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ORIGINAL RESEARCH

Correlation of NPDC1 Expression and Perineural Invasion Status with Clinicopathological Features in Patients with Colon Cancer

Jiannan Li[1,2], Yao Sun[2], Lanqing Cao[3], Fang Wang[1]

[1]Department of Medical Microbiology, College of Basic Medical Sciences, Jilin University, Changchun, People's Republic of China; [2]Department of Colorectal and Anal Surgery, Jilin University Second Hospital, Changchun, People's Republic of China; [3]Department of Pathology, Jilin University Second Hospital, Changchun, People's Republic of China

Correspondence: Fang Wang, Department of Medical Microbiology, College of Basic Medical Sciences, Jilin University, Changchun, People's Republic of China, Email wf@jlu.edu.cn

Background: Colon cancer is a prevalent gastrointestinal malignancy that often exhibits distant metastasis, hindering the effectiveness of surgical interventions. In addition to well-known hematogenous and lymphatic metastasis, perineural invasion (PNI) has emerged as a significant mode of distant metastasis in colon tumors. PNI is closely associated with oncologic pain in advanced cancer patients, but the underlying mechanisms and associated biomarkers, which might be the novel therapeutic targets, remain poorly understood.

Methods: In this study, we employed large databases and bioinformatics methods to identify genes strongly linked to PNI in colon cancer and investigated their involvement in tumor nerve invasion, progression mechanisms, and chemotherapy resistance. Immunohistochemical techniques were utilized to validate the expression of target genes in 384 colon cancer tissues, and their expression was correlated with clinicopathological characteristics and patient survival data in our hospital. Furthermore, we conducted a comprehensive literature review to explore the potential functions of the target genes and their associated genes.

Results: Our screening revealed a significant correlation between neural proliferation differentiation and control-1 (NPDC1) expression and patient prognosis, suggesting a potential association with neural infiltration in colon cancer. Additionally, NPDC1 may promote tumorigenesis, progression, and chemoresistance through various related pathways.

Conclusion: Our study provides novel insights into the utility of NPDC1 as a predictive marker for PNI status, disease-free survival, and overall survival in patients with colon cancer, highlighting the prevalence of NPDC1 overexpression in patients with PNI in colon cancer.

Keywords: colon cancer, neural proliferation differentiation and control-1, NPDC1, perineural invasion, PNI, clinicopathological features

Introduction

Colon cancer is one of the most prevalent digestive system tumors. Surgical interventions have shown positive outcomes in early resectable colon cancer; however, the cure rate remains inadequate for patients with advanced colon cancer and postoperative recurrence.[1,2] In addition to the traditional modes of tumor spread, such as direct infiltration, peritoneal dissemination, hematogenous metastasis, and lymphatic metastasis, perineural invasion (PNI) is considered a pathway for tumor spread in gastrointestinal tumors like colon cancer and may even be the sole route for certain distant metastases.[3,4] Hence, PNI is critical in patient survival, distant metastasis and recurrence risk, and overall survival duration in colon cancer. Therefore, more research into colon cancer and the biomarkers associated with PNI is essential for patient diagnosis, care, and prognosis.

The transcription factor E2F-1 and the cell cycle protein D1, which are known to be involved in neural proliferation, have been revealed to be tightly linked with neural proliferation differentiation and control-1 (NPDC1), a protein that is uniquely produced in the neuronal cells.[5–7] This shows that NPDC1 may control the cell cycle and cell differentiation

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processes. Meanwhile, research suggests that the intestinal neuropeptide secretion pathway, which in turn influences axonal regeneration, may be connected to the expression of NPDC1.[8] We, therefore, hypothesize that the expression of NPDC1 may be associated with the occurrence, growth, and neural infiltration of tumors connected to the intestine. However, no pertinent research examines the precise connection between the expression of NPDC1, the onset of colon cancer, and PNI. PNI is characterized as a tumor invasion of one or more of the three layers that make up the nerve structure (epineurium, perineurium, and endoneurium) if the tumors cover>33% of the nerve's perimeter.[9] PNI is a substantial predictor of poor patient outcomes and is an independent risk factor.[10,11] Earlier hypotheses proposed that tumor cells infiltrate along the "low-resistance space" around nerves, but subsequent research has revealed the complexity of this mechanism. It involves multiple interconnected steps, including neural proliferation, tumor cell invasion, and the tumor microenvironment.[9]

Three main variables that affect brain tumor invasion are neurotrophic factors, neurotransmitters, and chemokines. In addition, it has been demonstrated that aberrant gene expression is linked to the onset and progression of PNI in colon cancer.[12] Colon cancer PNI is closely correlated with upregulation of CTNNB1, high expression of ACTL6A, high expression of FOLR1, Pyruvate carboxylase, matrix metalloproteinase-11, etc.[13–17] Most contemporary clinicopathological experiments on tumor neural invasion are still in the verification stage. However, because PNI has a complicated connection involving the tumor, the nerve, and the microenvironment, the molecular mechanisms underlying its occurrence and progression remain unknown. This study explores the abovementioned difficulties by starting with the target gene *NPDC1*.

In this study, we screened for genes closely connected to colon cancer neuroinvasion using bioinformatics methods, huge databases, and clinical case information. Run gene function, survival, and pathway analyses on the NPDC1 target gene that has been found. The results of gene screening were further validated through pathological experiments and clinical data, providing valuable indicators and potential therapeutic targets for PNI in colon cancer.

Materials and Methods

This study complies with the Declaration of Helsinki. The data accessed from the various databases complied with relevant data protection and privacy regulations.

Bioinformatics Dataset

The Cancer Genome Atlas (TCGA) and Ensembl datasets were first selected as the core datasets. Then, we term so f bioinformatics. We were able to extract the gene expression information, immune infiltration information, and clinical information related to colon cancer from the TCGA database and obtain the target genes of this study by combining the nerve-related genes in the Ensembl dataset with the PNI information of colon cancer in the TCGA database as the core data.

Data Processing and Analysis

R, SPSS, and Cytoscape software were used for data processing. Fold change (FC) and p-value were the standard parameters for screening differentially expressed genes (DEG). Genes in the TCGA database were screened using the "DESeq2" R software package. We used FC values >1, log2FC >0.4, and $P < 0.05$ as cutoff values for data screening. Use terms like "neural proliferation", "chemokines", and "neurotrophic factors" to search the Ensemble database for genes associated with PNI.

Additionally, we discovered the target genes for this study by survival analysis. The "survival" package was used to perform survival analysis, and the "ggplot2" package was used for mapping.

Bioinformatics Exploration of Target Genes

After collecting the target genes, we extensively explored the existing large databases using bioinformatics tools. Mulberry plots were created based on the R package "ggalluvial" to visualize the gene expression patterns of the target genes in colon cancer samples with various stages, ages, and other clinical characteristics, as well as their association with patient survival. R software was utilized to perform the Kruskal–Wallis test, examining the

differences in target gene expression between colon cancer metastasis (M1), non-metastasis group (M0), and normal group. We examined the expression and prognosis of each CpG methylation of NPDC1 in the MethSurv website[18–20] since DNA methylation is a significant factor affecting gene expression. To further investigate this gene in several colon cancer cell lines in the CCLE database, we confirmed the levels of NPDC1 in GEPIA.[21–23] We confirmed the role of NPDC1 in the pathological staging, metastasis, and prognosis of patients with colon cancer using the Proteinatlas and cBioportal databases.[24]

We employed the "immunedeconv" R package to analyze immune infiltration, which integrates six cutting-edge algorithms, including TIMER, xCell, MCP-counter, CIBERSORT, EPIC, and quanTIseq. Immune checkpoint transcripts such as SIGLEC15, IDO1, and CD274 were examined.[25] Statistical analysis was conducted using R software. Univariate Cox regression analysis and forest plots were performed using the "forest plot" R package. Additionally, pan-cancer analysis was conducted to investigate whether these findings apply to other tumors.

Exploration of Gene Function and Related Signaling Pathways

The STRING database was utilized to investigate the relationship between the target genes and associated genes and the pathways in which they may function. The Cytoscape software was employed for this analysis.[26] The core genes were selected based on their degree value, serving as a reference.[27] To identify genes positively and negatively correlated with the target genes, the TCGA gene expression database was searched. Take Spearman's analysis to describe the correlations between quantitative variables. The multi-gene correlation plots were generated using the R package "pheatmap". As previously described, the Kaplan-Meier (KM) survival analysis was conducted to test the survival differences between two or more groups. RNAseq data and clinical information were obtained and retained from the TCGA dataset. The prognostic model was built using a multi-factor Cox regression analysis and R software's "surv" package. To assess the accuracy of the prediction model, timeROC analysis was also carried out.[28,29]

Collection and Processing of Clinicopathological Data

The research for this thesis was supported by the Ethics Committee of the Second Hospital of Jilin University (No. 2022141). The patient's case and tumor pathology samples were retrospectively collected from patients who underwent surgical resection for tumors at our hospital from 2009 to 2018. Patients who received neoadjuvant chemotherapy were excluded from the data collection. The tumor staging was performed according to the 5th edition colorectal tumor staging guidelines of the World Health Organization. Among the 384 patients, 339 had low histological grades, and 45 had high grades. The study included 150 female patients and 234 male patients. The last follow-up was conducted on December 2022, with a follow-up period of 60.9 months (range: 10.5–115.9 months). Clinical information, such as the patient's age at the time of initial diagnosis, gender, tumor grade, and lymph node metastasis, was obtained from the patient's pathology reports. Hematoxylin-eosin (H&E) staining and immunohistochemistry were performed on the collected pathological specimens. The immunohistochemical methods and scoring criteria used in this study were adopted from a previous study by Cao et al.[30] The presence of PNI was determined based on S-100 staining, which was verified in the study by Conte et al[31,32] Statistical analysis was conducted using SPSS software, with reference to the previous study by Cao et al.[30]

Results

Bioinformatics results

Acquisition of Target Genes

The crossover genes obtained from the Wayne diagram were considered candidate genes for this study. (Figure 1A). Among these genes, four target genes, including NPDC1, were identified. Survival analysis was performed on these four genes, and a forest plot was created to visualize the results (Figure 1B). We investigated the expression of CDKN2A and BRICD5 in colon cancer using the GEPIA data to validate further the crossover genes mentioned above. The results of

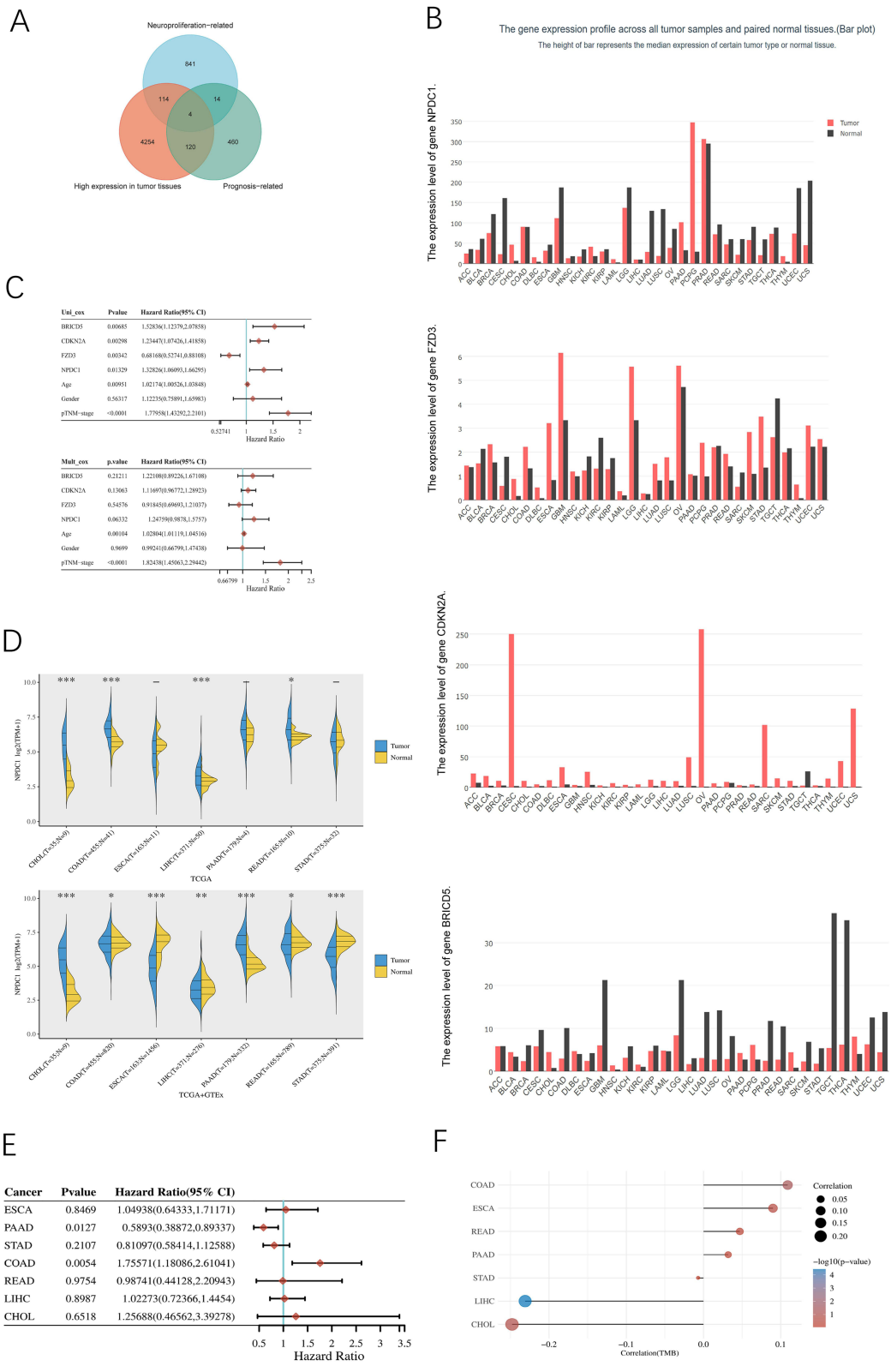

Figure 1 Exploration of neural infiltration-associated genes in colorectal cancer. (**A**) Combining the Ensembl database and The Cancer Genome Atlas database to obtain the Venn diagram of colon cancer neural invasion-related genes; (**B**) Expression validation of four crossover genes in the GEPIA database; (**C**) The survival analysis forest plot of crossover genes; (**D**) Expression level of neural proliferation differentiation and control-1 (NPDC1) in digestive system tumors database, Genotype Tissue Expression database; (**E**) Forest plot: The P value, risk coefficient and confidence interval of a gene in multiple tumors are analyzed by univariate Cox regression; (**F**) Tumor mutational burden of NPDC1 in the digestive system.

FZD in further validating the survival analysis were unsatisfactory. Therefore, we selected NPDC1 as the target gene of this study (Figure 1B and C).

Furthermore, immunoinfiltration and pan-cancer analyses were conducted to investigate the role of NPDC1. Compared to normal tissues, colon cancer, liver cancer, cholangiocarcinoma, and rectal cancer tissues exhibited higher levels of NPDC1 expression (Figure 1D). This higher expression of NPDC1 may have unfavorable effects on the prognosis of these cancers (Figure 1E). In addition, a correlation was discovered between the level of NPDC1 and tumor mutation burden (TMB) in colon cancer (Figure 1F, $p < 0.05$). In the CCLE database, we looked at the expression levels of NPDC1 in various colorectal cancer cell lines and the digestive system and head tumors (Figure 2A and B). The findings demonstrated that all of the cancers mentioned above had high levels of NPDC1 expression. We discovered that patients with colon cancer with significant NPDC1 methylation had a poor prognosis. With the expression levels of NPDC1 clustered together, we obtained a heatmap and predictive value for DNA methylation in colon cancer (Figure 2C and D).

The analysis of immune infiltration revealed that the expression level of NPDC1 was associated with various immune infiltrating cells in digestive system tumors (Figure 2E). Additionally, we found genes whose expression was associated positively and negatively with NPDC1. LAMP2 and ATP6AP2 showed a significant negative correlation, while HMG20B and GRIN1 showed a significant positive correlation (Figure 2F).

3.1.2 Relationship between the expression level of NPDC1 and the stage and prognosis of patients with colon cancer

We obtained clinical and survival data associated with NPDC1 expression levels from the TCGA database in patients with colon cancer. It was found that NPDC1 was significantly overexpressed in colon cancer tumor tissues (Figure 3A). We observed that as patients' ages and TNM stages advanced, they were more likely to exhibit high levels of NPDC1 expression. Furthermore, the Mulberry plot (Figure 3B) demonstrated no significance in genders. However, high NPDC1 expression exhibited significantly shorter overall survival times than those in the low expression group (Figure 3C). Moreover, the high expression level of NPDC1 showed some predictive value for colon cancer (Figure 3D). The prognostic differences between patients with different pathological stages were also shown (Figure 3E). The KM curves for NPDC1 with different expression levels displayed statistical differences (Figure 3F).

Gene Function and Pathway Exploration of NPDC1 and Its Associated Genes

In the STRING database, we explored the interaction network centered around NPDC1 and visualized the relationships using Cytoscape (Figure 4A). The associations between NPDC1 and its interacting genes, such as *JUN, E2F1*, and *CCDN1*, were mapped in Cytoscape. Furthermore, using ClueGO, we performed an enrichment analysis of these genes and identified 33 associated pathways (Figure 4B, C, D). These pathways include cell cycle protein-dependent protein serine/threonine kinase activity, cell cycle checkpoint signaling, DNA replication, fibroblast proliferation, positive transcriptional regulation of the RNA polymerase II promoter, and involvement in the cellular response to chemical stimuli. These pathways play significant roles in colon cancer. Based on the computed risk scores of the patients, we classified them into high- and low-risk groups (Figure 5A). Figure 5B shows the overall survival. Figure 5C shows a heat map showing the expression of the three prognostic genes. Using Cox regression analysis, we constructed risk models on genes with a degree value greater than 10. The optimal model has a Ri Riskscore = (0.3629) × NPDC1 + (−0.1046) × JUN + (−0.5438) × CCNB1 + (−0.0734) × CCND1 + (0.0078) × CDC6 + (0.0259) × CCND2 + (0.1744) × E2F1 + (0.3999) × CDK2 + (0.6057) × CCNA1. This model demonstrated that the mentioned gene set is a poor prognostic predictor in patients with colon cancer (Figure 5D). Additionally, KM survival indicates that the prognosis for the high-risk group is worse (Figure 5E). Through multi-way Cox regression analysis, we assessed the significance of NPDC1 as an independent prognostic factor (Figure 6A and B). Additionally, one-way Cox regression analysis revealed a significant association between NPDC1 expression and patient prognosis. The column line graphs in Figure 6C estimated the patient's survival rates for the next 1, 3, and 5 years.

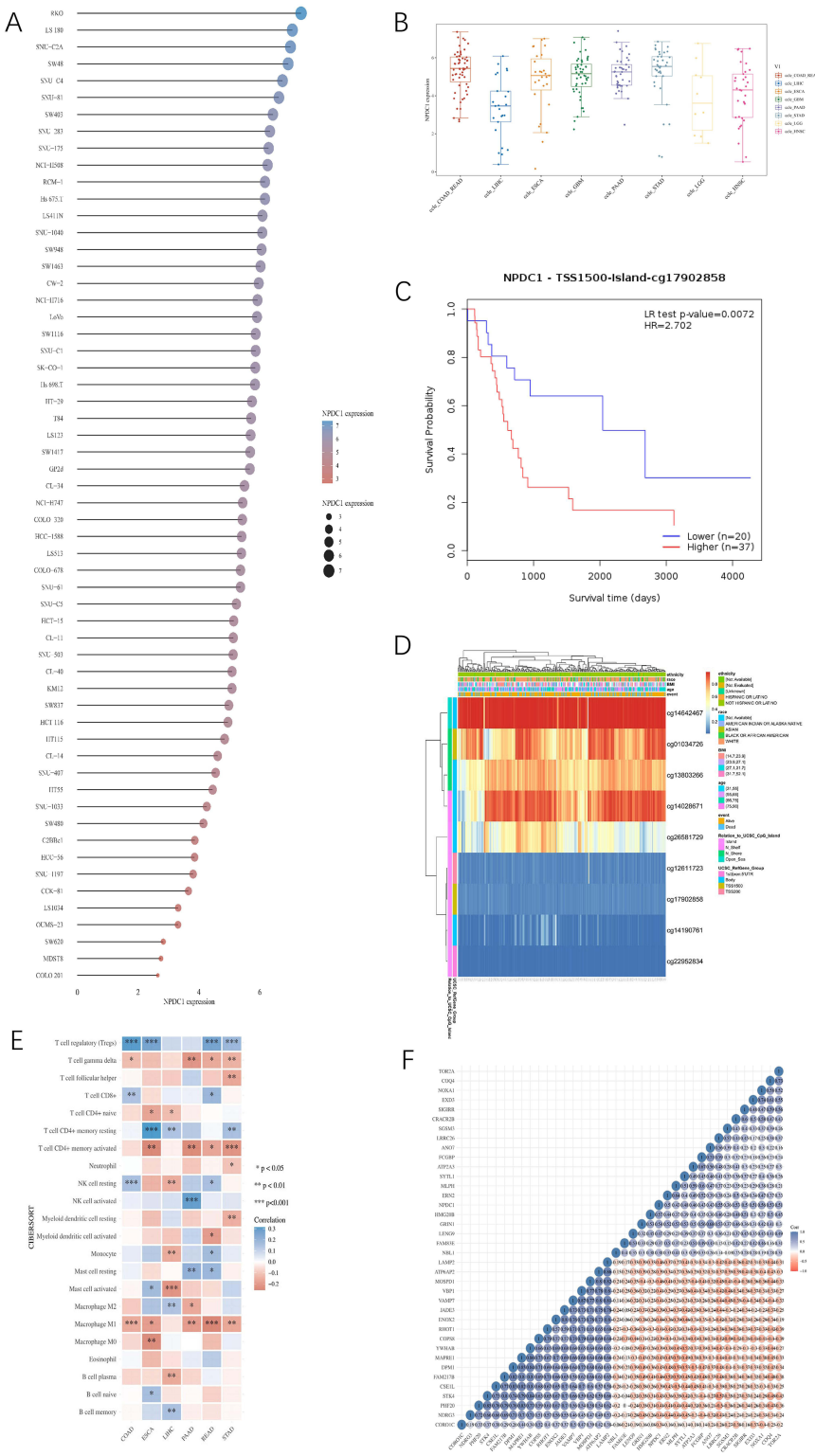

Figure 2 Pan-cancer analysis, DNA methylation, and immune infiltration assay of neural proliferation differentiation and control-1 (NPDC1). (**A**) The CCLE database shows the expression of NPDC1 in various colorectal cell lines. The horizontal coordinates in the graph represent the gene expression, its vertical coordinates represent various cell lines, the size of the dots in the graph represents the high or low expression, and its various colors represent the high or low expression; (**B**) Expression of NPDC1 in the digestive system tumors and some head tumors from the CCLE database; (**C**) Kaplan-Meier plot showing survival in the high and low methylation groups dichotomized by the MethSurv platform; (**D**) Heatmap of DNA methylation expression levels of the NPDC1 in colon cancer by the MethSurv platform; (**E**) Analysis of immune infiltration of NPDC1 in the digestive system tumors; Significance of the two sample groups was tested by Wilcoxon test; (**F**) Heat map of the relationship between genes positively and negatively correlated with NPDC1. *P < 0.05, **P < 0.01, ***P < 0.001.

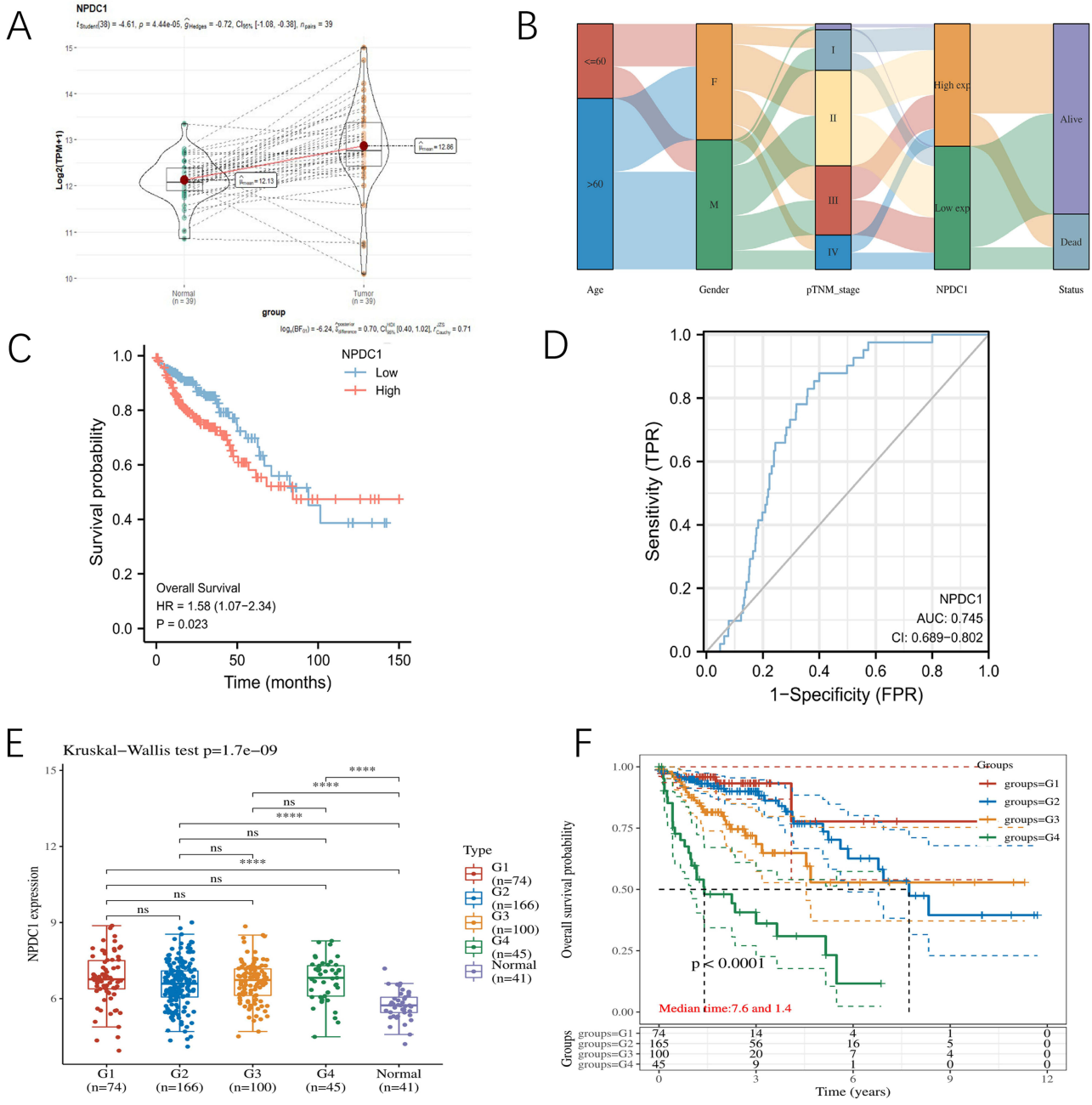

Figure 3 Significance of neural proliferation differentiation and control-1 (NPDC1) expression in colon cancer. (**A**) The levels of NPDC1 expression in different tissues; (**B**) Mulberry plots showing the correlation between NPDC1 expression levels and clinical information; (**C**) NPDC1 survival curves. (**D**) Receiver operating characteristic curve of the predictive power of the variable NPDC1 in predicting tumor and normal outcomes; (**E**) Expression of NPDC1 in various pathologically staged tissues; (**F**) Prognostic differences between patients with different pathological stages. ****P < 0.0001, ns indicates no significance.

Results of Clinicopathological Data Analysis

Characteristics of the Collected Patients

The median age of the 384 cases of colon cancer was 65.9 years, ranging from 24 to 93 years. We compared the characteristics of patients with positive and negative PNI to determine the association between PNI occurrence and relevant clinicopathological features. Among the patients, 57.9% had PNI positivity, with 150 (32.9%) among female patients and 234 (67.1%) among male patients. However, the percentage of positive patients was slightly higher in female patients, although this difference was not statistically significant (P = 0.449). We observed a significant correlation between PNI occurrence and tumor markers (CEA,

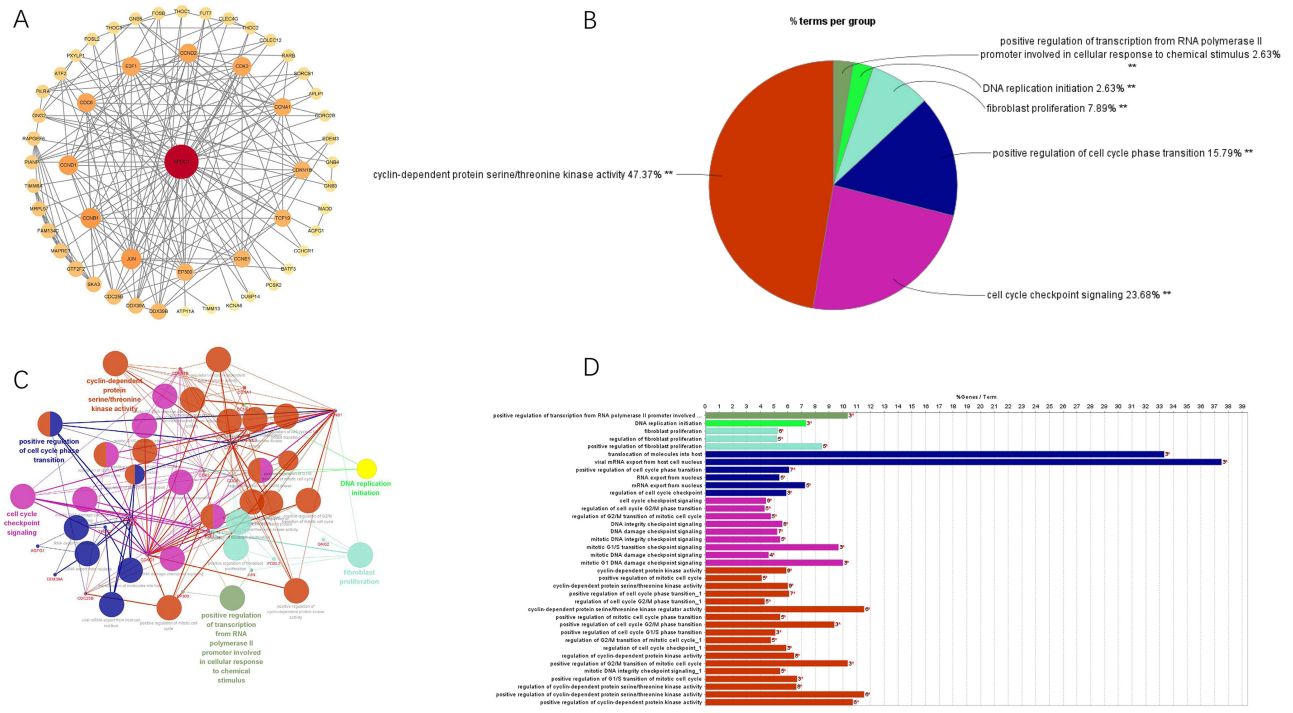

Figure 4 Exploration of neural proliferation differentiation and control-1 (NPDC1)-associated genes and analysis of related pathways. (**A**) Inter-gene relationship network map with NPDC1 as the core; (**B**) (**C**) and (**D**) Bar chart, pie chart and functional relationship of related pathways involved in the gene set with NPDC1 as the core. *P < 0.05, **P < 0.01.

CA125, and CA199). Furthermore, the likelihood of PNI occurrence was found to increase with the age of the patients (P = 0.013) (Table 1).

Association of the Occurrence of PNI with Clinicopathological Parameters in Patients with Colon Cancer

Regarding the association between PNI occurrence and clinicopathological parameters in patients with colon cancer, we found that patients with PNI had different TNM stages, lymph node metastasis status, and histological grading. The percentage of PNI-positive patients was 53.33% among highly differentiated tumors and 46.75% among those with low differentiation, showing a statistically significant difference (P = 0.004). The likelihood of PNI increased with advanced tumor infiltration depth and lymph node infiltration status (P < 0.001). Patients with stage III TNM staging had a significantly higher chance of developing PNI (P < 0.001). Additionally, we examined the relationship between PNI occurrence and microsatellite instability (MSI), oncogene P53, and Ki-67 status. The results showed no significant correlation between PNI and MSI, although the probability of PNI occurrence increased with higher Ki-67 values (P = 0.003) (Table 1).

Relationship Between NPDC1 Expression and PNI Status and Prognostic Survival of Patients

In terms of NPDC1 expression, we confirmed its level using immunohistochemical experiments. Compared to patients with low NPDC1 expression, patients with high NPDC1 expression had a significantly higher incidence of PNI (P < 0.001) (Table 1). Figure 7A and B illustrates low NPDC1 expression in patients without PNI immunohistochemical sections, while Figure 7C–E demonstrates high NPDC1 expression in nerve infiltrate-positive sections. These findings align with the results of the previous bioinformatics investigation, supporting the relationship between NPDC1 expression and PNI occurrence. Moreover, the data suggest that NPDC1 expression is detrimental to patient survival. Using KM curves, we analyzed the association between NPDC1 expression and disease-free survival (DFS) and overall survival (OS) in patients with colon cancer. The results indicated that NPDC1 expression was a prognostic risk factor for DFS (Figure 8A) and OS (Figure 8B). We verified the results of the clinicopathologic experiments using the Proteinatlas and cBioportal databases to validate the results. The results showed that the survival analysis was consistent with our findings (Figure 9A). However, the cBioportal

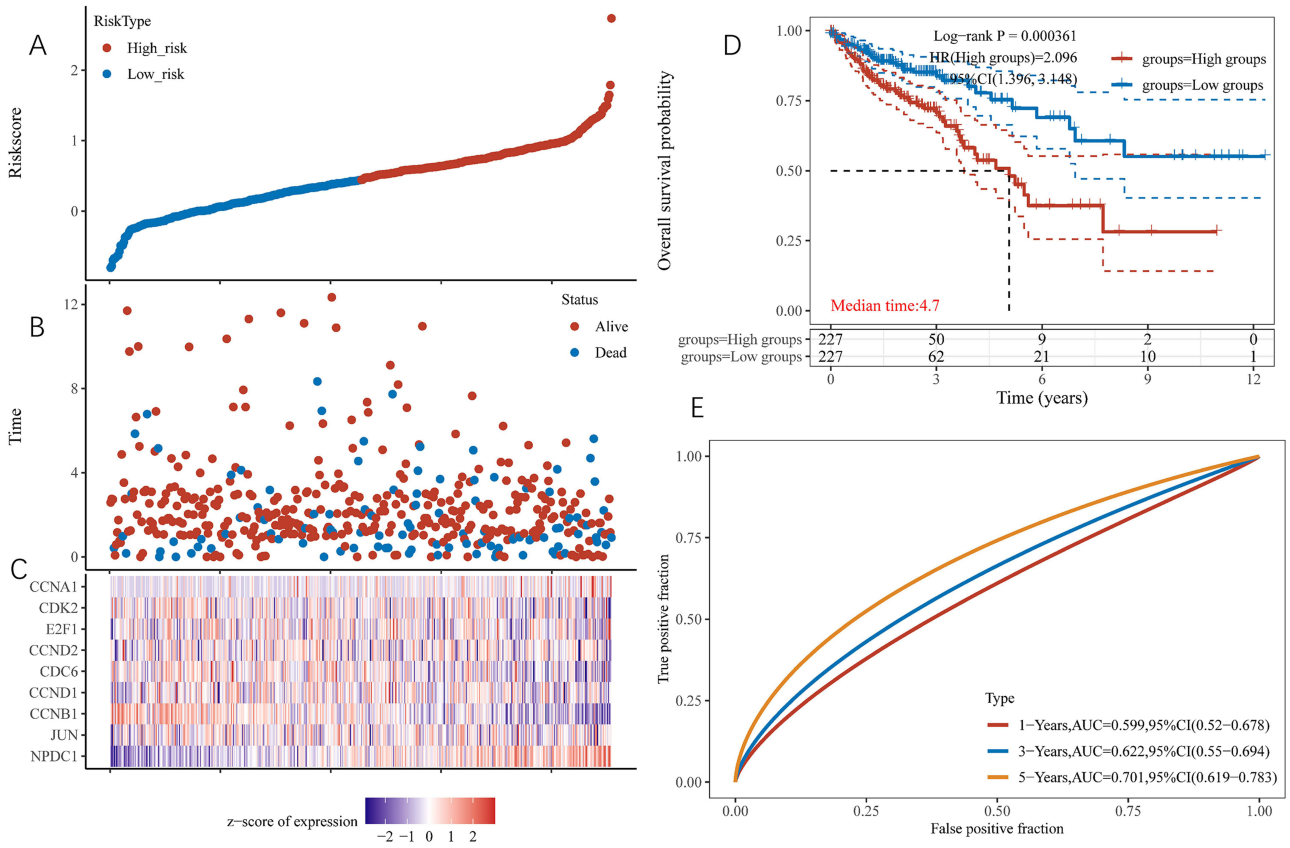

Figure 5 Prognostic value of neural proliferation differentiation and control-1 and its associated genes in patients with colon cancer. (**A**) Risk score curves; (**B**) Scatter plot distribution of survival time and survival status corresponding to different sample Riskscore; (**C**) Heat map of prognostic gene expression in high and low risk groups; (**D**) Kaplan-Meier survival curves of target genes; (**E**) Receiver operating characteristic curves of target gene models. The higher area values under the receiver operating characteristic curve correspond to higher predictive power.

database revealed no significant difference in the expression of NPDC1 between pathological stages, metastasis, and lymph node infiltration in patients with colon cancer (Figure 9B–D).

Discussion

PNI can occur in various malignancies, including colorectal, pancreatic, and head and neck cancers. The high expression of NPDC1 in tumor tissues has been associated with the clinical stage of tumors, tumor recurrence, distant metastasis, and survival prognosis. This study represents the first genetic correlation investigation focusing on PNI in colon cancer. To validate the findings obtained from bioinformatics analysis, we conducted additional validation using clinical data and pathological immunohistochemical analysis to demonstrate the association between NPDC1 and PNI in colon cancer.

Initially, we screened the Ensembl database to identify gene sets closely associated with nerve growth and proliferation. Through differential analysis of these gene sets in the TCGA colon cancer database, we identified gene sequences represented by NPDC1 that are potentially crucial in PNI development in colon cancer. Survival, immune infiltration, and gene expression analyses were performed to refine the selection and confirm NPDC1 as the optimal gene for this study.

Bioinformatics exploration also revealed that NPDC1 was highly expressed in tumor metastasis tissues, negatively impacting patient survival. Furthermore, we observed high NPDC1 expression in the liver, bile duct, and rectal cancer tissues. The analysis of immune infiltration indicated significant associations between NPDC1 expression levels and NK, T, and B cells, suggesting a potential relationship between NPDC1 expression and immune escape mechanisms of tumor cells. Given the significance of NPDC1, we focused on a gene set strongly associated

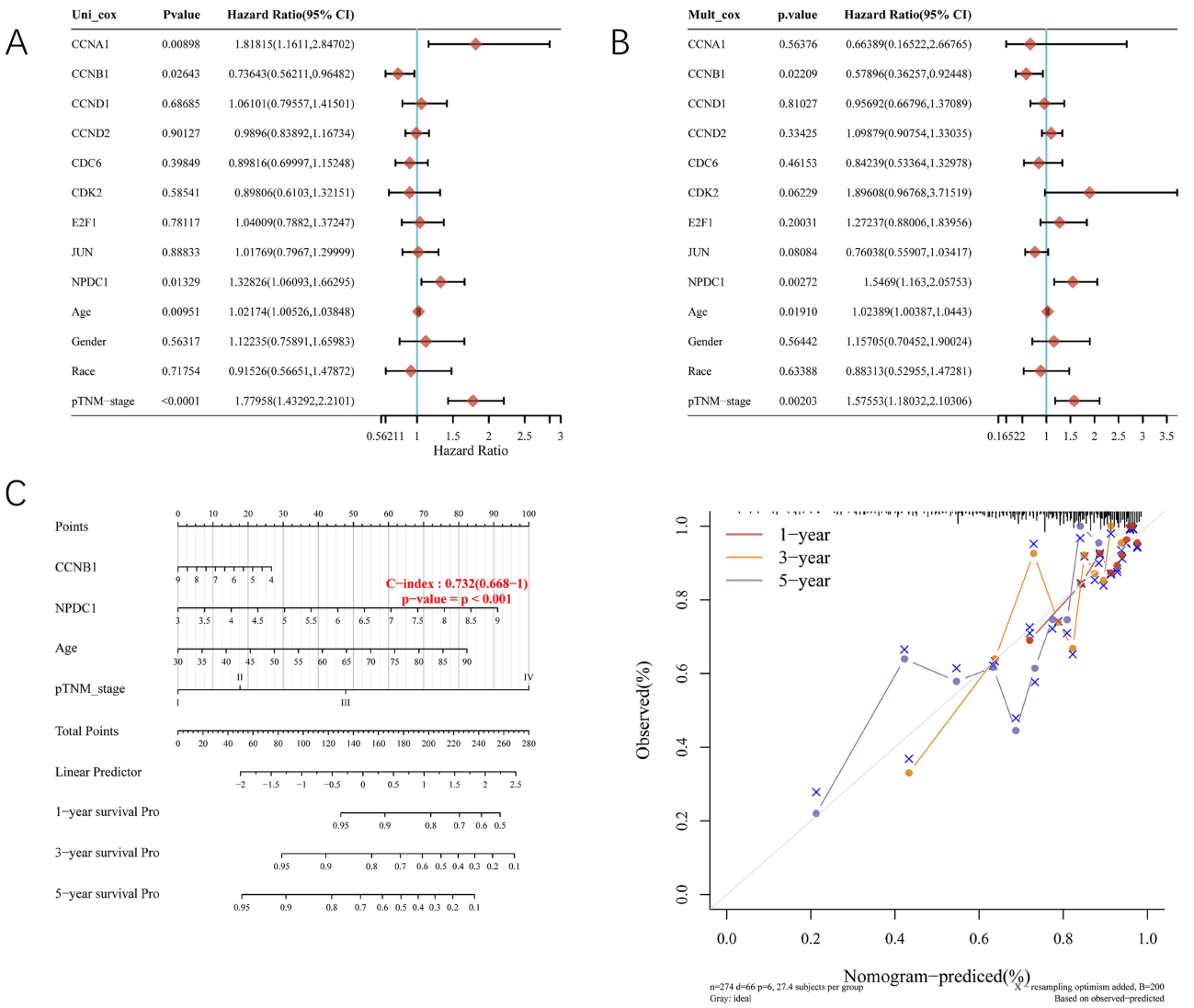

Figure 6 Univariate and multivariate survival analysis of neural proliferation differentiation and control-1-associated genes. (**A**) Univariate Cox regression analysis; (**B**) Multivariate regression analysis; (**C**) Calibration curves of 1-, 3-, and 5-year survival and the calibration curves for the survival nomogram model.

with its expression and identified potential interactions among these genes. We discovered a significant correlation between NPDC1 and genes involved in tumor cell proliferation, migration, immune infiltration, tumor stroma formation, and angiogenesis by protein-protein interaction and gene function enrichment analysis (Figure 4B–D). Functional enrichment analysis revealed their involvement in various activities, including cell cycle operation, cell migration, and kinase signaling pathways.[33,34] The epigenetic mechanism of DNA methylation significantly impacts the expression of the *NPDC1* gene.[35] The prognostic value of NPDC1 in a single CpG was significant in the development of colon cancer, which was revealed in the DNA methylation analysis of this study. Due to its level of expression, DNA methylation, immune infiltration, and potential gene-related pathways, NPDC1 is strongly shown to play a driving role in the development of colon cancer.

We selected a gene set with NPDC1 at its core to create a prognosis model. This model has some accuracy in predicting 5-year survival but is a poor prognostic factor for patients with colon cancer. However, the prediction of 1- and 3-year survival is poor. The ability to predict 1- and 3-year patient survival is poor. However, NPDC1 was still statistically significant in subsequent Cox single-factor and multi-factor regression. Additionally, we investigated the

Table 1 Correlation of PNI Status with Clinicopathological Features and NPDC1 Expression in Patients with Colon Cancer

Characteristics		n	PNI Status		*P*
			Negative n	Positive n	
Gender	Female	234	155	77	0.449
	Male	150	95	55	
NPDC1	Low expression	216	170	46	<0.001
	High expression	168	82	86	
Histology grade	Low-grade	339	231	108	0.004
	High-grade	45	21	24	
T stage	T1	8	8	0	<0.001
	T2	41	41	0	
	T3	261	162	99	
	T4	74	41	33	
N stage	N0	138	135	3	<0.001
	N1	137	64	73	
	N2	109	53	56	
TNM stage	I	43	43	0	<0.001
	II	95	92	3	
	III	246	117	129	
LVI	Absent	183	156	27	<0.001
	Present	201	96	105	
Necrosis Stages	Absent	215	173	42	<0.001
	Present	169	79	90	
MSI	Stable	353	232	121	0.892
	Unstable	31	20	11	
P53	Wild-type	129	82	47	0.546
	Mutant	255	170	85	
CEA	≤5 ng/mL	39	25	14	0.833
	>5 ng/mL	345	227	118	
CA125	≤35 U/mL	49	31	18	0.710
	>35 U/mL	335	221	114	
CA199	≤37 U/mL	25	16	9	0.860
	>37 U/mL	359	236	123	
Age		65.9 ± 11.7 [24–93]	67.1 ± 11.3 [24–93]	63.9 ± 12.1 [26–89]	0.013
TB		9.4 ± 6.4 [0–29]	8.4 ± 5.8 [0–21]	11.2 ± 6.9 [0–29]	<0.001
Ki-67 status (%)		84.5 ± 13.8 [37.5–99.5]	83.0 ± 14.2 [37.5–99.5]	87.4 ± 12.4 [52.5–99.5]	0.003

Note: Data are expressed as mean ± standard deviation.
Abbreviations: MSI, microsatellite instability; TB, tumor budding.

genes positively and negatively correlated with NPDC1 expression levels, potentially upstream and downstream regulatory genes for NPDC1. However, further research is needed to understand their relationship fully.

In addition to bioinformatics mining, we collected clinical information from relevant medical records to investigate the causes and development of PNI in colon cancer. We found a significant correlation between PNI and tumor TNM stage, histological grade, and lymph node metastasis status. Furthermore, we confirmed that PNI increased the expression of NPDC1 in tumor tissues (Table 1), thus validating the findings obtained from bioinformatic analysis. Our study demonstrated that higher TNM-stage tumors were more likely to exhibit PNI. We also observed a correlation between PNI and markers such as Ki-67, necrosis stages, and tumor budding (TB). Ki-67, an indicator of tumor cell proliferation,[36] was found to be associated with PNI. A high percentage of necrosis stages indicates a higher probability of aggressive tumor growth.[37] Moreover, TB, representing aggressive tumor growth, may be an independent predictor of

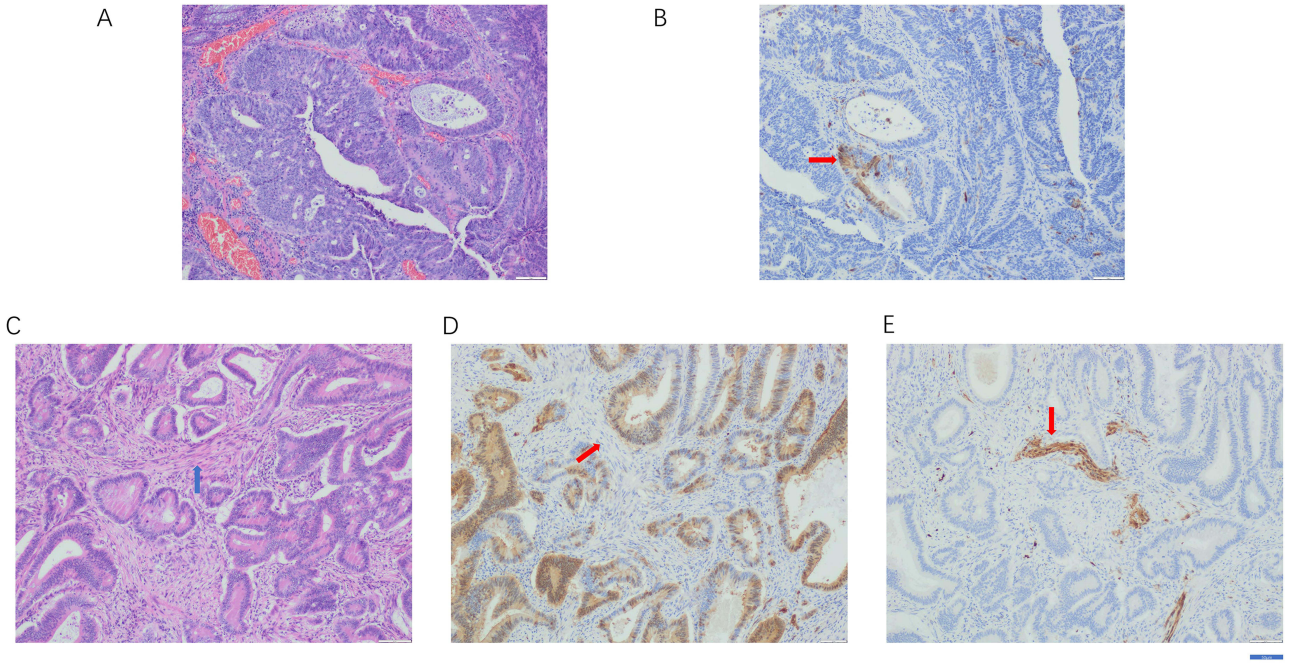

Figure 7 Immunohistochemical detection of neural proliferation differentiation and control-1 (NPDC1) in colon cancer tissues. (**A**) Representative example of Hematoxylin and eosin (H&E) staining of perineural invasion (PNI)-negative cancer tissue; (**B**) Representative examples of NPDC1 immunohistochemistry in PNI-negative cancer tissues, with red arrows pointing to NPDC1 expression; (**C**) Representative examples of H&E staining in PNI-positive cancer tissues, with blue arrows pointing to tumor cells encircling the nerves; (**D**) Representative examples of NPDC1 immunohistochemistry in PNI-positive cancer tissues, with red arrows pointing to nerve tissue; (**E**) S-100 staining of nerve tissue infiltrating into the interior of the tumor, with red arrows pointing to nerve tissue. Scale bars = 50 μm.

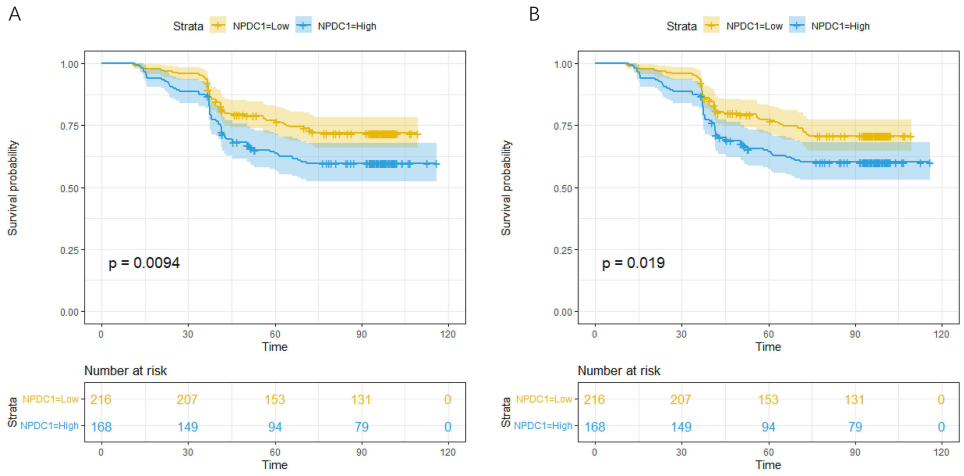

Figure 8 Kaplan-Meier curves of disease-free survival (**A**), overall survival (**B**), and neural proliferation differentiation and control-1 expression levels in patients with colon cancer.

lymph node metastasis in colon cancer.[38] The development of PNI in tumors coincided with tumor progression, and high NPDC1 expression was associated with advanced tumor stages and elevated markers. The results from the cBioportal database showed that the expression levels of NPDC1 did not significantly differ in lymph node metastasis or tumor stage in contrast to the findings from our clinicopathological experiments. We hypothesized that this might be due to the few samples we selected or geographical differences. However, since the expression of NPDC1 is associated with a decreased chance of survival for patients with colon cancer, we still believe it is crucial to continue researching this gene.

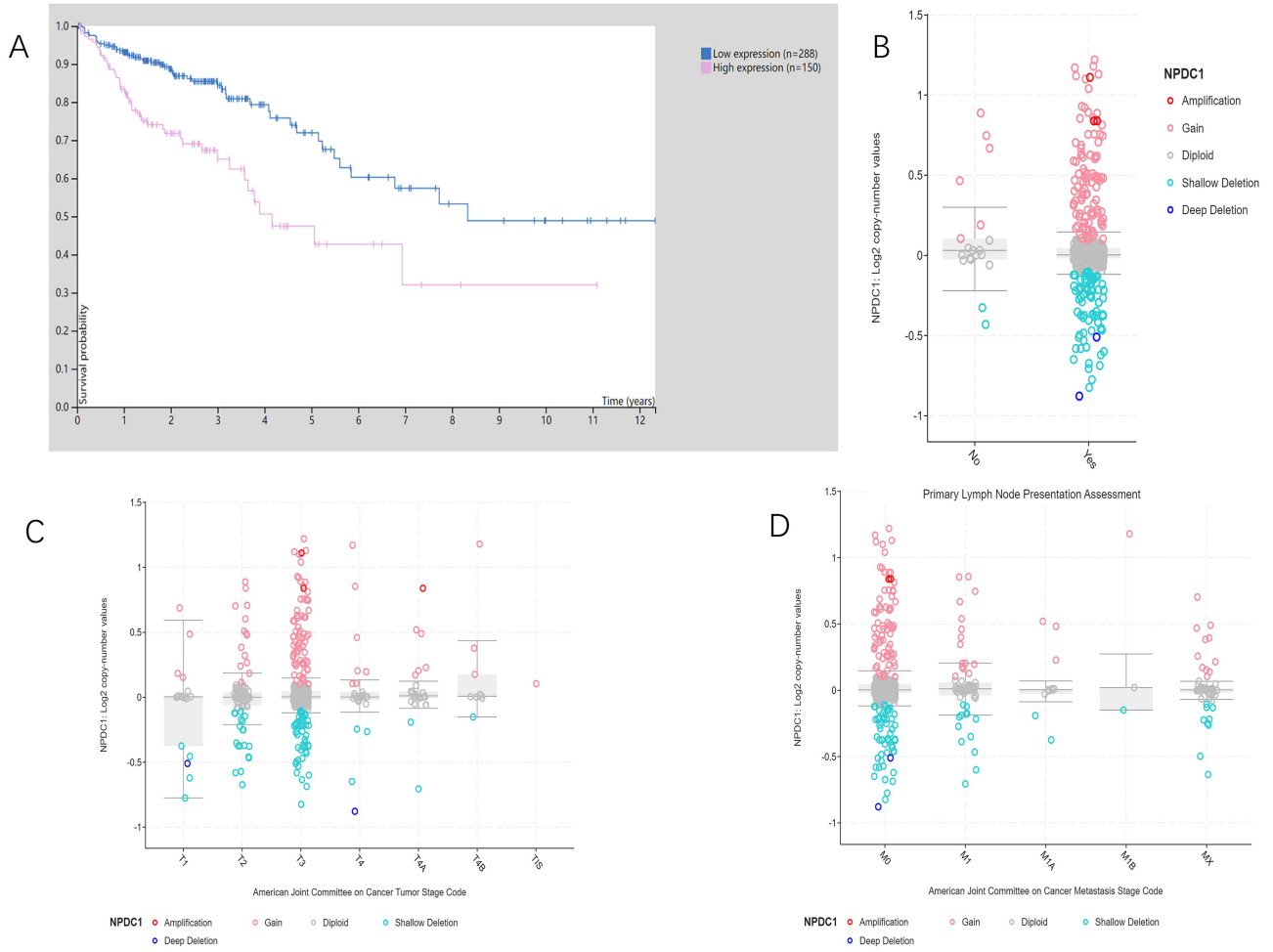

Figure 9 Validation of the significance of neural proliferation differentiation and control-1 (NPDC1) expression in other databases. (**A**) Proteinatlas database correlation between NPDC1 expression and patient survival. (**B**–**D**) cBioportal database clinical significance of NPDC1 expression, lymph node metastasis (**B**), tumor stage (**C**), and distant metastasis (**D**).

Conclusion

In conclusion, our study provides compelling evidence that the expression level of NPDC1 is directly associated with the development of PNI in colon cancer and serves as a poor prognostic indicator for patient survival. Furthermore, NPDC1 expression may offer insights into the risk of distant tumor metastasis, enabling risk stratification and informing decisions regarding adjuvant chemotherapy. This suggests that NPDC1 may facilitate tumor PNI and progression through these pathways. Our findings were corroborated by immunohistochemical experiments and data correlation analysis using samples from our institution's clinicopathological data. Our study provides valuable insights into the mechanisms underlying tumor PNI and chemoresistance, offering potential markers for PNI in colon cancer.

Abbreviations

PNI, Perineural invasion; NPDC1, Differentiation and control-1; LASSO, Least absolute shrinkage and selection operator; KM, Kaplan-Meier; TMB, Tumor mutation; MSI, Microsatellite instability; DFS, Disease-free survival; OS, Overall survival; FC, Fold change; DEG, Differentially expressed genes; H&E, Hematoxylin-eosin.

Data Sharing Statement

The datasets used and analyzed during the current study are available from the corresponding author upon reasonable request.

Ethics Approval and Consent to Participate

This study was approved by the Ethics Committee of Jilin University Second Hospital (No. 2022141). Written informed consent was obtained from all participants. All methods in the study followed relevant guidelines and regulations.

Consent for Publication

Written consent was obtained from patients for the publication of this study and accompanying images.

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Disclosure

The authors declare that they have no competing interests in this work.

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