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RESEARCH ARTICLE

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The prognostic effect of PNN in digestive tract cancers and its correlation with the tumor immune landscape in colon adenocarcinoma

Hui Zhang[1] | Ming Jin[1] | Meng Ye[2] | Yanping Bei[1] | Shaohui Yang[3] | Kaitai Liu[1]

[1]Department of Radiation Oncology, The Lihuili Hospital, Ningbo Medical Center, Ningbo, China

[2]Department of Oncology and Hematology, The Affiliated Hospital of Medical School of Ningbo University, Ningbo, China

[3]Department of General Surgery, The Lihuili Hospital, Ningbo Medical Center, Ningbo, China

Correspondence
Kaitai Liu, Department of Radiation Oncology, The Lihuili Hospital, Ningbo Medical Center, Ningbo, China.
Email: liukaitai@nbu.edu.cn

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Abstract

Background: The present study investigated the expression, mutation, and methylation profile of PNN and its prognostic value in digestive tract cancers. The disparities in signaling pathways and the immune landscape in colon adenocarcinoma (COAD) based on PNN expression were specifically explored.

Methods: The expression, mutation, methylation levels of PNN, and survival data in esophageal cancer, gastric adenocarcinoma, COAD, and rectal adenocarcinoma were evaluated using several bioinformatic databases. Gene Ontology (GO) enrichment analysis and gene set enrichment analysis (GSEA) were performed to investigate the enriched biological functions and pathways in COAD. Several acknowledged bioinformatic algorithms were employed to assess the correlation between PNN expression and the tumor immune landscape in COAD.

Results: PNN was upregulated and remarkably related to tumor stage in digestive tract cancers. High expression of PNN was positively associated with poor progression-free survival and overall survival time, specifically in COAD. PNN expression was identified as an independent prognostic factor in COAD. GO and GSEA analyses revealed that PNN participates in multiple biological processes underlying carcinogenicity in COAD. Further investigation showed that PNN expression was significantly associated with tumor-infiltrating immune cells, immune cell functions, and several immune checkpoints in COAD. The PNN low expression group had a lower tumor immune dysfunction and exclusion (TIDE) score and a higher immunophenoscore (IPS), indicating a better response to immunotherapy.

Conclusion: PNN was highly expressed in digestive tract cancers and could act as an independent prognostic factor and a response predictor for immunotherapy in COAD.

KEYWORDS
colon adenocarcinoma, immune landscape, PNN, prognosis, The Cancer Genome Atlas

Hui Zhang and Ming Jin contributed equally to this work.

1 | INTRODUCTION

The PNN gene encodes the protein pinin, a desmosome-associated molecule that was originally found to play an essential role in epithelial cell–cell adhesion.[1] In recent years, PNN has been confirmed to be involved in the development of malignant tumors, although the results are still controversial. Shi et al. reported that PNN was expressed at relatively low levels in several cancer samples and cancer cell lines. Increasing PNN expression could significantly inhibit cancer cell proliferation.[2] The results indicated that PNN might act as an antioncogene in malignant tumors. In contrast, some studies observed that PNN was more highly expressed in tumor samples than in corresponding normal tissues and was associated with a poor prognosis in several cancer types, including colorectal cancer, hepatocellular carcinoma, and ovarian carcinoma.[3-5] Likewise, a recent study revealed the oncogenic role of PNN in OS-RC-2 and Caki-1, two human renal clear cell lines, according to the positive effects on cell proliferation and migration.[6] Besides, PNN was suggested to be involved in mRNA processing as well as transcriptional regulation of E-cadherin via its binding to CtBP, a transcriptional corepressor with tumorigenic potential that targets the promoter of E-cadherin, and PNN is also a transcriptional activator binding to the E-box 1 core sequence of the E-cadherin promoter gene, which plays essential role in tumorigenesis.[7] Therefore, the role of PNN in cancer requires further study.

Digestive tract cancers are common malignancies and account for a large proportion of cancer deaths worldwide.[8] Although some helpful diagnostic and predictive biomarkers have been identified, more reliable and effective molecular markers are expected for clinical management in digestive tract cancers. Mini Enrico et al. found that stage III colorectal patients with higher PNN expression benefit less from fluorouracil-based chemotherapy, resulting in an unfavorable disease-free survival outcome.[9] The results indicated that PNN might act as a predictive biomarker for the clinical benefit of adjuvant chemotherapy in those patients. However, to date, no studies have systematically explored the PNN profile and its prognostic value in digestive tract cancers, and the effect of PNN expression on the tumor immunity of colon adenocarcinoma (COAD) has not been reported. Therefore, in the present study, we comprehensively illuminated the expression profile, mutation features, and methylation status of PNN and its prognostic value in four digestive tract cancers, including esophageal cancer (ESCA), gastric adenocarcinoma (STAD), COAD, and rectal adenocarcinoma (READ), by analyzing The Cancer Genome Atlas (TCGA) and several acknowledged open-access bioinformatics databases. Furthermore, the disparities in signaling pathways and the immune landscape in colon adenocarcinoma (COAD) based on different PNN expression levels were specifically explored.

2 | MATERIALS AND METHODS

2.1 | Data resource

Data on PNN mRNA expression in four digestive tract cancers, including ESCA, STAD, COAD, READ, and corresponding normal tissues in TCGA, were acquired from the Genomic Data Commons (GDC) Data Portal (https://portal.gdc.cancer.gov). High-throughput sequencing (HTSeq) gene transcript data with normalization in fragments per kilobase of transcript per million mapped reads (FPKM) were downloaded by the Genomic Data Commons (GDC) Data transfer tool. In addition, the clinicopathologic data for four types of cancer, such as age, sex, pathological stage, tumor (T) status, node (N) status and metastasis (M) status, and survival information, were extracted from TCGA database. Since no personal identifying information was used in the current study, it was granted an exemption from ethics approval from the Institutional Review Board of the Lihuili Hospital, Ningbo Medical Center.

2.2 | Expression profile of PNN in digestive tract cancers

The mRNA expression levels of PNN in four digestive tract cancers were extracted and structured from the HTSeq data by using Perl software. We assessed the differential expression of PNN in ESCA, STAD, COAD, and READ compared with corresponding normal tissues by the *limma* package.[10] In addition, we further analyzed the disparities in PNN expression levels according to different clinical stages in four digestive tract cancers. The results are represented by box plots, which were generated with the *ggpubr* package in R software.

2.3 | DNA methylation and PNN expression in digestive tract cancers

The Illumina Human Methylation 450K data of TCGA-EACA, TCGA-STAD, TCGA-COAD, and TCGA-READ samples were obtained from the open-access exploration platform (https://xena.ucsc.edu). The DNA methylation status of cg sites in the promoter region of PNN in four digestive tract cancers was recognized. Subsequently, we investigated the associations between PNN expression and DNA methylation in four digestive tract cancers by utilizing the Pearson correlation. The file used for annotating the information on cg sites was obtained from the official website of Illumina.[11] The R package *corrplot* was employed for the analyses.

2.4 | Prognosis value of PNN in digestive tract cancers

The effects of PNN mRNA expression on prognosis were evaluated according to progression-free survival (PFS) and overall survival (OS) by the Kaplan–Meier method. Thereafter, the independent prognostic values of PNN expression in ESCA, STAD, COAD, and READ patients were evaluated using univariate and multivariate Cox regression analyses. All analyses were conducted by the *survival* and *survminer* packages of R software, and the forest plot was drawn by the package *ggplot*.

2.5 | CpG site methylation of PNN and its prognostic effect in digestive tract cancers

We evaluated the DNA methylation of PNN CpG sites and its prognostic value for OS in ESCA, STAD, COAD, and READ by the excellent online MethSurv database. MethSurv is an online bioinformatics platform for multivariable prognosis assessment according to massive DNA methylation data (https://biit.cs.ut.ee/methsurv/).[12]

2.6 | Genetic mutations of PNN and its prognostic effect in digestive tract cancers

We explored the genetic mutation features of PNN in ESCA, STAD, and colorectal cancer (CRC) by utilizing the open-access cBioPortal database (v3.6.12; http://www.cbioportal.org). cBioPortal is a pre-eminent public online tool for exploring, analyzing, and visualizing comprehensive cancer genomics data.[13] Data in TCGA PanCancer Atlas of ESCA, STAD, and CRC were involved in the study, with selected genomic profiles as follows: mutations, structural variant, putative copy-number alterations from Genomic Identification of Significant Targets in Cancer (GISTIC), and mRNA Expression z-scores relative to diploid samples (RNASeqV2RSEM). In addition, the correlations between genetic mutations of PNN and OS were assessed. The z-score threshold was set to ±1.8.

2.7 | External validation of the prognostic value in COAD

To verify the prognostic value of PNN expression in COAD, we obtained microarray profiles of COAD from the GEO database (Gene Expression Omnibus, http://www.ncbi.nlm.nih.gov/geo/). GSE17536 and GSE29623 were collected, and survival analyses according to PNN expression were performed.

2.8 | Establishment of a prognostic nomogram in COAD

We developed a nomogram incorporating PNN expression and clinicopathological characteristics for better prognosis prediction in patients with COAD by the *rms* package of R software.[14] The 1-, 3-, and 5-year OS rates served as endpoints in the nomogram. The clinicopathological characteristics included age, sex, and stage. ROC curves were constructed to evaluate the predictive ability of the 1-, 3-, and 5-year OS rates, and a calibration chart was drawn to evaluate the accuracy of the nomogram.[15]

2.9 | Functional enrichment analysis in COAD

To evaluate the underlying gene functions and signaling pathways by which PNN participated in tumorigenesis of COAD, we performed Gene Ontology (GO) analysis by utilizing the open-access online Metascape database (http://metascape.org).[16] Before this, we recognized the top 50 similar genes of PNN in COAD through an interactive open-access bioinformatics platform: gene expression profiling interactive analysis (GEPIA) (http://gepia.cancer-pku.cn). In the present GO analysis, we only considered human species, and the enrichment analysis was conducted with the custom settings of thresholds in "min overlap 3," "p value 0.05," and "min enrichment 3." Furthermore, we performed gene set enrichment analysis (GSEA) to unfold the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways related to PNN expression in COAD. GSEA software (version 4.0.1) was downloaded from the website (http://software.broadinstitute.org/gsea/index.jsp), and the annotated gene set file (c2.cp.kegg.v7.0.symbols.gmt) was acquired from the MSig database.[17] The median value of gene expression was taken as the cutoff point, by which the software divided all samples into high and low groups. The model of "high vs. low" and a random combination of at least 1,000 permutations were selected for analysis. A false discovery rate (FDR) <0.05 was the criterion for the identification of the enriched pathways.

2.10 | PNN expression and immune landscape in COAD

According to the median value of PNN expression, the COAD samples were divided into two groups: high and low expression. Several acknowledged algorithms, including TIMER,[18] CIBERSORT,[19] CIBERSORT-ABS, quanTIseq,[20] MCPcounter,[21] xCELL,[22] and EPIC,[23] were applied to estimate the relationships between PNN expression and tumor-infiltrating immune cells (TIICs). The tumor microenvironment (TME) can define the immune phenotypes of cancers and influence the prognosis of patients. Furthermore, the ESTIMATE

algorithm, a method that calculates immune, stromal, and ESTIMATE scores based on the expression of related molecular biomarkers in immune and stromal cells, was employed to assess the TME status of COAD.[24] Single-sample gene set enrichment analysis (ssGSEA) was further applied to explore the immune-related functional disparities in different PNN expression groups by using the R package *gsva*.[25] Potential immune checkpoint genes (ICGs) retrieved from a previous study were also assessed in different PNN expression groups, and the results are presented as box diagrams. Moreover, the Tumor Immune Dysfunction and Exclusion (TIDE) score and Immunophenoscore (IPS) were calculated to predict the potential clinical immunotherapy response of patients. TIDE is an algorithm for predicting the clinical response to immune checkpoint inhibitors (ICIs) by using gene expression profiles.[26] IPS is a bioinformatics method to quantitatively score the tumor immunogenicity range from 0 to 10, which has been verified for inferring the clinical response to treatment of ICIs.[27] Generally, a lower TIDE score and higher IPS indicate a better response to immunotherapy.

2.11 | Statistical analysis

Perl software 5.32 was used to extract and structure the HTSeq FPKM data, DNA methylation data, and GSEA preparation documents. R 4.0.3 software with specific packages was used to perform analyses for differential gene expression, Pearson correlation, prognostic value evaluation, and nomogram development. The comparisons of intergroup variables were conducted by using the chi-square test with SPSS software 20.0 (IBM). $p < 0.05$ was considered to be statistically significant.

3 | RESULTS

3.1 | Expression features of PNN in digestive tract cancers

The target gene transcript data included in this study were as follows: 162 ESCA and 11 adjacent normal samples, 375 STAD and 32 adjacent normal samples, 480 COAD and 41 adjacent normal samples, and 167 READ and 10 adjacent normal samples. PNN mRNA expression was significantly upregulated in four types of digestive tract cancer samples compared with corresponding normal tissues (Figure 1A). Correlations between PNN expression and tumor clinical stages in ESCA, STAD, COAD, and READ were analyzed. In general, the expression of PNN was positively associated with tumor stage in digestive tract cancers (Figure 1B). Patients with advanced clinical stage tended to have higher PNN expression.

3.2 | Relationships between DNA methylation and PNN expression

Studies have suggested that gene promoter region methylation could affect gene expression, contributing to the progression of human cancer. Current research assessed the effect of promoter DNA methylation on PNN expression in four types of digestive tract cancers using Pearson correlation. We found a significant negative correlation between PNN expression and promoter region methylation levels in digestive tract cancers, especially in STAD (Figure 2A–D). This result indicated that in STAD, abnormal methylation of the promoter region might be one of the important causes of PNN

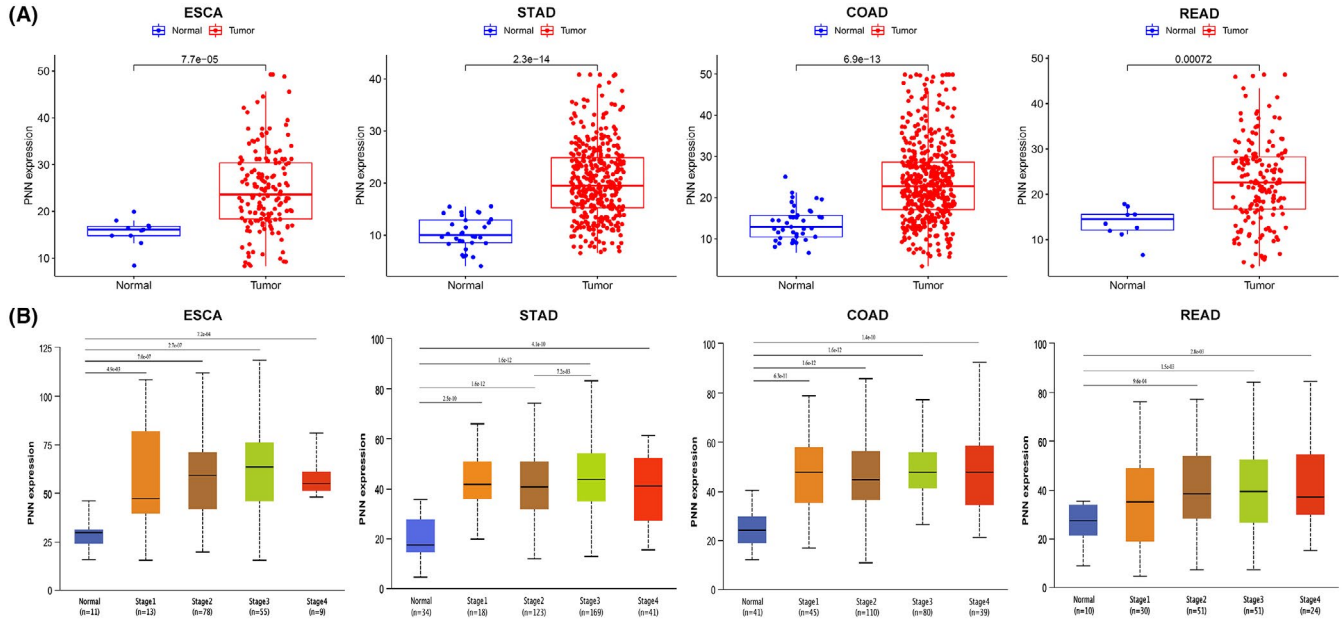

FIGURE 1 (A) The expression level of PNN in ESCA, STAD, colon adenocarcinoma (COAD), READ, and corresponding normal tissues. (B) Correlations between PNN expression and tumor stages in ESCA, STAD, COAD, and READ. The expression of PNN was positively associated with tumor stage in digestive tract cancers

overexpression, but in other digestive tract cancers, other regulatory mechanisms may influence PNN expression.

3.3 | The prognostic value of PNN in digestive tract cancers

Kaplan–Meier analyses indicated that PNN played a disparate prognostic role in different types of digestive tract cancers (Figure 3A,B). In ESCA, the high expression group had worse PFS ($p = 0.044$), and although the trend in OS was similar, there was no significant difference ($p = 0.076$). In STAD, patients with high PNN expression had a better trend in prognosis, but unfortunately, there was no statistically significant difference observed. In COAD, higher PNN expression was significantly associated with poorer OS ($p = 0.003$) and poorer PFS ($p = 0.009$). In patients with READ, a higher expression level of PNN was correlated with longer OS ($p = 0.02$), but there was no significant difference in PFS ($p = 0.082$).

We further explored the genetic alterations in PNN and their effects on prognosis in patients with digestive tract cancers by utilizing the cBioPortal database (Figure S1). The proportions of various genetic alterations of PNN in different digestive tract cancers were similar: 6% in ESCA, 8% in STAD, and 6% in CRC. Notably, the types of genetic alterations were diverse. Amplification and deep deletion were more common in ESCA, and missense mutation and truncating mutation were more frequent in STAD and CRC. There was no significant correlation between PNN genetic alterations and OS in these cancers.

Moreover, we investigated the DNA methylation of PNN CpG sites and the corresponding prognostic effects in four digestive tract cancers using the MethSurv database. The results were illustrated in Table 1. We observed that cg18648343, cg12087797, cg15592059, and cg20337385 were remarkably associated with prognosis in patients with STAD. In COAD, cg15592059, cg24034629, and cg10250651 were indicated as significant factors for prognosis. For READ, the meaningful cg sites in prognosis included cg02969452, cg18648343, and cg12087797. However, no statistically significant DNA methylation CpG sites were observed for predicting OS in ESCA.

Finally, we evaluated the independent prognostic effect of PNN expression in COAD by univariate and multivariate Cox regression analyses. After controlling the clinical parameters, univariate Cox

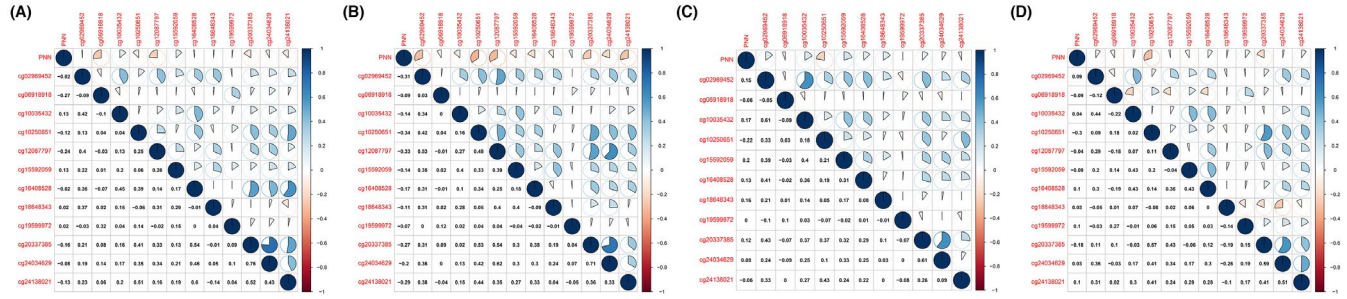

FIGURE 2 Pearson correlation between methylation and PNN expression in (A) ESCA, (B) STAD, (C), colon adenocarcinoma (COAD), and (D) READ. A significant negative correlation between PNN expression and promoter region methylation levels, especially in STAD

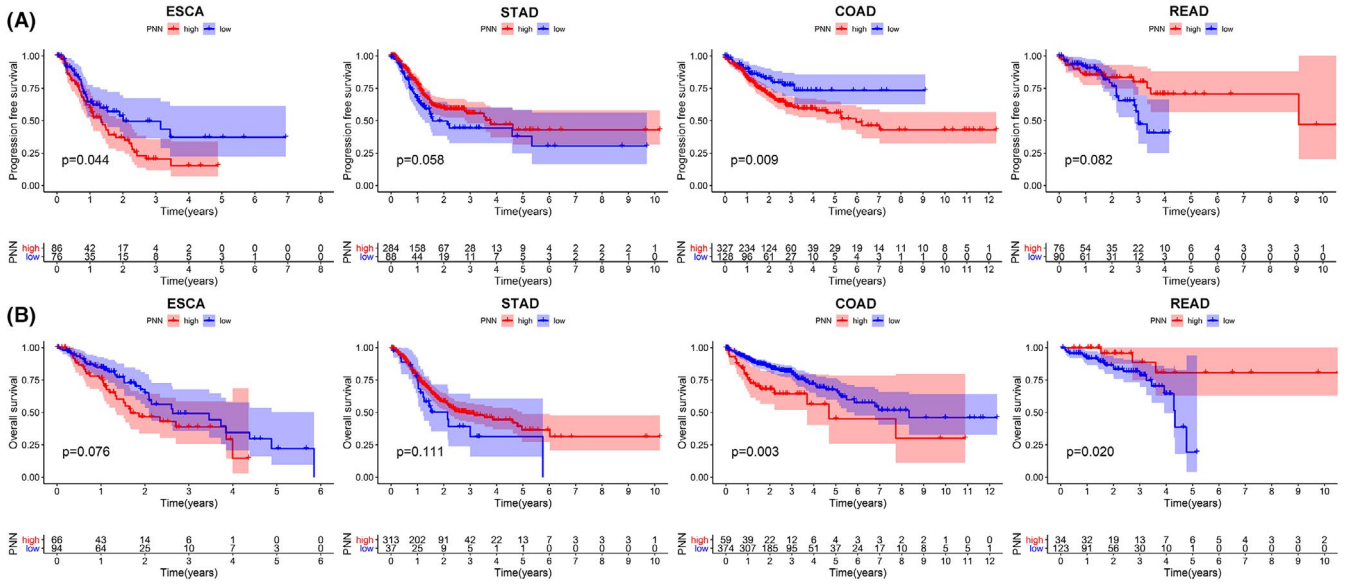

FIGURE 3 Prognostic value of PNN expression in four digestive tract cancers. (A) PFS. (B) OS

TABLE 1 The prognostic effect of CpGs in PNN

Tumor	Gene-CpG	HR	*p*-value
Esophageal carcinoma	PNN−5′UTR;1stExon-Island-cg02969452	0.641	0.06
	PNN−5′UTR;1stExon-Island-cg18648343	0.769	0.3
	PNN-TSS200-Island-cg10035432	1.205	0.41
	PNN-Body-Island-cg12087797	0.664	0.083
	PNN-Body-Island-cg15592059	1.204	0.42
	PNN-Body-Island-cg20337385	1.046	0.84
	PNN-Body-Island-cg24034629	0.646	0.076
	PNN-Body-S_Shore-cg03045079	1.417	0.2
	PNN-Body-S_Shelf-cg06918918	0.792	0.31
	PNN-TSS1500-N_Shore-cg10250651	0.839	0.5
	PNN-TSS1500-N_Shore-cg16408528	0.837	0.53
	PNN-TSS1500-N_Shore-cg19599972	0.863	0.52
	PNN-TSS1500-N_Shore-cg24138021	0.869	0.55
Stomach adenocarcinoma	PNN−5′UTR;1stExon-Island-cg02969452	0.816	0.22
	PNN−5′UTR;1stExon-Island-cg18648343	1.494	0.014*
	PNN-TSS200-Island-cg10035432	0.815	0.22
	PNN-Body-Island-cg12087797	0.639	0.0075*
	PNN-Body-Island-cg15592059	1.558	0.013*
	PNN-Body-Island-cg20337385	1.402	0.043*
	PNN-Body-Island-cg24034629	1.284	0.13
	PNN-Body-S_Shore-cg03045079	1.295	0.18
	PNN-Body-S_Shelf-cg06918918	0.743	0.13
	PNN-TSS1500-N_Shore-cg10250651	1.299	0.15
	PNN-TSS1500-N_Shore-cg16408528	1.066	0.7
	PNN-TSS1500-N_Shore-cg19599972	0.778	0.19
	PNN-TSS1500-N_Shore-cg24138021	1.214	0.29
Colon adenocarcinoma	PNN−5′UTR;1stExon-Island-cg02969452	1.589	0.079
	PNN−5′UTR;1stExon-Island-cg18648343	1.098	0.7
	PNN-TSS200-Island-cg10035432	1.214	0.42
	PNN-Body-Island- cg15592059	0.583	0.025*
	PNN-Body-Island-cg20337385	1.068	0.81
	PNN-Body-Island-cg24034629	0.423	0.0078*
	PNN-Body-S_Shore-cg03045079	0.793	0.35
	PNN-Body-S_Shelf-cg06918918	0.832	0.51
	PNN-TSS1500-N_Shore-cg10250651	0.595	0.041*
	PNN-TSS1500-N_Shore-cg16408528	0.796	0.38
	PNN-TSS1500-N_Shore-cg19599972	1.36	0.21
	PNN-TSS1500-N_Shore-cg24138021	1.245	0.37
Rectum adenocarcinoma	PNN−5′UTR;1stExon-Island-cg02969452	0.154	0.016*
	PNN−5′UTR;1stExon-Island-cg18648343	3.34	0.034*
	PNN-TSS200-Island-cg10035432	0.412	0.082
	PNN-Body-Island-cg12087797	0.318	0.048*
	PNN-Body-Island-cg15592059	1.515	0.4
	PNN-Body-Island-cg20337385	0.674	0.44
	PNN-Body-Island-cg24034629	0.756	0.58

TABLE 1 (Continued)

Tumor	Gene-CpG	HR	*p*-value
	PNN-Body-S_Shore-cg03045079	0.716	0.5
	PNN-Body-S_Shelf-cg06918918	1.677	0.3
	PNN-TSS1500-N_Shore-cg10250651	0.385	0.055
	PNN-TSS1500-N_Shore-cg16408528	0.42	0.14
	PNN-TSS1500-N_Shore-cg19599972	0.304	0.071
	PNN-TSS1500-N_Shore-cg24138021	0.424	0.087

*$p < 0.05$.

TABLE 2 Univariate cox regression analysis of PNN expression as survival predictors in COAD

	Univariate analysis		
Parameter	Hazard Ratio	95% CI	*p* value
Age	1.023	1.005–1.042	0.012*
Gender	1.162	0.769–1.757	0.476
T stage	2.777	1.842–4.187	<0.001*
N stage	2.550	1.673–3.886	<0.001*
M stage	3.519	2.312–5.356	<0.001*
PNN expression	2.064	1.256–3.392	0.004*

*$p < 0.05$.

regression analysis suggested that low PNN expression had significantly better outcomes in both survival ($p = 0.004$) and recurrence ($p = 0.016$) (Tables 2,3). When multivariate analysis with Cox regression was performed, PNN expression was confirmed as an independent prognostic factor for predicting OS in patients with COAD ($p = 0.041$, HR = 1.7, 95% CI 1.02–2.8, Figure 4).

3.4 | Validating the prognostic value of PNN expression in COAD

To verify our results, we further explored the prognostic value of PNN in COAD based on the GEO database. Two GEO datasets, GSE17536 ($n = 177$) and GSE29623 ($n = 65$), with COAD patients were collected in our study, and consistent results were observed. Patients with higher PNN expression were significantly associated with poorer PFS and OS time, both in the GSE17536 and GSE29623 datasets (Figure 5).

3.5 | Establishment of a prognostic nomogram in COAD

A hybrid prognostic nomogram incorporating PNN expression and common clinicopathological characteristics was successfully established for predicting the 1-, 3-, and 5-year overall survival probability, which might be promisingly applied in the clinical evaluation of patients with COAD (Figure 6A). ROC curves and calibration plots of the nomogram indicated an excellent predictive capacity and performance in predicting 1-, 3-, and 5-year overall survival in patients with COAD (Figure 6B,C).

3.6 | Functional enrichment analysis in COAD

Because PNN expression was identified as an independent prognostic factor for recurrence and survival outcome specifically in COAD, we further explored the biological functions of PNN by GO analysis based on Metascape in COAD. In this research, GO pathway and process enrichment analyses included molecular functions (MFs, functional set), biological processes (BPs, pathway), and cellular components (CCs, structural complex). The top 15 clusters are displayed in Figure 7A. CCs included GO: 0016607 (nuclear speck) and GO: 0000226 (microtubule cytoskeleton organization); MFs included GO:0006397 (mRNA processing), GO:1903313 (positive regulation of mRNA metabolic process), GO: 0031124 (mRNA 3'-end processing), GO: 0018023 (peptidyl-lysine trimethylation), and GO: 0006354 (DNA-templated transcription, elongation); BPs included GO: 0033044 (regulation of chromosome organization), GO: 0009314 (response to radiation), GO: 0051056 (regulation of small GTPase mediated signal transduction), and GO: 0061136 (regulation of proteasomal protein catabolic process).

GSEA was performed to evaluate the underlying signaling pathways involved in the carcinogenesis of PNN in COAD. The study indicated that high PNN expression was positively associated with "spliceosome," "basal transcription factors," "WNT signaling pathway," "ERBB signaling pathway," "mTOR signaling pathway," and "Adherens junction" (Figure 7B).

3.7 | Associations between PNN and tumor immune landscape in COAD

In the last few years, increasing research has revealed the crucial relationships between the immune microenvironment and cancer progression. In the current study, we synthetically investigated the effects of PNN expression on tumor-infiltrating immune cells in COAD by using the TIMER algorithms CIBERSORT, CIBERSORT-ABS, QUANTISEQ, MCPCOUNTER, XCELL, and EPIC, which are

shown in a heatmap (Figure 8A). The results illustrated that the tumor-infiltrating immune cells were significantly different in the two PNN expression level groups. The low PNN expression group had significantly positive associations with CD8+ T cells and neutrophils but had markedly negative correlations with CD4+ T cells, T cell regulatory cells, macrophages, and dendritic cells. Immune-related functional analyses showed that checkpoint (inhibition), cytolytic (activity), APC coinhibition, APC costimulation, HLA, CCR, inflammation promotion, MHC class I, parainflammation, T cell costimulation, T cell coinhibition, and type I/II INF response were significantly different between the low and high expression groups (Figure 8B). The low expression group had significantly upregulated expression of GZMA, TNF, LAG3, HAVCR2, and PDCD1 (Figure 8C). In addition, ESTIMATE analysis demonstrated that the low expression group had a higher immune score and ESTIMATE score, indicating a higher tumor purity in the high expression group (Figure 8D). The TIDE and IPS analyses showed a lower TIDE score and higher IPS in the low expression group, including "CTLA4_neg PD1_pos," "CTLA4_pos PD1_neg," and "CTLA4_pos PD1_pos," suggesting that low PNN expression might indicate a better clinical response to ICI treatment (Figure S2).

TABLE 3 Univariate Cox regression analysis of PNN expression as recurrence predictors in COAD

	Univariate analysis		
Parameter	Hazard Ratio	95% CI	*p* value
Age	1.000	0.986–1.015	1.000
Gender	1.192	0.831–1.711	0.339
T stage	2.750	1.947–3.883	<0.001*
N stage	2.551	1.772–3.672	<0.001*
M stage	3.088	2.145–4.445	<0.001*
PNN expression	1.767	1.112–2.806	0.016*

*$p < 0.05$.

4 | DISCUSSION

PNN was initially reported as a novel factor involved in the mature desmosomes of epithelial cells.[1] Studies have revealed that PNN participates in apoptosis, proliferation, and migration regulation by affecting mRNA splicing and gene transcription.[28] PNN was once described as a potential cancer suppressor factor in RCC via PNN/DRS/memA, and upregulated expression of PNN resulted in inhibition of cell growth.[2] Conversely, PNN has been found to increase cell growth. High PNN expression had a negative effect on survival in breast cancer cells. With an increasing number of studies, the biofunction of PNN has been gradually disclosed. Previous research has revealed that PNN is overexpressed in nasopharyngeal cancer and is associated with poor overall survival.[29] A similar finding was also reported in a hepatocellular carcinoma (HCC) study; an elevated level of PNN was correlated with aggressive characteristics and poor overall survival. In addition, suppression of PNN expression inhibits HCC cell proliferation and cell viability but promotes glucose deprivation-induced apoptosis.[4] However, few studies have systematically explored the PNN profile in digestive tract cancers to date. In our study, we comprehensively explored the expression of PNN in digestive tract cancers. Our findings showed that PNN was highly expressed in ESCA, STAD, COAD, and READ compared with corresponding normal tissues. We further analyzed the PNN expression status in different clinical stages for each type of digestive tract cancer. The study demonstrated that PNN was overexpressed in all stages of tumors compared with corresponding normal tissues. In addition, we observed that advanced-stage tumors tended to have higher PNN expression in digestive tract cancers.

Studies have shown that abnormal DNA methylation participates in gene expression. DNA methylation can be used as a biomarker for cancer diagnosis and prognosis.[30] For example, Li et al. found that abnormal DNA methylation of the MCC gene was associated with the progression of esophageal adenocarcinoma via epigenetic regulation.[31] Homma et al. revealed that promoter region

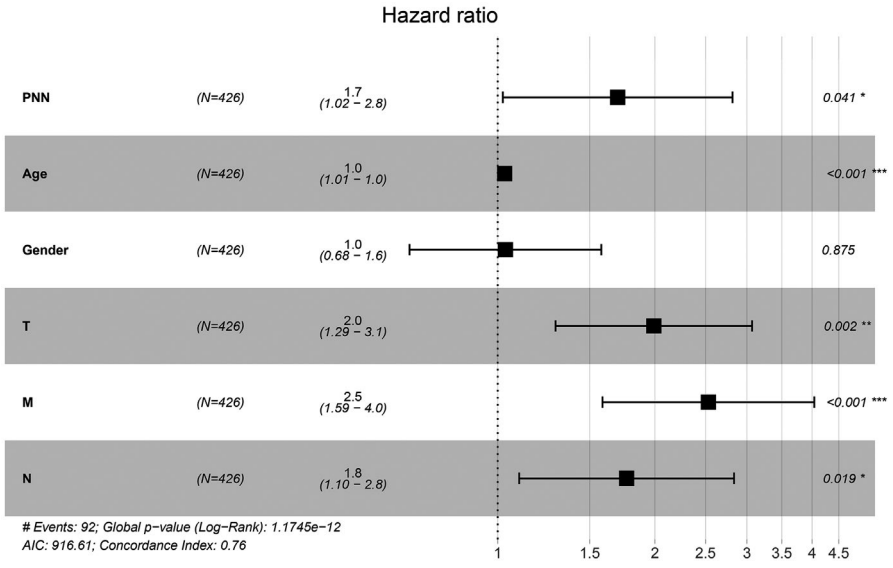

FIGURE 4 The results of multivariate Cox regression analyses of significant prognosis in patients with colon adenocarcinoma (COAD), which are represented in a forest plot. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$

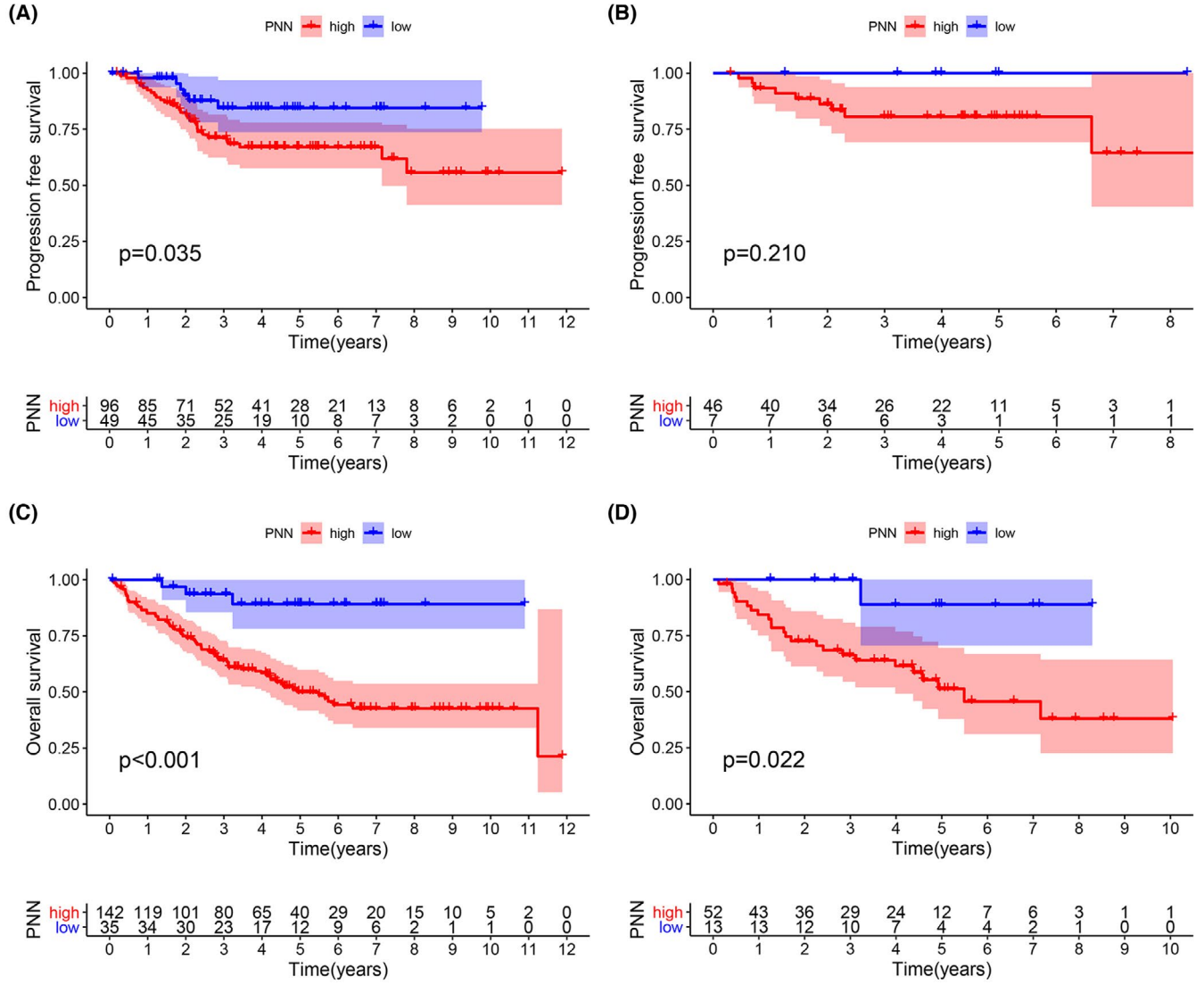

FIGURE 5 Survival curves of different PNN expression groups in colon adenocarcinoma (COAD) based on the GSE17536 dataset (A,C) and GSE29623 dataset (B,D)

hypermethylation resulted in frequent gene silencing of RUNX3 in gastric cancer occurrence and development.[32] Melotte et al. suggested that N-Myc downstream-regulated gene 4 (NDRG4) promoter methylation could be a potential biomarker for the detection of colorectal cancer.[33] Liang et al. found that some methylation-regulated differentially expressed genes play an important role in colon cancer (CC) progression.[34] Wang et al. reported that hypomethylated and hypermethylated differentially methylated CpG sites could be used as diagnostic and prognostic biomarkers in CC.[35] Thus, in the present study, we analyzed the correlations between PNN mRNA expression and the DNA methylation level of cg sites in promoter regions in different digestive tract cancers. The results showed that PNN expression was significantly negatively associated with the DNA methylation level in gastric cancer. This intensively indicated that abnormal methylation of the promoter region is one of the important causes of PNN gene overexpression in STAD. Moreover, our study revealed that methylation of several PNN CpG sites showed significantly positive prognostic effects in STAD, COAD, and READ, such as cg12087797 for STAD, cg15592059, cg24034629, cg10250651 for COAD, and cg02969452, cg12087797 for READ. These results may provide a clue that PNN promoter region methylation could be a candidate prognostic biomarker in patients with these cancers.

The prognostic value of PNN expression has been investigated in several cancers. Upregulated PNN was found to be related to cellular proliferation, invasion, and metastasis in colorectal cancer.[3] Upregulated PNN was confirmed as an independent adverse prognostic factor in hepatocellular carcinoma patients.[4] In addition, an association between the overexpressed level of PNN and aggressive behavior and poor prognosis in patients with ovarian cancer and nasopharyngeal cancer has also been reported.[5,29] In the current study, we found that PNN high expression had significantly poor OS and DFS in colon cancer, which was verified based on GEO datasets. Further analysis confirmed that PNN expression

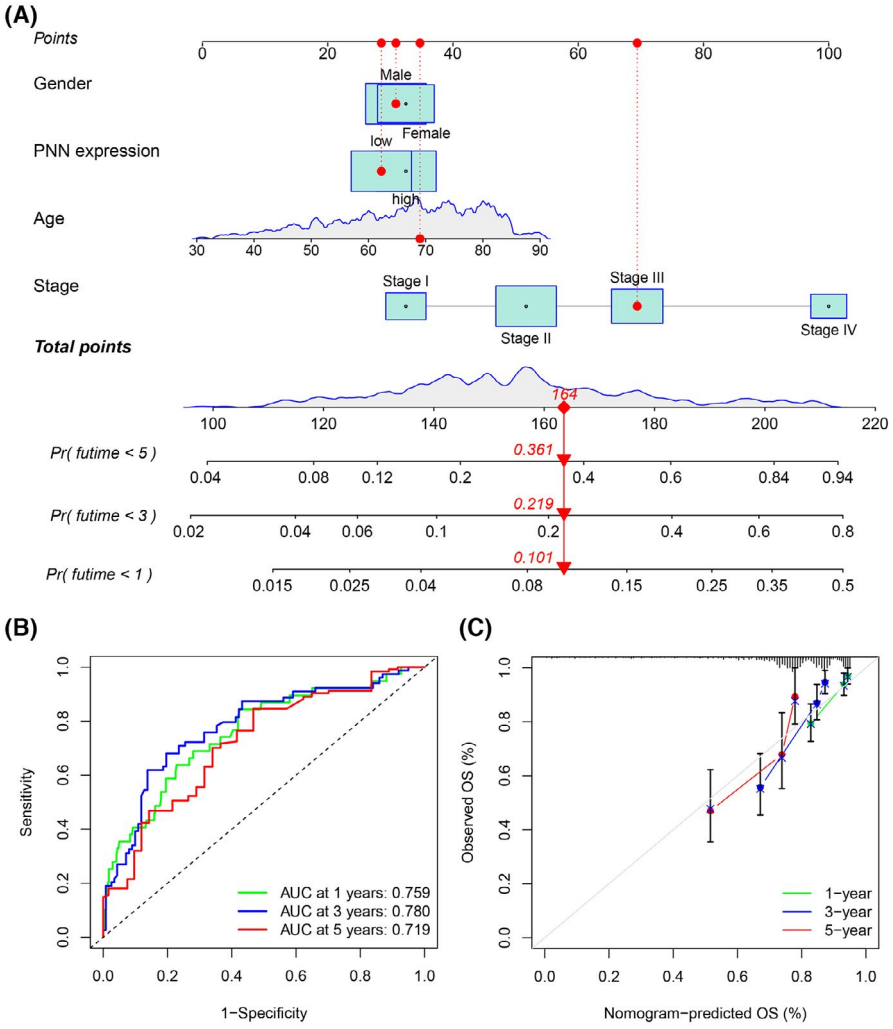

FIGURE 6 (A) A prognostic nomogram for patients with colon adenocarcinoma (COAD). (B) ROC curves showing the capability of the nomogram in predicting 1-, 3-, and 5-year OS. (C) Calibration plot showing that the nomogram-predicted survival probabilities correspond closely to the observed proportions

was an independent prognostic factor for predicting OS in colon cancer. Additionally, we found that high expression of PNN was significantly related to poorer PFS in esophageal cancer. However, in contrast, upregulated PNN expression was markedly related to longer OS and tended to have longer PFS in rectal cancer. However, beyond that, our study rejected the independent prognostic effect of PNN in esophageal, gastric, and rectal cancer. Since previous studies have not subdivided colorectal cancer, the controversial results may be attributed to heterogeneity of the tumor site or insufficient sample numbers of rectal cancer in TCGA. Since our study validated the powerful efficiency of the prognostic value of PNN in patients with COAD, a promising prognostic nomogram incorporating PNN expression and common clinicopathological characteristics was successfully established for predicting the 1-, 3-, and 5-year overall survival probability, which had excellent predictive capacity and performance. It might be well applied in clinical evaluation.

It is commonly known that the prognosis and drug response of colorectal cancer patients are closely associated with specific gene mutation statuses, such as KRAS, BRAF, and PIK3CA.[36,37] Thus, we further explored whether PNN mutation features affect the prognosis of digestive tract cancers. Our results turned out to be disappointing; although the types of genetic alterations were diverse, there were no significant correlations between the PNN mutation status and overall survival in different digestive tract cancers.

The molecular mechanism of PNN has been illustrated by several studies; however, it remains uncertain. Activation of the ERK signaling pathway was observed in colorectal cancer cells and HCC cells associated with PNN overexpression.[3,4] A recent study reported that PNN was highly expressed in osteosarcoma and facilitated cell proliferation, invasion, and adhesion through inhibition of microRNA (miR)-330-3p by circular RNA cir_0032463.[38] In human corneal epithelial cells, PNN plays a key role in cell–cell adhesion by inducing desmoglein-2 (DSG2) and E-cadherin (E-ca), while downregulation of PNN reduces E-cadherin and interrupts cell–cell adhesion.[39,40] In previous studies, E-cadherin was proposed as a tumor suppressor gene clinically; however, in invasive ductal breast cancer, E-cadherin was found to promote metastasis.[41,42] Thus, the role of PNN in regulating E-cadherin expression and tumor invasion remains controversial. Another study showed that PNN was upregulated in prostate cancer tissues and accelerated cell invasion with downregulation of E-cadherin. A mechanistic study demonstrated that PNN promotes tumor proliferation by activating CREB via the PI3K/AKT and ERK/MAPK pathways.[43] In

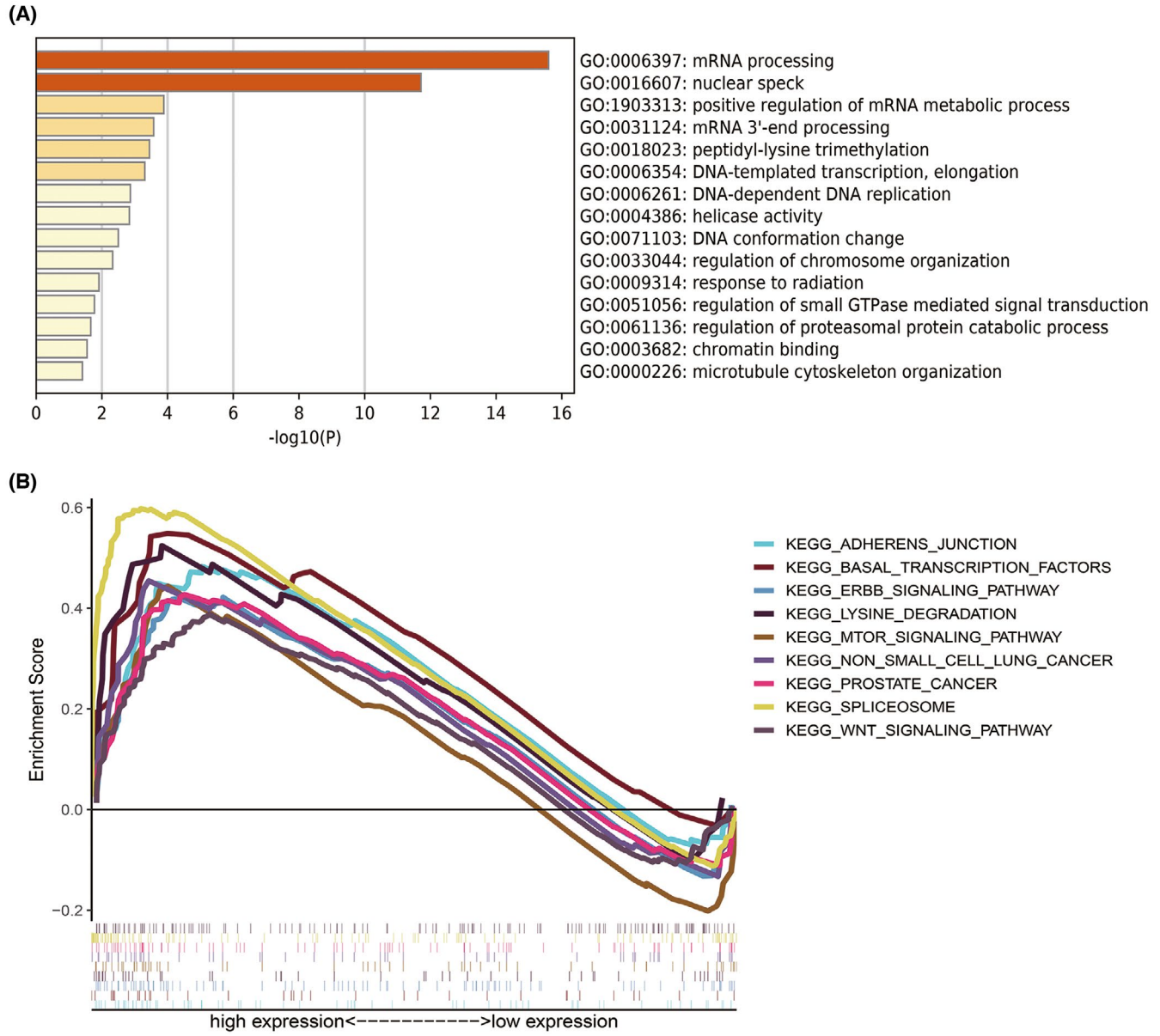

FIGURE 7 (A) GO analysis of PNN in colon adenocarcinoma (COAD). (B) GSEA of PNN in COAD

our study, the results of functional enrichment analysis demonstrated that PNN was involved in "spliceosome," "Adherens junction," and mRNA processing." KEGG analysis showed that PNN affects cell–cell adhesion and tumor invasion and metastasis via a variety of signaling pathways (e.g., WNT signaling pathway, ErbB signaling pathway, and mTOR signaling pathway). The WNT signaling pathway is known as one of the most important signaling pathways, and its activation is very common during the development of many tumors by facilitating cell differentiation, polarization, and migration.[44] ErbB belongs to the receptor tyrosine kinase receptor family and includes four distinct members: EGFR (also known as ErbB-1/HER1), ErbB-2 (HER2), ErbB-3 (HER3), and ErbB-4 (HER4). The ErbB pathway is one of the most extensively studied areas of signal transduction and best exemplifies the pathogenic power of aberrations in biological information transfer.[45,46] The mTOR signaling pathway is frequently activated in cancer and regulates cell growth and various cellular metabolic processes.[47]

Until now, few studies have investigated the effect of PNN expression on tumor-infiltrating immune cells and the tumor microenvironment. A study reported that PNN was strongly related to the T cell receptor signaling pathway in renal cell carcinoma and had a positive correlation with TIICs.[6] In the present study, we found that the tumor-infiltrating immune cells were significantly different in the two PNN expression level groups. The low PNN expression group had significantly positive associations with CD8+ T cells and neutrophils but had markedly negative correlations with CD4+ T cells, T cell regulatory cells, macrophages, and dendritic cells. A study analyzing the prognostic landscape of infiltrating immune cells across human cancers showed that CD8+ T cells were regarded as one of the top favorable prognostic T cell signatures in pancancer and

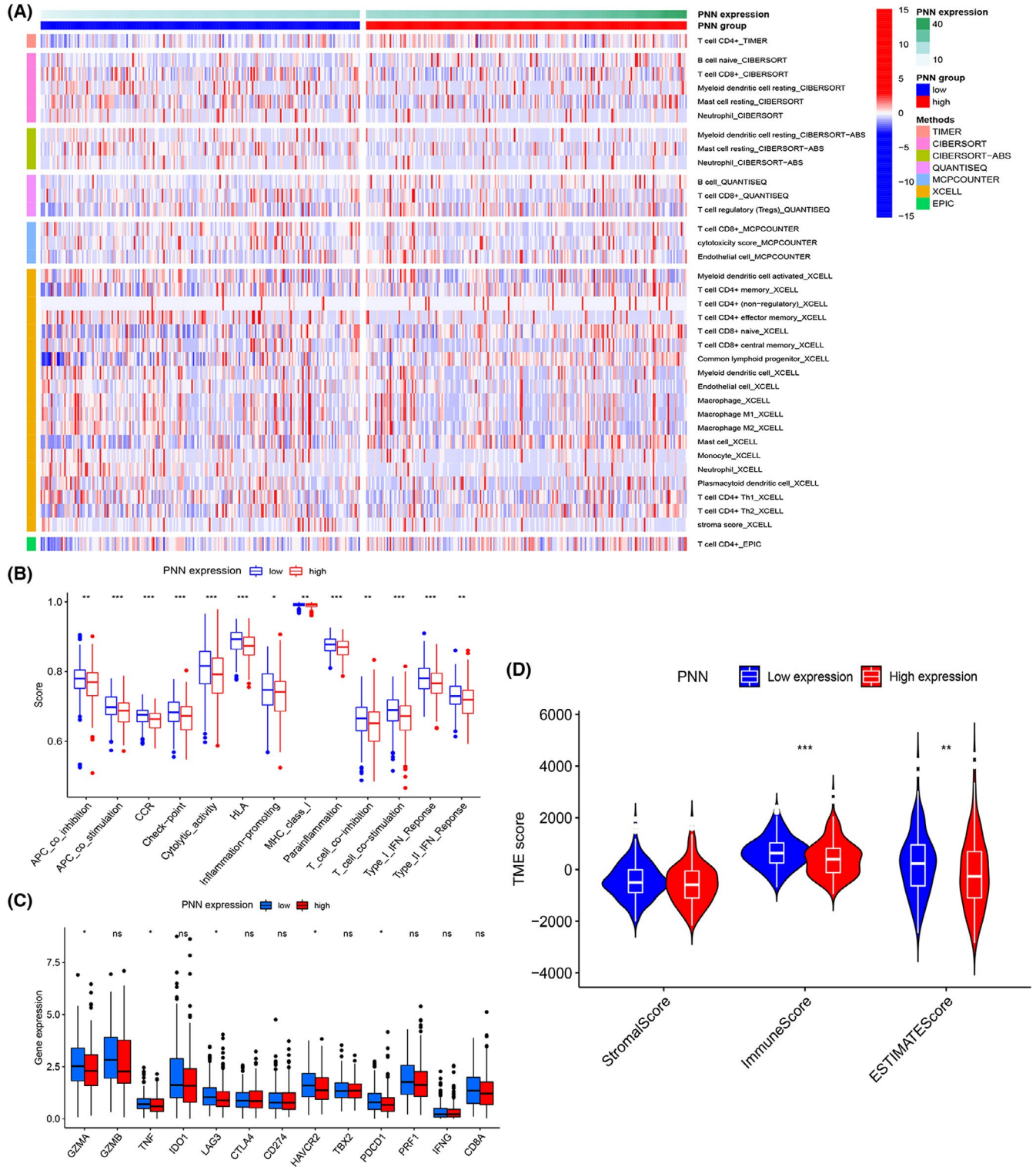

FIGURE 8 (A) Heatmap for tumor-infiltrating immune cells in colon adenocarcinoma (COAD) by using different algorithms among the low and high PNN expression groups. (B) Immune-related functional analyses between the low and high PNN expression groups in COAD. (C) The expression of immune checkpoint genes between the low and high PNN expression groups in COAD. (D) ESTIMATE analysis between the low and high PNN expression groups in COAD

solid tumors.[48] In addition, ESTIMATE analysis demonstrated that the low expression group had a higher immune score and ESTIMATE score, indicating a higher tumor purity in the high expression group. Moreover, immune-related functions were significantly different between the low and high expression groups. Our study showed that abnormally high expression of PNN can regulate the immune microenvironment of colon cancer, reduce the invasion of killer immune cells, and increase the invasion of regulatory immune cells,

leading to an increase in tumor cells. We found that low PNN expression significantly upregulated the expression of GZMA, TNF, LAG3, HAVCR2, and PDCD1. GZMA belongs to the serine protease family, is mainly expressed by cytotoxic cells (natural killer cells and cytolytic CD8+ T cells), and is involved in the regulation of the inflammatory response.[49] It is well known that inflammation is closely connected with tumorigenesis; for example, patients suffering from ulcerative colitis have a higher risk of colorectal cancer (CRC). Llipsy et al. found that GZMA plays a crucial role in inflammatory CRC. GZMA mRNA expression was significantly elevated in CRC tissue, and treatment with the GZMA inhibitor serpinb6b reduced the incidence of tumors in animal trials.[50] These findings provide information that GZMA may be a therapeutic target for CRC. Tumor necrosis factors (TNFs), including TNF-α and TNF-β, are mainly expressed on active macrophages and lymphocytes. TNF-α is also a potent proinflammatory cytokine that plays a critical role in the inflammatory response.[51] LAG3, also called CD223, is a receptor expressed on a natural killer cell line and has been highly considered a next-generation immune checkpoint due to its substantial prognostic value. Numerous studies have shown that LAG3 acts a remarkable synergy with PD-1 in promoting the immune escape of cancer cells in various cancer types, such as gastric cancer, renal cell carcinoma, and colorectal cancer.[52,53] Due to the striking therapeutic effects of the simultaneous blockade of LAG3 and PD-1 in melanoma patients, an increasing number of pharmaceutical companies are encouraged to invest in drug research. For example, early clinical data of BMS's LAG3 targeting antibody relatlimab showed an improved OS when combined with the PD-1 inhibitor nivolumab.[54] HAVCR2, also known as TIM3, was identified as a molecule expressed by interferon-γ (IFNγ)-producing CD4+ T cells, CD8+ T cells, and many other cell types. Many studies have reported that TIM3 can act on dysfunctional or "exhausted" T cells in chronic viral infections and cancer. Many clinical trials combining blockade of TIM3 with other checkpoint inhibitors, such as PD-1, PD-L1, and LAG3, are ongoing.[55] The TIDE and IPS analyses showed a lower TIDE score and higher IPS in the low expression group, suggesting that low expression of PNN might indicate a better clinical response to ICI treatment. This result is logically consistent with the above ICG analyses.

There were several limitations in this study. First, we only obtained the results through bioinformatics and database analysis, and further experimental verification is required. Second, the limited sample size of the subgroup may affect the results. Moreover, since APC, P53, and KRAS are common mutated genes in colon cancer, the correlations between PNN status and these genes worth further exploration. Finally, the prognostic nomogram for patients with COAD needs more clinical verification. However, our study has convincing power for its larger sample-based study using the TCGA database.

5 | CONCLUSION

In conclusion, our bioinformatics analyses demonstrated that PNN was highly expressed in digestive tract cancers and could act as an independent prognostic factor and a response predictor for ICI treatment in COAD. Our results have promising clinical application prospects and deserve further study.

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CONFLICT OF INTEREST

The authors declare that they have no competing interests.

AUTHOR CONTRIBUTIONS

KTL, HZ, and MJ conceived and designed the study. MJ, HZ, MY, and YPB performed the analyses. MY and SHY prepared all tables and figures. KTL, HZ, and MJ wrote the main manuscript. KTL and HZ contributed to the revised manuscript. All authors approved the final version of the manuscript.

ETHICAL APPROVAL

TCGA and GEO belong to public databases. The patients involved in the database have obtained ethical approval. Users can download relevant data for free for research and publish relevant articles. Our study is based on open-source data, so there are no ethical issues and other conflicts of interest. It was granted an exemption from ethics approval from the Institutional Review Board of the Lihuili Hospital, Ningbo Medical Center.

DATA AVAILABILITY STATEMENT

The data used for bioinformatics analyses in this study are freely available on the TCGA program website (https://xena.ucsc.edu; https://portal.gdc.cancer.gov/), GEO database website (Gene Expression Omnibus, http://www.ncbi. nlm.nih.gov/geo/), and other open-access bioinformatics databases. The interpretation and reporting of these data are the sole responsibility of the authors.

ORCID

Kaitai Liu https://orcid.org/0000-0003-1757-5591

REFERENCES

1. Ouyang P, Sugrue S. Characterization of pinin, a novel protein associated with the desmosome-intermediate filament complex. *J Cell Biol*. 1996;135(4):1027-1042.
2. Shi Y, Ouyang P, Sugrue S. Characterization of the gene encoding pinin/DRS/memA and evidence for its potential tumor suppressor function. *Oncogene*. 2000;19(2):289-297.
3. Wei Z, Ma W, Qi X, et al. Pinin facilitated proliferation and metastasis of colorectal cancer through activating EGFR/ERK signaling pathway. *Oncotarget*. 2016;7(20):29429-29439.
4. Yang X, Sun D, Dong C, Tian Y, Gao Z, Wang L. Pinin associates with prognosis of hepatocellular carcinoma through promoting cell proliferation and suppressing glucose deprivation-induced apoptosis. *Oncotarget*. 2016;7(26):39694-39704.

5. Zhang Y, Kwok JS, Choi PW, et al. Pinin interacts with C-terminal binding proteins for RNA alternative splicing and epithelial cell identity of human ovarian cancer cells. *Oncotarget*. 2016;7(10):11397-11411.
6. Jin M, Li D, Liu W, Wang P, Xiang Z, Liu K. Pinin acts as a poor prognostic indicator for renal cell carcinoma by reducing apoptosis and promoting cell migration and invasion. *J Cell Mol Med*. 2021;25(9):4340-4348.
7. Alpatov R, Shi Y, Munguba GC, et al. Corepressor CtBP and nuclear speckle protein Pnn/DRS differentially modulate transcription and splicing of the E-cadherin gene. *Mol Cell Biol*. 2008;28(5):1584-1595.
8. Sung H, Ferlay J, Siegel RL, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*. 2021;71(3):209-249.
9. Mini E, Lapucci A, Perrone G, et al. RNA sequencing reveals PNNandKCNQ1OT1as predictive biomarkers of clinical outcome in stage III colorectal cancer patients treated with adjuvant chemotherapy. *Int J Cancer*. 2019;145(9):2580-2593.
10. Ritchie ME, Phipson B, Wu D, et al. limma powers differential expression analyses for RNA-sequencing and microarray studies. *Nucleic Acids Res*. 2015;43(7):e47.
11. Meyer M, Kircher M. Illumina sequencing library preparation for highly multiplexed target capture and sequencing. *Cold Spring Harbor Protoc*. 2010;2010(6):pdb.prot5448.
12. Modhukur V, Iljasenko T, Metsalu T, Lokk K, Laisk-Podar T, Vilo J. MethSurv: a web tool to perform multivariable survival analysis using DNA methylation data. *Epigenomics*. 2018;10(3):277-288.
13. Gao J, Aksoy BA, Dogrusoz U, et al. Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. *Sci Signal*. 2013;6(269):pl1.
14. Tang Y, Li C, Zhang YJ, Wu ZH. Ferroptosis-related long non-coding RNA signature predicts the prognosis of head and neck squamous cell carcinoma. *Int J Biol Sci*. 2021;17(3):702-711.
15. Iasonos A, Schrag D, Raj GV, Panageas KS. How to build and interpret a nomogram for cancer prognosis. *J Clin Oncol*. 2008;26(8):1364-1370.
16. Zhou Y, Zhou B, Pache L, et al. Metascape provides a biologist-oriented resource for the analysis of systems-level datasets. *Nat Commun*. 2019;10(1):1523.
17. Subramanian A, Tamayo P, Mootha VK, et al. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. *Proc Natl Acad Sci*. 2005;102(43):15545-15550.
18. Li T, Fan J, Wang B, et al. TIMER: a web server for comprehensive analysis of tumor-infiltrating immune cells. *Can Res*. 2017;77(21):e108-e110.
19. Newman AM, Liu CL, Green MR, et al. Robust enumeration of cell subsets from tissue expression profiles. *Nat Methods*. 2015;12(5):453-457.
20. Plattner C, Finotello F, Rieder D. Deconvoluting tumor-infiltrating immune cells from RNA-seq data using quanTIseq. *Methods Enzymol*. 2020;636:261-285.
21. Becht E, Giraldo NA, Lacroix L, et al. Estimating the population abundance of tissue-infiltrating immune and stromal cell populations using gene expression. *Genome Biol*. 2016;17(1):218.
22. Aran D, Hu Z, Butte AJ. xCell: digitally portraying the tissue cellular heterogeneity landscape. *Genome Biol*. 2017;18(1):220.
23. Racle J, de Jonge K, Baumgaertner P, Speiser D, Gfeller D. Simultaneous enumeration of cancer and immune cell types from bulk tumor gene expression data. *eLife*. 2017;6:e26476.
24. Yoshihara K, Shahmoradgoli M, Martinez E, et al. Inferring tumour purity and stromal and immune cell admixture from expression data. *Nat Commun*. 2013;4:2612.
25. Yi M, Nissley DV, McCormick F, Stephens RM. ssGSEA score-based Ras dependency indexes derived from gene expression data reveal potential Ras addiction mechanisms with possible clinical implications. *Sci Rep*. 2020;10(1):10258.
26. Jiang P, Gu S, Pan D, et al. Signatures of T cell dysfunction and exclusion predict cancer immunotherapy response. *Nat Med*. 2018;24(10):1550-1558.
27. Charoentong P, Finotello F, Angelova M, et al. Pan-cancer immunogenomic analyses reveal genotype-immunophenotype relationships and predictors of response to checkpoint blockade. *Cell Rep*. 2017;18(1):248-262.
28. Hsu SY, Mukda S, Leu S. Expression and distribution pattern of Pnn in ischemic cerebral cortex and cultured neural cells exposed to oxygen-glucose deprivation. *Brain Sci*. 2020;10(10):708.
29. Tang T, Yang L, Cao Y, et al. LncRNA AATBC regulates Pinin to promote metastasis in nasopharyngeal carcinoma. *Mol Oncol*. 2020;14(9):2251-2270.
30. Koch A, Joosten SC, Feng Z, et al. Analysis of DNA methylation in cancer: location revisited. *Nat Rev Clin Oncol*. 2018;15(7):459-466.
31. Li D, Zhang L, Liu Y, et al. Specific DNA methylation markers in the diagnosis and prognosis of esophageal cancer. *Aging*. 2019;11(23):11640-11658.
32. Homma N, Tamura G, Honda T, et al. Spreading of methylation within RUNX3 CpG island in gastric cancer. *Cancer Sci*. 2006;97(1):51-56.
33. Melotte V, Lentjes MH, van den Bosch SM, et al. N-Myc downstream-regulated gene 4 (NDRG4): a candidate tumor suppressor gene and potential biomarker for colorectal cancer. *J Natl Cancer Inst*. 2009;101(13):916-927.
34. Liang Y, Zhang C, Dai D-Q. Identification of differentially expressed genes regulated by methylation in colon cancer based on bioinformatics analysis. *World J Gastroenterol*. 2019;25(26):3392-3407.
35. Wang Y, Zhang M, Hu X, Qin W, Wu H, Wei M. Colon cancer-specific diagnostic and prognostic biomarkers based on genome-wide abnormal DNA methylation. *Aging*. 2020;12(22):22626-22655.
36. Oh JE, Kim MJ, Lee J, et al. Magnetic resonance-based texture analysis differentiating KRAS mutation status in rectal cancer. *Cancer Res Treat*. 2020;52(1):51-59.
37. Derbel O, Wang Q, Desseigne F, et al. Impact of KRAS, BRAF and PI3KCA mutations in rectal carcinomas treated with neoadjuvant radiochemotherapy and surgery. *BMC Cancer*. 2013;13:200.
38. Qin G, Wu X. Hsa_circ_0032463 acts as the tumor promoter in osteosarcoma by regulating the miR-330-3p/PNN axis. *Int J Mol Med*. 2021;47(5):92.
39. Alpatov R, Munguba GC, Caton P, et al. Nuclear speckle-associated protein Pnn/DRS binds to the transcriptional corepressor CtBP and relieves CtBP-mediated repression of the E-cadherin gene. *Mol Cell Biol*. 2004;24(23):10223-10235.
40. Joo J, Alpatov R, Munguba G, Jackson M, Hunt M, Sugrue S. Reduction of Pnn by RNAi induces loss of cell-cell adhesion between human corneal epithelial cells. *Mol vis*. 2005;11:133-142.
41. Leu S. The role and regulation of Pnn in proliferative and non-dividing cells: form embryogenesis to pathogenesis. *Biochem Pharmacol*. 2021;192:114672.
42. Padmanaban V, Krol I, Suhail Y, et al. E-cadherin is required for metastasis in multiple models of breast cancer. *Nature*. 2019;573(7774):439-444.
43. Meng X, Zhang H, Ren Y, et al. Pinin promotes tumor progression via activating CREB through PI3K/AKT and ERK/MAPK pathway in prostate cancer. *Am J Cancer Res*. 2021;11(4):1286-1303.
44. Taciak B, Pruszynska I, Kiraga L, Bialasek M, Krol M. Wnt signaling pathway in development and cancer. *J Physiol Pharmacol*. 2018;69(2):185-196.
45. Normanno N, De Luca A, Bianco C, et al. Epidermal growth factor receptor (EGFR) signaling in cancer. *Gene*. 2006;366(1):2-16.
46. Citri A, Yarden Y. EGF-ERBB signalling: towards the systems level. *Nat Rev Mol Cell Biol*. 2006;7(7):505-516.

47. Mossmann D, Park S, Hall MN. mTOR signalling and cellular metabolism are mutual determinants in cancer. *Nat Rev Cancer*. 2018;18(12):744-757.
48. Gentles AJ, Newman AM, Liu CL, et al. The prognostic landscape of genes and infiltrating immune cells across human cancers. *Nat Med*. 2015;21(8):938-945.
49. Arias M, Martinez-Lostao L, Santiago L, Ferrandez A, Granville DJ, Pardo J. The untold story of granzymes in oncoimmunology: novel opportunities with old acquaintances. *Trends Cancer*. 2017;3(6):407-422.
50. Santiago L, Castro M, Sanz-Pamplona R, et al. Extracellular granzyme A promotes colorectal cancer development by enhancing gut inflammation. *Cell Rep*. 2020;32(1):107847.
51. Horiuchi T, Mitoma H, Harashima S, Tsukamoto H, Shimoda T. Transmembrane TNF-alpha: structure, function and interaction with anti-TNF agents. *Rheumatology*. 2010;49(7):1215-1228.
52. Woo SR, Turnis ME, Goldberg MV, et al. Immune inhibitory molecules LAG-3 and PD-1 synergistically regulate T-cell function to promote tumoral immune escape. *Cancer Res*. 2012;72(4):917-927.
53. Lecocq Q, Keyaerts M, Devoogdt N, Breckpot K. The next-generation immune checkpoint LAG-3 and its therapeutic potential in oncology: third time's a charm. *Int J Mol Sci*. 2021;22(1):75.
54. Baraniskin A, Van Laethem JL, Wyrwicz L, et al. Clinical relevance of molecular diagnostics in gastrointestinal (GI) cancer: European Society of Digestive Oncology (ESDO) expert discussion and recommendations from the 17th European Society for Medical Oncology (ESMO)/World Congress on Gastrointestinal Cancer, Barcelona. *Eur J Cancer*. 2017;86:305-317.
55. Wolf Y, Anderson AC, Kuchroo VK. TIM3 comes of age as an inhibitory receptor. *Nat Rev Immunol*. 2020;20(3):173-185.

SUPPORTING INFORMATION

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