype [20]. Similarly, PCAT6 overexpression in cholangiocarcinoma (CCA) has been linked to the modulation of macrophage function in affected patients, suggesting that it may be a viable immunotherapeutic target in this oncogenic setting [21]. However, few studies have examined the relevance of PCAT6 in CRC. This study was thus conducted to compare PCAT6 expression levels in CRC tissues and to explore its associated prognostic and therapeutic utility. Overall, we found that PCAT6 is upregulated in CRC tumors relative to healthy normal tissue levels, suggesting that it may play a tumorigenic role in affected patients.

Herein, we analyzed RNA-seq data pertaining to CRC patients derived from the TCGA database, revealing significant PCAT6 upregulation in CRC tumors relative to normal tissue samples. Such upregulation was tied to certain characteristics of advanced disease (lymphatic invasion, pathological stage, TNM stage, tumor type) and to a poorer overall patient prognosis. GSEA results revealed PCAT6 upregulation to be linked to biological activities including base excision repair, G2/M DNA damage checkpoint, cellular senescence, DNA methylation, and RNA-mediated gene silencing, all of which are linked to tumor cell invasion, proliferation, and metastasis [22–27]. PCAT6 may thus represent a promising new therapeutic and prognostic target in CRC patients.

In ovarian cancer cells, PCAT6 has been found to be upregulated and to enhance migratory, invasive, and proliferative activity levels by inhibiting the tumor suppressor gene PTEN in a manner closely tied to distant metastasis or lymph node metastasis [28]. Similar findings have also been reported in intrahepatic cholangiocarcinoma, with PCAT6 upregulation being linked to more advanced disease [29]. Higher PCAT6 levels have also been reported in glioblastoma cells and tissues, functioning through a positive feedback mechanism to regulate the progression of this cancer type [30]. In line with these prior reports, we herein found PCAT6 to be upregulated in CRC and to be correlated with patient TNM stage, lymph node metastasis, and clinical stage, suggesting that it may be a clinically relevant indicator of CRC development and progression.

DNA methylation is a common epigenetic modification that can control associated transcriptional activity. By regulating DNA methyltransferase activity and associated enzymes or by modulating substrate availability, dietery compounds can impact DNA methylation. These epigenetic modifications are thought to be directly relevant to carcinogenic processes, and offer substantial therapeutic potential [31]. Patterns of DNA methylation in thyroid cancer have been previously explored as promising prognostic and therapeutic targets with the potential to benefit patients with thyroid cancer [32]. Increased signals associated with cancer gene-silencing activities for certain miRNAs are increased, enhancing cancer cell migration and proliferation [33]. This suggests can influence CRC cells through pathways related to DNA methylation and RNA-mediated gene silencing.

The present analyses further revealed close correlations between PCAT6 expression levels and immune cell infiltration in CRC tumors. Specifically, PCAT6 expression levels were moderately to strongly positively correlated with NK and pDC cell infiltration, in addition to being significantly correlated with Treg, Th17,

and NK CD56^{bright} cell infiltration. This suggests that PCAT6 may shape intratumoral immune responses in CRC. In contrast to the robust relationship observed for pDCs, aDCs were only weakly correlated with PCAT6 expression, suggesting a link between this lncRNA and tumor-associated DC polarization Moreover, PCAT6 levels were closely correlated with expression levels of many NK cell-related markers, suggesting a link between this lncRNA and these important cytolytic cells. These correlative relationships suggest that PCAT6 can affect immune cell recruitment and infiltration in CRC tumors.

The results of this study offer new insights into the functional importance of PCAT6 in CRC. However, further work will be needed to validate and expand upon these results. For example, additional analyses of clinical factors associated with changes in PCAT6 expression levels warrant further in-depth study. In addition, the data analyzed herein were derived from multiple laboratories, potentially resulting in inconsistent sample and data processing. Moreover, the number of healthy controls in this study differed significantly from the number of CRC samples, necessitating follow-up studies in which sample sizes are more appropriately balanced. Even so, as a multi-center study, this analysis may overcome some of the limitations of single-center studies. As this was a retrospective study, data pertaining to certain interventions were missing and certain information was insufficiently specific. Further prospective work will overcome these potential analytical biases. Additionally, this was an RNA-seq based analysis, and correlations between PCAT6 expression and proteomic changes thus warrant further study to clarify the direct mechanisms whereby this lncRNA influences CRC onset and progression. Future cell line- and animal model-based studies may offer greater insight into how PCAT6 shapes to oncogenic processes highlighted in this report.

Conclusion

In summary, the results of this study suggest that the upregulation of PCAT6 is closely tied to the progression of CRC, immune cell infiltration, and worse patient survival. This lncRNA may thus promote dysregulated immune and inflammatory responses, driving oncogenic progression. While these results offer new insight into the pathological basis of CRC, additional prospective analyses and randomized clinical trials will be essential to further clarify the underlying molecular mechanisms and clinical relevance in patients with this deadly cancer type.

Declarations

All procedures were conducted in accordance with the Declaration of Helsinki.

Consent for publication

LncRNA PCAT6 as a predictor of poor colorectal cancer patient prognosis: A TCGA dataset analysis (Not applicable).

Ethics approval and consent to participate:

This study does not contain any studies with human participants or animals performed by any of the authors.

Availability of data and materials:

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article.

Competing interests:

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Authors' contributions:

Conceptualization and design of the study: Dengqun Sun. Details of the experimental design: Lin Han and Yanjun Sun. Carrying out the experiment: Lin Han. Implementation of the scenario: Yanjun Sun, and Lin Han. Funding: Dengqun Sun. Preparation of the data: Lin Han. Analysis: Dengqun Sun and Lin Han. Writing of the paper: Lin Han, Dengqun Sun, and Yanjun Sun. All authors reviewed the manuscript.

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Tables

TABEL 1 | TCGA colorectal cancer patient characteristics.

Clinical characteristic	Level	Overall(644)
T stage (%)	T1	20 (3.1%)
	T2	111 (17.3%)
	T3	436 (68%)
	T4	74 (11.5%)
N stage (%)	N0	368 (57.5%)
	N1	153 (23.9%)
	N2	119 (18.6%)
M stage (%)	M0	475 (84.2%)
	M1	89 (15.8%)
Pathologic stage (%)	Stage I	111 (17.8%)
	Stage II	238 (38.2%)
	Stage III	184 (29.5%)
	Stage IV	90 (14.4%)
CEA level (%)	<=5	261 (62.9%)
	>5	154 (37.1%)
Lymphatic invasion (%)	No	350 (60.1%)
	Yes	232 (39.9%)
History of colon polyps (%)	No	377 (67.9%)
	Yes	178 (32.1%)
Colon polyps present (%)	No	224 (69.3%)
	Yes	99 (30.7%)
Neoplasm type (%)	Colon adenocarcinoma	478 (74.2%)
	Rectum adenocarcinoma	166 (25.8%)
Gender (%)	Female	301 (46.7%)
	Male	343 (53.3%)
Race (%)	Asian	12 (3%)
	Black or African American	69 (17.5%)
	White	313 (79.4%)
Age (median [IQR])		68 (58, 76)

IQR, InterQuartile Range.

TABEL 2 | LncRNA PCAT6 expression associated with clinical pathological characteristics (logistic regression).

Characteristics	Total(N)	Odds ratio in PCAT6 expression (OR)	*P*-value
T stage (T3&T4 vs. T1&T2)	641	1.449 (0.985-2.140)	0.061
N stage (N1&N2 vs. N0)	640	1.809 (1.319-2.488)	<0.001
M stage (M1 vs. M0)	564	2.065 (1.300-3.330)	0.002
CEA level (>5 vs. <=5)	415	1.884 (1.260-2.831)	0.002
Perineural invasion (Yes vs. No)	235	1.909 (1.057-3.499)	0.034
History of colon polyps (Yes vs. No)	555	0.721 (0.503-1.031)	0.074
Colon polyps present (Yes vs. No)	323	0.674 (0.417-1.084)	0.105
Lymphatic invasion (Yes vs. No)	582	1.947 (1.392-2.733)	<0.001
Pathologic stage (Stage III&Stage IV vs. Stage I&Stage II)	623	1.942 (1.410-2.681)	<0.001
Neoplasm type (Rectum adenocarcinoma vs. Colon adenocarcinoma)	644	2.143 (1.493-3.097)	<0.001

Table 3 | Univariate and multivariate Cox proportional hazards regression analysis of PCAT6 expression.

Characteristics	Total(N)	Univariate analysis		Multivariate analysis	
		Hazard ratio (95% CI)	P value	Hazard ratio (95% CI)	P value
T stage (T1&T2 vs. T3&T4)	640	2.468 (1.327-4.589)	**0.004**	0.258 (0.044-1.517)	0.134
Nstage (N0 vs. N1&N2)	639	2.627 (1.831-3.769)	**<0.001**	4.661 (0.804-27.013)	0.086
M stage (M0 vs. M1)	563	3.989 (2.684-5.929)	**<0.001**	5.691 (1.220-26.546)	**0.027**
CEA level (<=5 vs. >5)	414	2.620 (1.611-4.261)	**<0.001**	1.178 (0.296-4.687)	0.816
Perineural invasion (No vs. Yes)	235	1.692 (0.907-3.156)	0.099	0.958 (0.295-3.106)	0.942
Lymphatic invasion(No vs. Yes)	581	2.144 (1.476-3.114)	**<0.001**	2.085 (0.505-8.617)	0.310
Pathologic stage (Stage I&Stage II vs. Stage III&Stage IV)	622	2.988 (2.042-4.372)	**<0.001**		
Colon polyps present (No vs. Yes)	323	1.250 (0.743-2.103)	0.401		
History of colon polyps (No vs. Yes)	554	0.789 (0.496-1.257)	0.319		
Race (Asian vs. Black or African American&White)	394	0.840 (0.205-3.438)	0.809		
Gender (Female vs. Male)	643	1.054 (0.744-1.491)	0.769		
Weight (<=90 vs. >90)	348	0.742 (0.412-1.339)	0.322		
Height (<170 vs. >=170)	329	0.779 (0.473-1.281)	0.324		
Age (<=65 vs. >65)	643	1.939 (1.320-2.849)	**<0.001**	9.813 (2.525-38.135)	**<0.001**
PCAT6 (Low vs. High)	643	1.540 (1.079-2.199)	**0.017**	6.892 (1.713-27.727)	**0.007**

Table 4 | Gene sets enriched in phenotype high

MSigDB collection	Gene set name	NES	p.adj	FDR
c2.cp.v7.0.symbols.gmt	REACTOME_DNA_METHYLATION	-1.957	0.048	0.046
	REACTOME_GENE_SILENCING_BY_RNA	-1.682	0.048	0.046
	REACTOME_CHROMATIN_MODIFYING_ENZYMES	-1.423	0.048	0.046
	REACTOME_CELLULAR_SENESCENCE	-1.586	0.048	0.046
	REACTOME_G2_M_DNA_DAMAGE_CHECKPOINT	-1.631	0.048	0.046
	REACTOME_BASE_EXCISION_REPAIR	-1.735	0.048	0.046

NES = normalized enrichment score; p.adj = adjust p-value; FDR = false discovery rate.

Figures

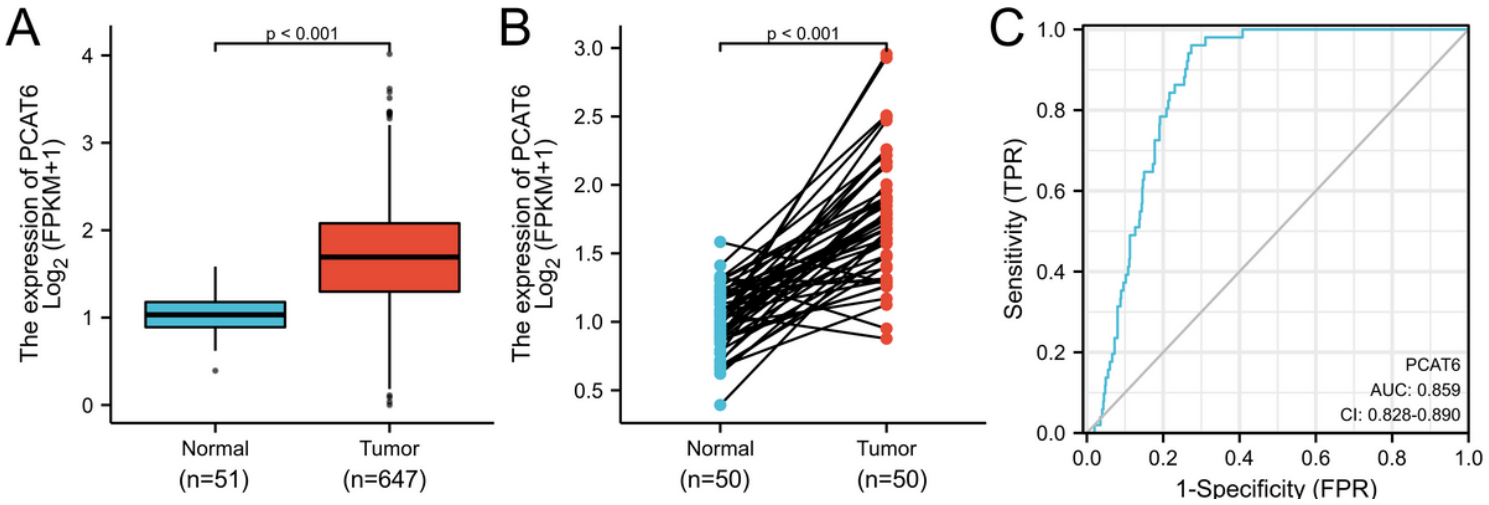

Figure 1

The expression and diagnostic value of PCAT6 in colorectal tissues. (A) PCAT6 showed significantly higher expression in cancer tissues than in normal tissues. (B) PCAT6 was prominently higher expressed in colorectal cancer (P < 0.001) compared with 50 pairs non-cancerous adjacent tissues. (C) Receiver Operating Characteristic Curve, FPR, False Positive Rate; TPR, True Positive Rate; CI, Confidence interval.

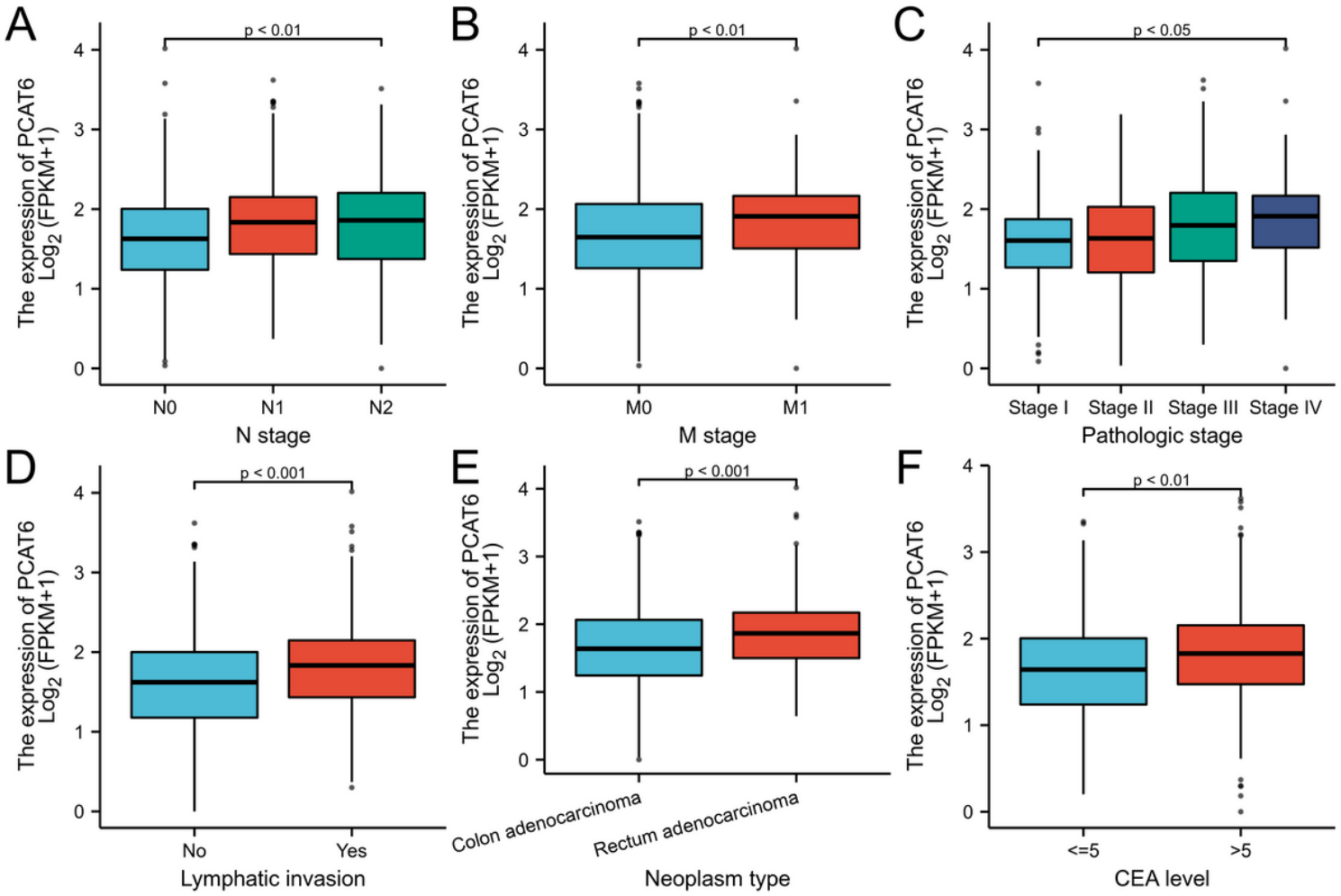

Figure 2

Association with LncRNA PCAT6 expression and clinicopathologic characteristics. (A) N stage, (B) M stage, (C) Clinical stage, (D) lymphatic invasion, (E) Neoplasm type,(F) CEA level. N, lymph node metastasis; M, distant metastasis, CEA, carcinoembryonic antigen.

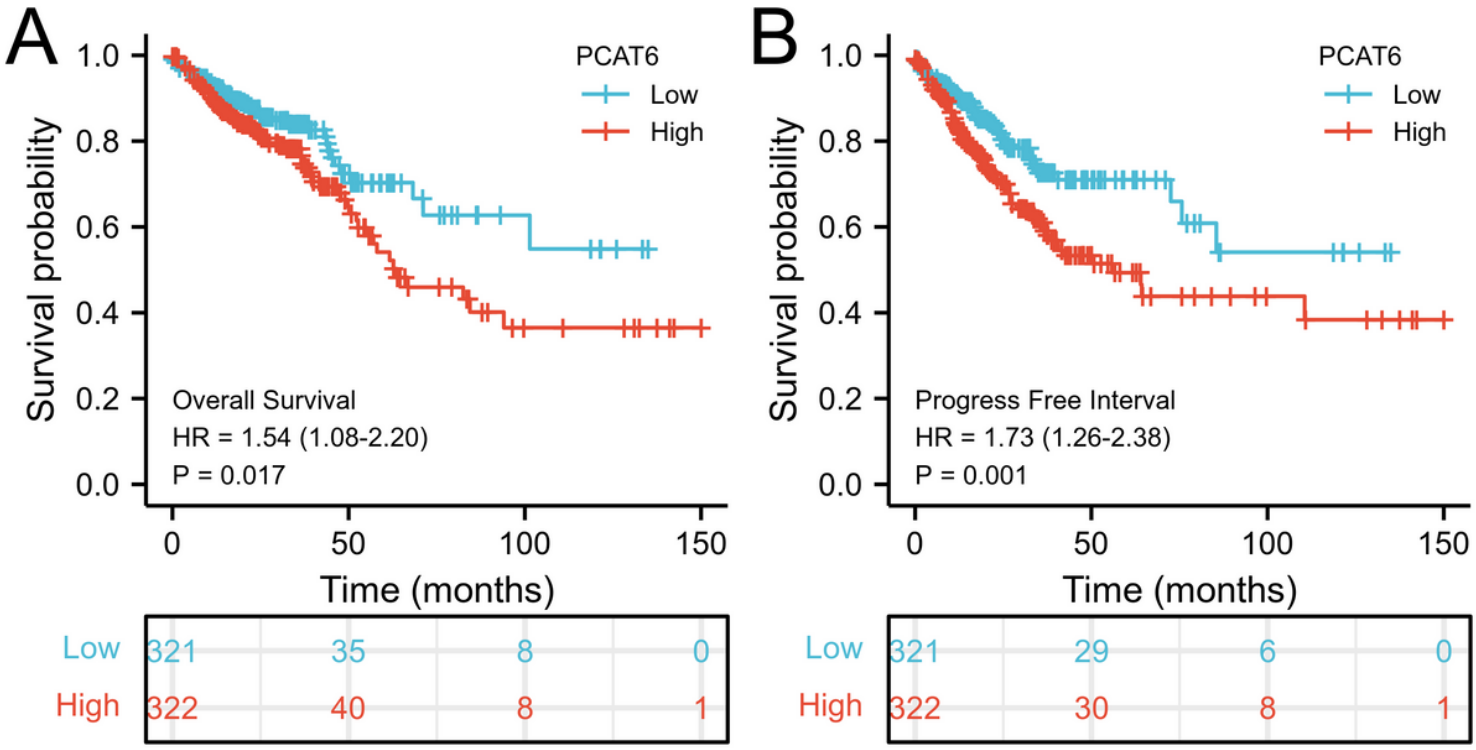

Figure 3

High expression of LncRNA PCAT6 is associated with poor OS and PFI in patients with colorectal cancer. (A) Effect of PCAT6 expression on OS of CRC patients in TCGA cohort. (B) Effect of PCAT6 expression on PFI of CRC patients in TCGA cohort. TCGA, The Cancer Genome Atlas; OS, overall survival; PFI, progression free interval; CRC, colorectal cancer.

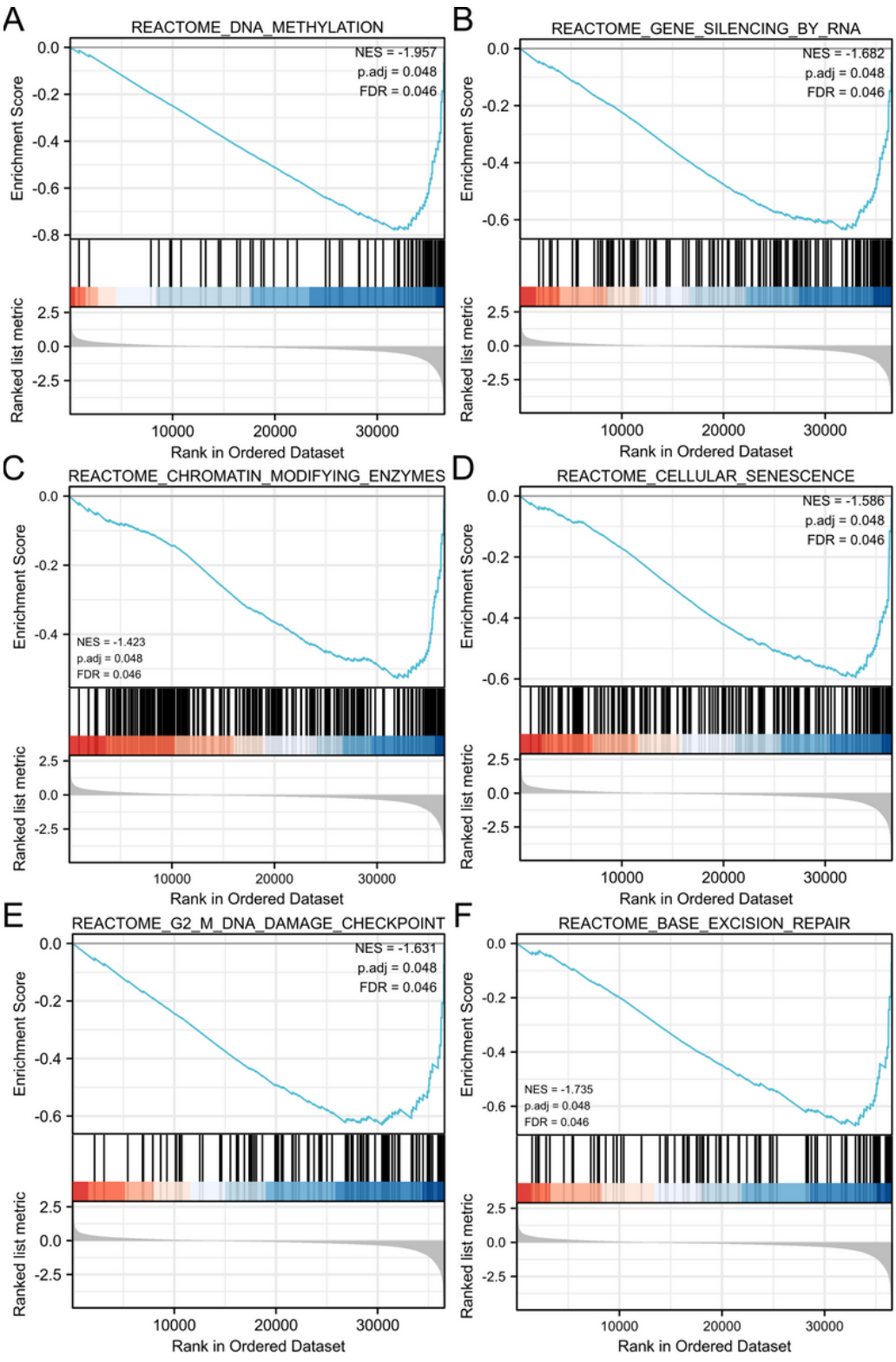

Figure 4

Enrichment plots from gene set enrichment analysis (GSEA). GSEA results showing DNA methylation (A), gene silencing by RNA (B), chromatin modifying enzymes (C), cellular senescence (D), G2 M DNA damage checkpoint (E), base excision repair (F) differentially enriched in IR4435-2HG-related colorectal cancer. NES, normalized ES; FDR, false discovery rate.

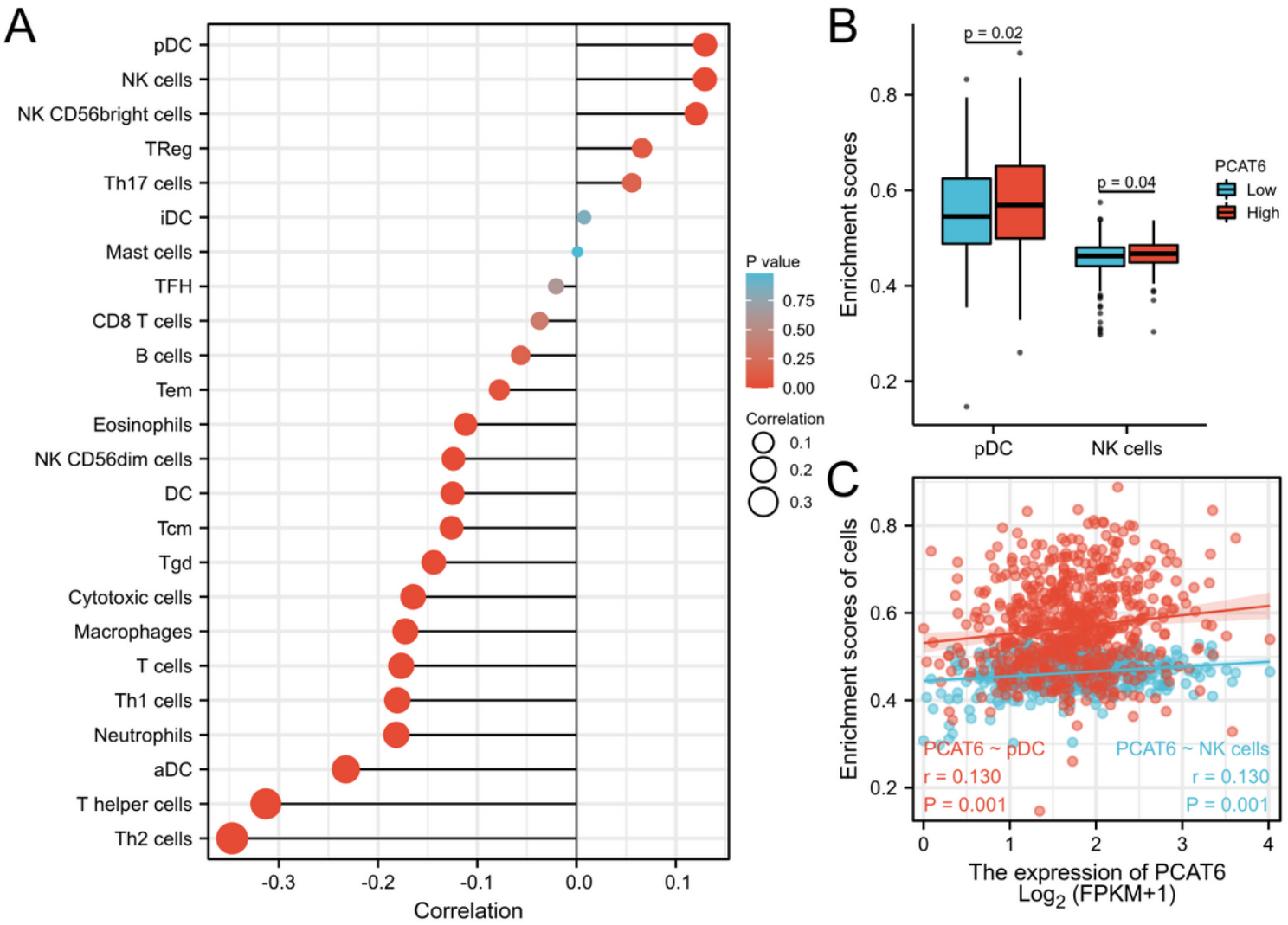

Figure 5

Immune Cell Infiltration Analysis of PCAT6 in the CRC. (A) The forest plot shows the correlation between PCAT6 expression level and 24 immune cells; (B) the enrichment scores of PCAT6 expression in pDC and NK cells; (C) The correlation between PCAT6 expression and pDC; the correlation between PCAT6 expression and NK cells. CRC, colorectal cancer.