Original Article

High expression of NXPH4 correlates with poor prognosis, metabolic reprogramming, and immune infiltration in colon adenocarcinoma

Zhe Sun[1#], Haodi Wang[1#], Yao Xu[2#], Yichi Liu[1], Lu Wang[1], Ruijie Zhou[2], Runlong Zhou[2], Wenjian Ma[1,3], Tongcun Zhang[1,2]

[1]College of Biotechnology, Tianjin University of Science and Technology, Tianjin, China; [2]Institute of Biology and Medicine, College of Life Sciences and Health, Wuhan University of Science and Technology, Wuhan, China; [3]Qilu Institute of Technology, Jinan, China

Contributions: (I) Conception and design: Z Sun, Y Xu; (II) Administrative support: T Zhang; (III) Provision of study materials or patients: Y Xu; (IV) Collection and assembly of data: H Wang, Y Liu, L Wang; (V) Data analysis and interpretation: Z Sun, Ruijie Zhou, Runlong Zhou; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

[#]These authors contributed equally to this work.

Correspondence to: Wenjian Ma, PhD. College of Biotechnology, Tianjin University of Science and Technology, #29 the 13th Avenue, TEDA, Tianjin 300457, China; Qilu Institute of Technology, Jinan, China. Email: ma_wj@tust.edu.cn; Tongcun Zhang, PhD. College of Biotechnology, Tianjin University of Science and Technology, #29 the 13th Avenue, TEDA, Tianjin 300457, China; Institute of Biology and Medicine, College of Life Sciences and Health, Wuhan University of Science and Technology, Wuhan, China. Email: zhangtongcun@wust.edu.cn.

Background: Colon adenocarcinoma (COAD) is a prevalent gastrointestinal malignant disease with high mortality rate, and identification of novel prognostic biomarkers and therapeutic targets is urgently needed. Although neurexophilin 4 (NXPH4) has been investigated in several tumors, its role in COAD remains unclear. The aim of this study was to explore the prognostic value and potential functions of NXPH4 in COAD.

Methods: The expression of NXPH4 in COAD were analyzed using The Cancer Genome Atlas (TCGA) and datasets from the Gene Expression Omnibus (GEO) database. The prognostic value of NXPH4 was determined using Kaplan-Meier analysis and Cox regression analysis. To investigate the possible mechanism underlying the role of NXPH4 in COAD, Gene Ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG), and gene set enrichment analysis (GSEA) were employed. The correlation between NXPH4 expression and immune cell infiltration levels was examined thorough single-sample gene set enrichment analysis (ssGSEA). Furthermore, the competing endogenous RNA (ceRNA) regulatory network that may be involved in NXPH4 in COAD was predicted and constructed through a variety of databases.

Results: NXPH4 expression was significantly higher in COAD tissue compared with normal colon tissues. Meanwhile, high expression of NXPH4 was associated with poor prognosis in COAD patients. GO-KEGG and GSEA analyses indicated that NXPH4 was associated with glycolysis and hypoxia pathway, and may promote COAD progression and metastasis by modulating metabolic reprogramming. ssGSEA analysis demonstrated that NXPH4 expression also associated with immune infiltration. Furthermore, we identified various microRNAs (miRNAs) and long noncoding RNAs (lncRNAs) as upstream regulators of NXPH4 in COAD.

Conclusions: The present study revealed that high expression of NXPH4 is associated with tumor progression, metabolic reprogramming, and immune infiltration. These findings suggest that NXPH4 could serve as a reliable prognostic biomarker and a promising therapeutic target in COAD.

Keywords: Neurexophilin 4 (NXPH4); colon adenocarcinoma (COAD); prognosis; metabolic reprogramming; immune infiltration

Submitted Dec 06, 2023. Accepted for publication Mar 15, 2024. Published online Apr 29, 2024.
doi: 10.21037/jgo-23-956
View this article at: https://dx.doi.org/10.21037/jgo-23-956

Introduction

Colon adenocarcinoma (COAD), an aggressive primary intestinal malignancy, is the third most prevalent malignancy and the second main cause of cancer-related mortality worldwide (1,2). Despite advances in screening and treatment, 5-year survival rate remains low, mainly due to late-stage diagnosis and high frequency of metastasis and recurrence (3-5). The molecular mechanisms driving COAD progression are still poorly understood and there is a lack of robust diagnostic and prognostic biomarkers, significantly hindering clinical management of the disease (6). Elucidating the molecular alterations driving COAD and identifying novel biomarkers could lead to improved diagnosis, prognosis prediction, and development of targeted therapies, which are urgently needed to improve outcomes for COAD patients.

Neurexophilin 4 (NXPH4) is a secretory protein that belongs to the Neurexophilin family, which also includes NXPH1, NXPH2, and NXPH3. The protein family numbers share a common structure consisting of five domains and the mature peptide molecular weight is 29 KD (7,8). NXPH can bind to neuronal membrane proteins (NMPs) Neurexin Iα and is highly expressed in the nervous system, which is responsible for feeding, emotion, balance, and movement (9). Loss of NXPH4 has been shown to impair cerebellar Golgi-granule inhibitory neurotransmission and synapse number (10). These findings suggest that NXPH4 plays a primary role in modulating specific synapse function and cerebellar motor control.

Interestingly, recent studies have also implicated NXPH4 in the development and progression of several malignant tumors including bladder cancer (11-13), hepatocellular carcinoma (14,15), and non-small cell lung cancer (16). Additionally, it was reported that NXPH4 promotes gemcitabine resistance in bladder cancer (13). However, the biological functions, clinical significance, and underlying molecular mechanisms of NXPH4 in COAD remain largely unknown and unexplored.

In the present study, we comprehensively analyzed NXPH4 expression and its potential clinical relevance in COAD using data from The Cancer Genome Atlas (TCGA) database. We investigated genes co-expressed with NXPH4 in COAD and explored their potential biological functions and associated signaling pathways. Furthermore, we analyzed correlation between NXPH4 and genes related to glycolysis, hypoxia, and immune infiltration to uncover mechanisms involved in COAD progression. Through a series of correlation and expression analyses, we screened microRNAs (miRNAs) and long noncoding RNAs (lncRNAs) that are correlated with NXPH4 expression in COAD, and eventually constructed a competing endogenous RNA (ceRNA) network. Our multipronged *in silico* analysis establishes NXPH4 as a candidate biomarker for COAD diagnosis and prognosis, and provides a foundation for future exploration of novel therapeutic strategies. We present this article in accordance with the TRIPOD reporting checklist (available at https://jgo.amegroups.com/article/view/10.21037/jgo-23-956/rc).

Highlight box

Key findings

- High expression of neurexophilin 4 (NXPH4) resulted in the poor prognosis in colon adenocarcinoma (COAD) patients.
- High expression of NXPH4 correlated with metabolic reprogramming, and immune infiltration in COAD.

What is known and what is new?

- NXPH4 played important roles in tumor progression of several malignant tumors, including bladder cancer, hepatocellular carcinoma, and non-small cell lung cancer.
- We established NXPH4 as a candidate biomarker for COAD diagnosis and prognosis. Additionally, we explored the role of NXPH4 in metabolic reprogramming and immune infiltration in COAD.

What is the implication, and what should change now?

- The potential molecular mechanisms and upstream noncoding RNA network of NXPH4 in COAD should be further studied by *in vitro* and *in vivo* experiments.

Methods

Data acquisition

Expression data of NXPH4 gene and clinical information of COAD patients were collected from TCGA through the Genomic Data Commons (GDC) data portal (17). We acquired both raw RNA-sequencing data and corresponding clinical information of tumor tissues and adjacent tissues for 33 different types of cancer. From TCGA COAD dataset, we extracted data from 521 samples, which included 41 para-cancerous tissues and 480 tumor tissues. Additionally, the NXPH4 expression data were obtained from GSE71187 (18) and GSE44076 (19) datasets in the Gene Expression Omnibus (GEO) database (20) to verify the NXPH4 expression level in tumor and normal tissues. The study was

conducted in accordance with the Declaration of Helsinki (as revised in 2013).

Survival analysis

The COAD patients were classified into high-expression and low-expression groups according to the median expression level of NXPH4. Kaplan-Meier survival analysis was performed to investigate the differential survival outcomes including overall survival (OS), disease-specific survival (DSS), and progression-free interval (PFI) of TCGA COAD cohort. The survival package was applied for statistical analysis of survival data, and the survminer package was employed for mapping survival curves. The Kaplan-Meier plotter database (21) was also utilized to evaluate the correlation between NXPH4 expression and survival outcomes in patients with colon cancer. In addition, independent prognostic factors were identified by univariate and multivariate Cox regression analysis through survival package.

Diagnostic value analysis

The receiver operating characteristic (ROC) curve was utilized to evaluate the diagnostic value of NXPH4 in COAD using pROC package. The area under the curve (AUC) of 0.5–0.7 denoted low accuracy, AUC of 0.7–0.9 denoted certain accuracy, and AUC above 0.9 denoted high accuracy.

Construction and evaluation of clinical nomogram

We selected independent prognostic factors based on the multivariate Cox analysis to construct nomogram to evaluate the 1-, 3-, and 5-year OS probability of COAD patients. We also generated calibration plot to validate the efficiency of the nomogram. Nomogram and calibration plot were generated by the survival package and rms package (22). Furthermore, we utilized the timeROC package (23) to perform time-dependent survival ROC curve analysis to evaluate the prognostic value of NXPH4 expression in predicting 1-, 3-, and 5-year OS in COAD patients.

Functional enrichment analysis

We explored the differentially expressed genes (DEGs) between low- and high-NXPH4 expression groups in COAD using DESeq2 package (24). The volcano plot was drawn by the ggplot2 package with the threshold of $|\log 2 \text{ (fold change)}| > 1.0$ and $P<0.05$. The co-expression genes of NXPH4 were screened using limma package. The correlation between the NXPH4 expression level and co-expression genes were examined by Spearman's correlation coefficients and the Z-test ($|\text{Spearman's correlation coefficient}| > 0.3$, and $P<0.001$). Additionally, we employed the ClusterProfiler package for Gene Ontology (GO) analysis, which included the study of biological processes (BP), cellular components (CCs), and molecular functions (MFs), as well as the Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis (25). The ggplot2 package was employed for visualization. Adjusted $P<0.05$ was considered to be statistically significant in the enrichment results.

Protein-protein interaction (PPI) network

Gene Multiple Association Network Integration Algorithm (GeneMANIA) (26), and Search Tool for the Retrieval of Interacting Genes (STRING) (27) were used to explore proteins that interact with NXPH4 and to construct PPI networks.

Gene set enrichment analysis (GSEA)

The ClusterProfiler package was applied to conduct GSEA between low- and high-NXPH4 expression groups (25). A reference gene set, h.all.v7.5.1.symbols.gmt, was downloaded from the Molecular Signatures Database (MSigDB). Adjusted $P<0.05$ and false discovery rate (FDR) <0.25 were defined to be statistically significant.

Immune infiltration analysis

To evaluate correlations between NXPH4 expression and immune cell infiltration, the single-sample gene set enrichment analysis (ssGSEA) was applied using the GSVA package (28). Tumor infiltration status of 24 immune cell types was included for analysis (29). In addition, infiltrating immune cells with the different expression groups of NXPH4 were analyzed by the Wilcoxon rank sum test, and the correlation between NXPH4 expression and infiltration levels of immune cells was analyzed using Spearman's correlation analysis.

TISIDB database analysis

The TISIDB database serves as a web-based tool for

identifying the connections between tumors and the immune system (30). Utilizing the "immunomodulator" module offered by the TISIDB database, we examined the link between NXPH4 expression and the expression levels of immunostimulators and immunoinhibitors. Additionally, we utilized the "chemokine" module to assess the correlation between NXPH4 expression and the expression levels of chemokines and chemokine receptors.

Upstream miRNAs and lncRNAs analysis

ENCORI database (31) and TargetScan database (32) were used to predict upstream potential miRNAs of NXPH4 and potential lncRNAs of miRNAs. ENCORI was also used to analyze the correlation between miRNAs, lncRNAs, and NXPH4 expression in COAD. The ceRNA network was visualized using the igraph and ggraph packages.

Statistical analysis

All statistical analyses were conducted using the R programming language (version 4.2.1) and R package ggplot2 (version 3.3.6). The expression of NXPH4 was analyzed by Wilcoxon rank sum test in unpaired samples, and paired *t*-test in paired samples. The correlation between quantitative variables was assessed using Spearman's correlation analysis. Statistical significance was represented by P<0.05 (*), P<0.01 (**), and P<0.001 (***).

Results

NXPH4 expression in pan-cancer

To gain an initial understanding of NXPH4 expression patterns across cancer types, we performed a pan-cancer analysis using mRNA expression data from the TCGA database. Our analysis revealed that NXPH4 expression was significantly increased in 17 of 33 cancer tissues compared to normal tissues (*Figure 1A*), and in 14 of the 23 paired cancer tissues and adjacent normal tissues (*Figure 1B*). We next evaluated whether NXPH4 overexpression correlates with patient prognosis by conducting Kaplan-Meier survival analysis. Intriguingly, higher NXPH4 expression was associated with significantly worse OS in several cancers, including adrenocortical carcinoma (ACC), bladder urothelial carcinoma (BLCA), breast invasive carcinoma (BRCA), COAD, kidney renal papillary cell carcinoma (KIRP), liver hepatocellular carcinoma (LIHC), rectum adenocarcinoma (READ), uterine corpus endometrial carcinoma (UCEC), and uveal melanoma (UVM) (*Figure 1C*). Together, these pan-cancer analyses provide an initial indication that NXPH4 may have an oncogenic role, which warrants further mechanistic exploration in COAD.

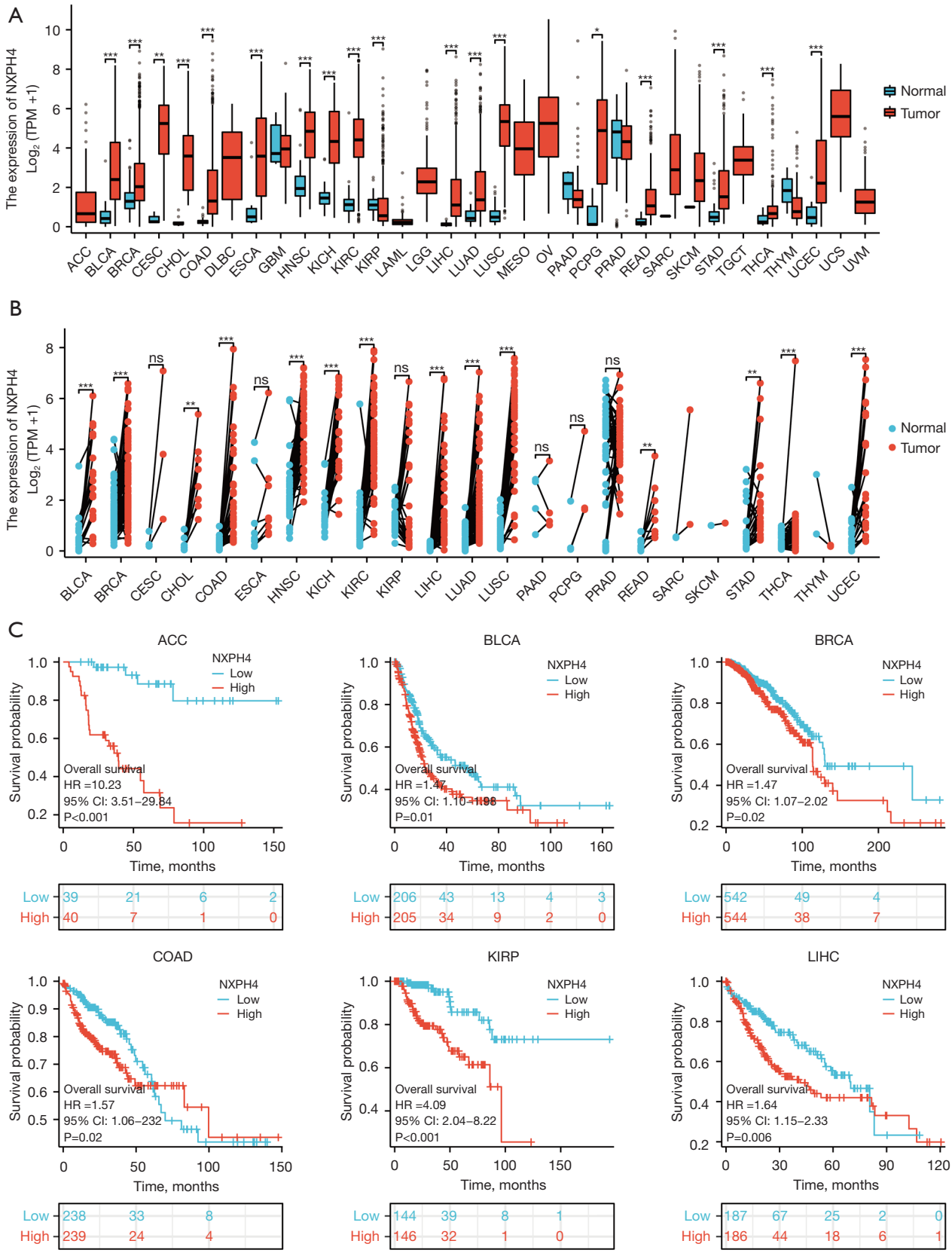
NXPH4 expression in COAD patients

After observing NXPH4 overexpression profile across multiple cancers, we focused our analysis on COAD specifically. The TCGA data collection and analysis flowchart is presented in Figure S1. Using TCGA data, we found NXPH4 was significantly upregulated in 480 COAD tumor samples compared to 41 normal colon tissues (*Figure 2A*). This overexpression was validated in 41 paired COAD tumor and adjacent normal tissues (*Figure 2B*). To further confirm this pattern, we analyzed two additional colon cancer datasets from the GSE71187 and GSE44076 of GEO database. Consistently, both showed higher NXPH4 expression in COAD patients relative to adjacent normal tissues (*Figure 2C,2D*).

To further understand the relationship between NXPH4 expression and clinicopathological features in COAD, we analyzed the data from 478 patients in the TCGA database by dividing them into high and low NXPH4 expression groups (*Table 1*). Intriguingly, high mRNA expression of NXPH4 positively correlated with advanced T stage (*Figure 2E*), lymphatic invasion (*Figure 2F*), and certain histological subtypes (*Figure 2G*), suggesting an association with aggressive disease characteristics. Together, concordant upregulation of NXPH4 in multiple COAD patient datasets and its links to unfavorable clinical features support an oncogenic role for NXPH4 in colon cancer.

Diagnostic and prognostic value of NXPH4 in COAD patients

To evaluate the potential association between NXPH4 expression and aggressive clinical features in COAD, we conducted an assessment of the correlation between NXPH4 mRNA expression levels and the prognosis of COAD patients in TCGA database using the Kaplan-Meier analysis. The results showed that COAD patients with higher NXPH4 expression correlated with reduced OS (*Figure 3A*), DSS (*Figure 3B*), and PFI (*Figure 3C*). We further explored the correlation between NXPH4 expression levels and the survival of colon cancer patients



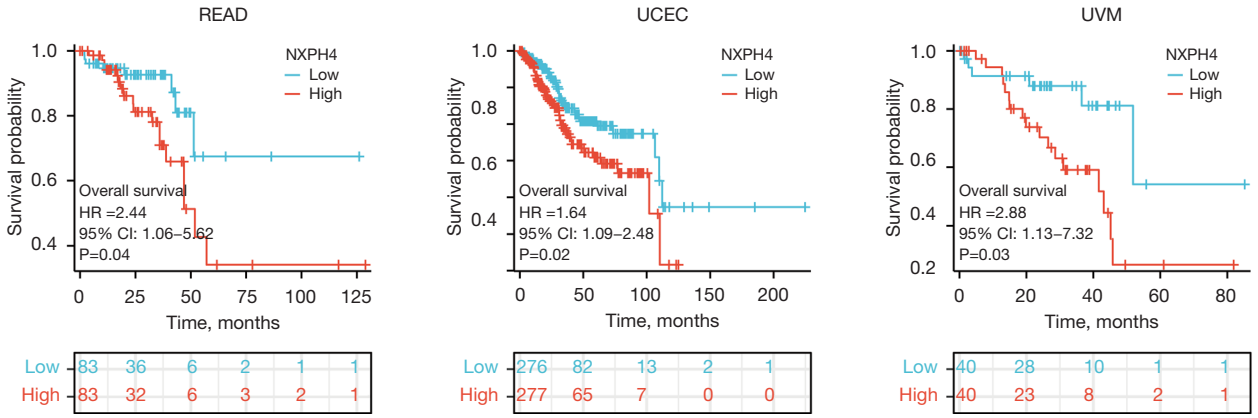


Figure 1 Expression status and prognostic value of NXPH4 in pan-cancer. (A,B) Expression of NXPH4 in different tumor tissues and normal tissues according to the TCGA database. (C) Prognostic analysis of NXPH4 expression levels in different human cancers. *, P<0.05; **, P<0.01; ***, P<0.001; ns, no significance. NXPH4, neurexophilin 4; TPM, transcripts per million; ACC, adrenocortical carcinoma; HR, hazard ratio; BLCA, bladder urothelial carcinoma; BRCA, breast invasive carcinoma; COAD, colon adenocarcinoma; KIRP, kidney renal papillary cell carcinoma; LIHC, liver hepatocellular carcinoma; READ, rectum adenocarcinoma; UCEC, uterine corpus endometrial carcinoma; UVM, uveal melanoma; TCGA, The Cancer Genome Atlas.

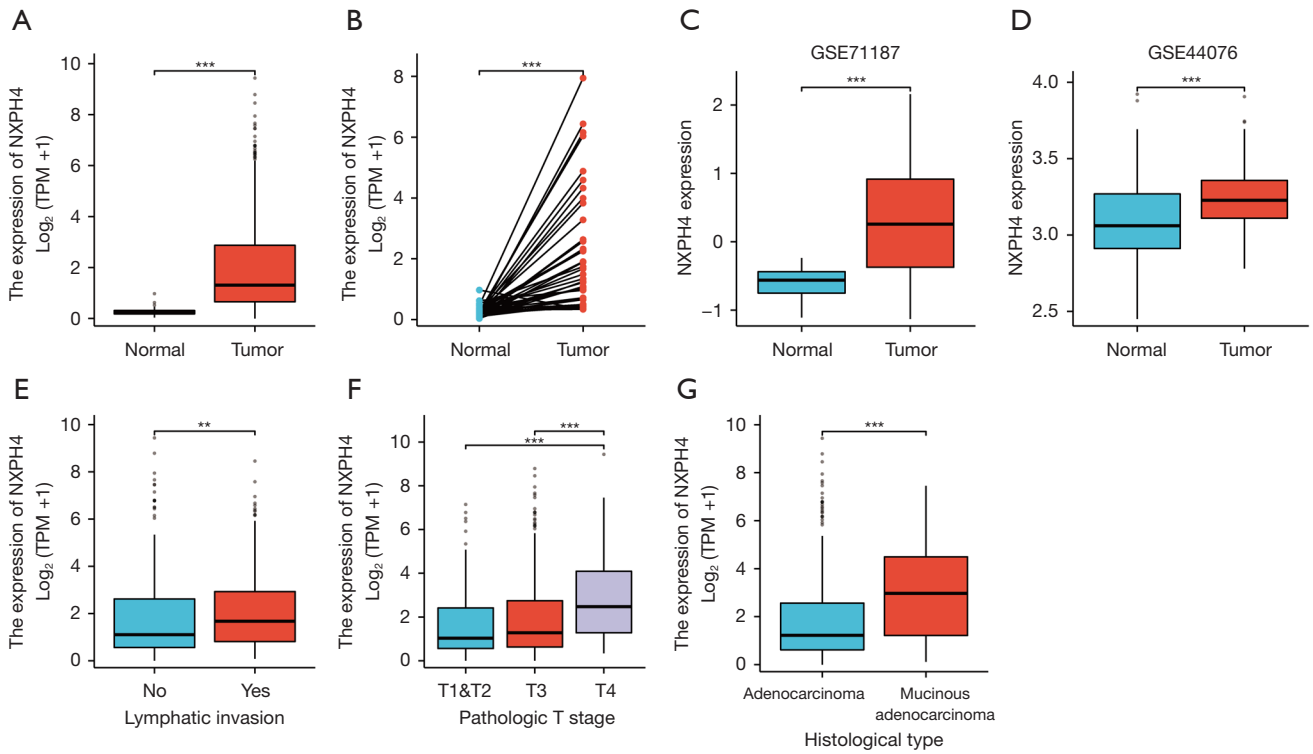


Figure 2 NXPH4 expression in COAD patients. (A,B) Expression of NXPH4 in COAD tissues and adjacent normal colon tissues according to the TCGA database. (C,D) Expression of NXPH4 in COAD tissues and normal colon tissues according to the GEO database. (E-G) Correlation between NXPH4 expression and clinical parameters in COAD. **, P<0.01; ***, P<0.001. NXPH4, neurexophilin 4; TPM, transcripts per million; COAD, colon adenocarcinoma; TCGA, The Cancer Genome Atlas; GEO, Gene Expression Omnibus.

Table 1 Clinical characteristics of COAD patients

Characteristics	Low expression of NXPH4	High expression of NXPH4	P value
N	239	239	
Pathologic T stage, n (%)			0.01
T1&T2	58 (12.2)	36 (7.5)	
T3&T4	181 (37.9)	202 (42.3)	
Pathologic N stage, n (%)			0.09
N0	151 (31.6)	133 (27.8)	
N1&N2	88 (18.4)	106 (22.2)	
Pathologic M stage, n (%)			0.03
M0	182 (43.9)	167 (40.2)	
M1	25 (6.0)	41 (9.9)	
Pathologic stage, n (%)			0.054
Stage I&Stage II	144 (30.8)	124 (26.6)	
Stage III&Stage IV	89 (19.1)	110 (23.6)	
Age, n (%)			0.71
≤65 years	99 (20.7)	95 (19.9)	
>65 years	140 (29.3)	144 (30.1)	
CEA level, n (%)			0.69
≤5 ng/mL	96 (31.7)	100 (33.0)	
>5 ng/mL	55 (18.2)	52 (17.2)	

COAD, colon adenocarcinoma; NXPH4, neurexophilin 4; CEA, carcinoembryonic antigen.

using the Kaplan-Meier plotter database. The results demonstrated that increased NXPH4 expression was significantly associated with both poor OS (Figure S2A) and relapse-free survival (RFS) (Figure S2B). These data indicated that NXPH4 expression levels could serve as an independent prognostic biomarker. An ideal biomarker for clinical implementation should have both prognostic and diagnostic value. To further investigate the clinical utility of NXPH4, we assessed its diagnostic accuracy for COAD using ROC curve analysis. The AUC was 0.937 [95% confidence interval (CI): 0.910–0.963] (*Figure 3D*), indicting excellent performance as a diagnostic marker. Together, these results suggest that NXPH4 can serve as a sensitive index for predicting both the prognosis of COAD patients and can act as an effective diagnostic biomarker in COAD.

Prognostic prediction model development of NXPH4 in COAD patients

To identify risk factors associated with OS in COAD patients, TCGA-COAD dataset was used to perform univariate and multivariate Cox regression analyses. Univariate Cox regression analysis revealed that T stage, N stage, M stage, age, carcinoembryonic antigen (CEA) level, and NXPH4 expression were factors that influenced OS. Multivariate Cox regression analysis showed that NXPH4 expression (P=0.03), age, and N stage were independent risk factors for OS (*Table 2*). Consequently, NXPH4 could serve as an independent prognostic factor for predicting OS in COAD patients.

To translate these findings into a clinical tool, a nomogram based on various clinical features, including NXPH4 was constructed to predict 1-, 3-, and 5-year OS in COAD patients (*Figure 4A*). The C-index of the nomogram was 0.821 (95% CI: 0.789–0.853), indicating good predictive accuracy on the survival probability over a given period. The calibration plot showed a relative satisfactory predictive accuracy of the nomogram (*Figure 4B*). The bias-corrected line in the calibration plot closely followed the ideal curve, indicating significant correlation between the observed and predicted values. Subsequently, the time-dependent ROC curve was plotted to examine the prognostic value of NXPH4 expression in the prediction of 1-, 3-, and 5-year OS. The results also demonstrated favorable discrimination, with AUC values of 0.629, 0.614, and 0.563 at 1-, 3-, and 5-year, respectively (*Figure 4C*). Overall, this modeling provides proof-of-concept for incorporating NXPH4 expression data into quantitative risk stratification for COAD patients, though additional independent validation is still needed to support clinical implementation.

Functional enrichment analysis of NXPH4 in COAD

To elucidate the BPs and pathways associated with NXPH4 in COAD, we compared the transcriptional profiles of tumors with high versus low NXPH4 expression. The COAD patients were divided into high- and low-NXPH4 expression groups based on the median NXPH4 expression value, using the criterion of |log 2 (fold change)| >1.0 and P<0.05. We identified 1,342 DEGs, with 648 up-regulated and 694 down-regulated (*Figure 5A*). To pinpoint genes

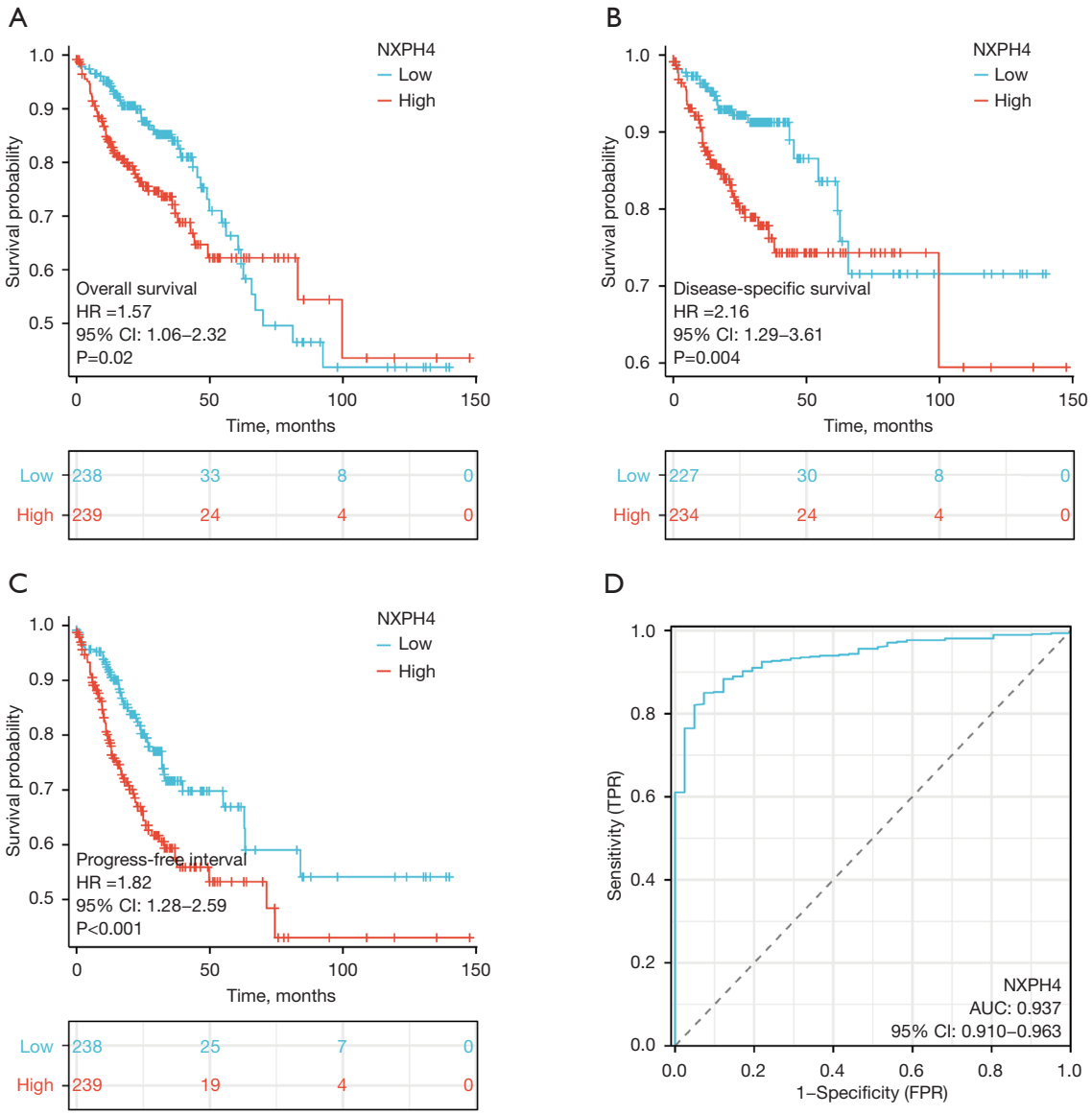


Figure 3 Prognostic and diagnostic value of NXPH4 in COAD. (A-C) Kaplan-Meier survival curve analysis of OS, DSS and PFI in COAD cohort. (D) ROC curve of NXPH4 expression in COAD cohort. NXPH4, neurexophilin 4; HR, hazard ratio; FPR, false positive rate; TPR, true positive rate; AUC, area under the curve; CI, confidence interval; COAD, colon adenocarcinoma; OS, overall survival; DSS, disease-specific survival; PFI, progression-free interval; ROC, receiver operating characteristic.

most correlated with NXPH4 in COAD, the top 20 genes positively and negatively correlated with NXPH4 expression were presented in a heatmap (*Figure 5B*,*5C*).

GO and KEGG enrichment analysis of the identified DEGs revealed several interesting biological themes. The top enrichment results were displayed in the form of bubble plots (*Figure 5D*), and the following BPs were found to be significantly affected: endocrine hormone secretion, antimicrobial humoral response, and neuropeptide signaling pathway. The most enriched MFs were neuropeptide binding and receptor activity, highlighting neuroendocrine signaling associations. KEGG pathway analysis also showed enrichment for neuroactive ligand-receptor interactions, maturity onset diabetes of the young, and fat digestion/absorption. These results provide clues into the functional roles of NXPH4 in COAD, which may be associated with

Table 2 Univariate and multivariate Cox regression analysis of the correlation between OS and NXPH4 expression

Characteristics	Total, N	Univariate analysis		Multivariate analysis	
		Hazard ratio (95% CI)	P value	Hazard ratio (95% CI)	P value
Pathologic T stage	476				
T1&T2	94	Reference		Reference	
T3&T4	382	3.072 (1.423–6.631)	0.004	2.684 (0.615–11.721)	0.19
Pathologic N stage	477				
N0	283	Reference		Reference	
N1&N2	194	2.592 (1.743–3.855)	<0.001	2.435 (1.111–5.333)	0.03
Pathologic M stage	414				
M0	348	Reference		Reference	
M1	66	4.193 (2.683–6.554)	<0.001	1.790 (0.786–4.079)	0.17
Age	477				
≤65 years	194	Reference		Reference	
>65 years	283	1.610 (1.052–2.463)	0.03	2.953 (1.401–6.224)	0.004
CEA level	302				
≤5 ng/mL	195	Reference		Reference	
>5 ng/mL	107	3.128 (1.788–5.471)	<0.001	1.982 (0.951–4.131)	0.07
Histological type	472				
Adenocarcinoma	402	Reference		–	–
Mucinous adenocarcinoma	70	1.269 (0.753–2.139)	0.37	–	–
Colon polyps present	249				
No	162	Reference		–	–
Yes	87	1.324 (0.738–2.373)	0.35	–	–
Anatomic neoplasm subdivision	442				
Descending colon	20	Reference		–	–
Ascending colon	88	2.170 (0.650–7.243)	0.21	–	–
Sigmoid colon	157	1.232 (0.372–4.077)	0.73	–	–
Transverse colon	40	1.400 (0.361–5.429)	0.63	–	–
Cecum	111	2.078 (0.635–6.803)	0.23	–	–
Hepatic flexure	26	1.441 (0.344–6.040)	0.62	–	–
NXPH4	477				
Low	238	Reference		Reference	
High	239	1.570 (1.062–2.319)	0.02	2.044 (1.070–3.905)	0.03

OS, overall survival; NXPH4, neurexophilin 4; CI, confidence interval; CEA, carcinoembryonic antigen.

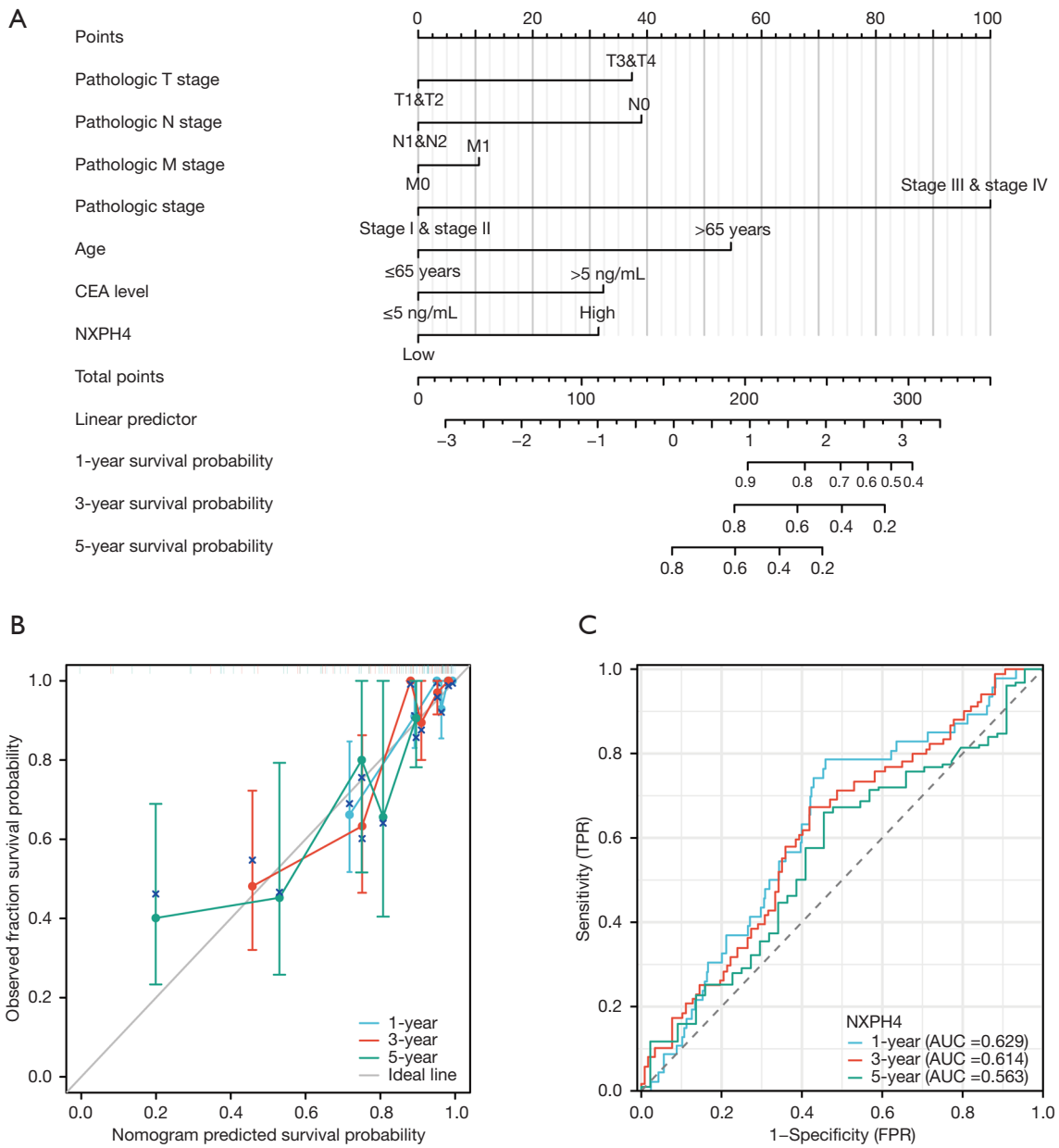


Figure 4 Prognostic prediction model of NXPH4 in COAD patients. (A) Nomogram for 1-, 3- and 5-year OS of COAD patients. (B) Calibration plots for 1-, 3- and 5-year OS prediction. (C) Time-dependent ROC curves and AUC values for 1-, 3- and 5-year OS prediction. CEA, carcinoembryonic antigen; NXPH4, neurexophilin 4; FPR, false positive rate; TPR, true positive rate; AUC, area under the curve; COAD, colon adenocarcinoma; OS, overall survival; ROC, receiver operating characteristic; AUC, area under the curve.

neuroendocrine signaling, hormone responses, and digestive processes through modulating gastrointestinal pathways.

Construction of the PPI network of NXPH4

To gain further insights into the interacting partners and signaling networks involving NXPH4 in COAD, we constructed PPI networks using GeneMANIA and STRING databases. The resulting PPI network revealed that NXPH4 had close connections with other members of the neurexophilin family, including NXPH1, NXPH2, NXPH3, NXPE1, NXPE2, NXPE3, and NXPE4. Additional PPI

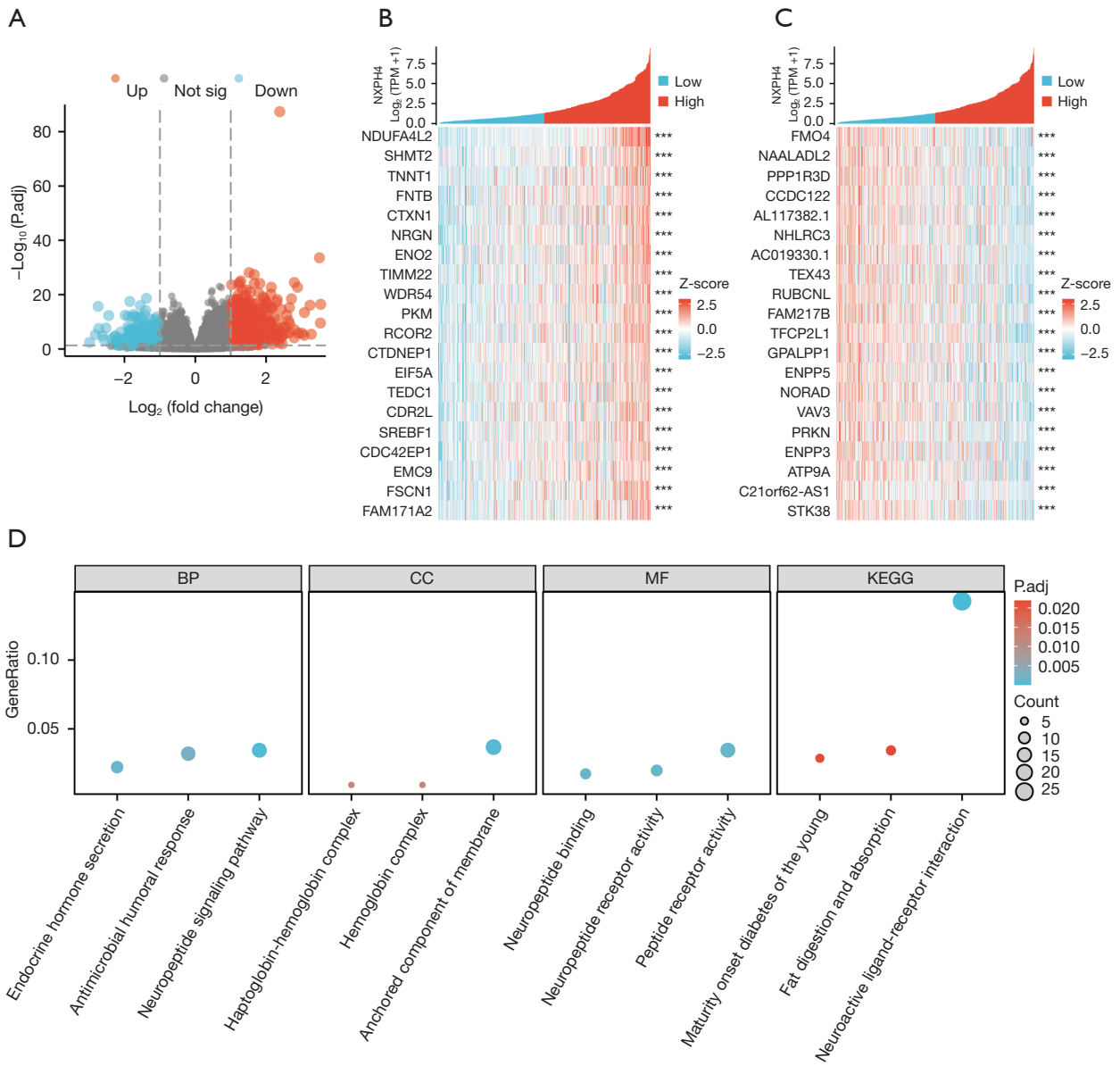


Figure 5 Functional enrichment analysis of NXPH4 in COAD. (A) Volcano plot of DEGs between the high and low NXPH4 expression groups. (B,C) Heat map showing the top 20 positively and negatively co-expressed genes with NXPH4 in COAD. (D) GO-KEGG enrichment analysis of NXPH4 expression-correlated DEGs. ***, P<0.001. NXPH4, neurexophilin 4; TPM, transcripts per million; BP, biological process; CC, cellular component; MF, molecular function; KEGG, Kyoto Encyclopedia of Genes and Genomes; COAD, colon adenocarcinoma; DEGs, differentially expressed genes; GO, Gene Ontology.

nodes included other neurexins (NRXN1, NRXN2, NRXN3), and related transmembrane proteins latrophilin-1 (LPHN1) and latrophilin-2 (LRRTM2) (*Figure 6A,6B*). Mapping this NXPH4-centered interaction network provides clues into its binding partners and points to potential involvement in neurexin-mediated cell adhesion and signaling. The neurexin family has established roles in the nervous system (33), but emerging links to cancer warrant further exploration. Elucidating the proteins that cooperate with NXPH4 may uncover new mediators of its oncogenic functions.

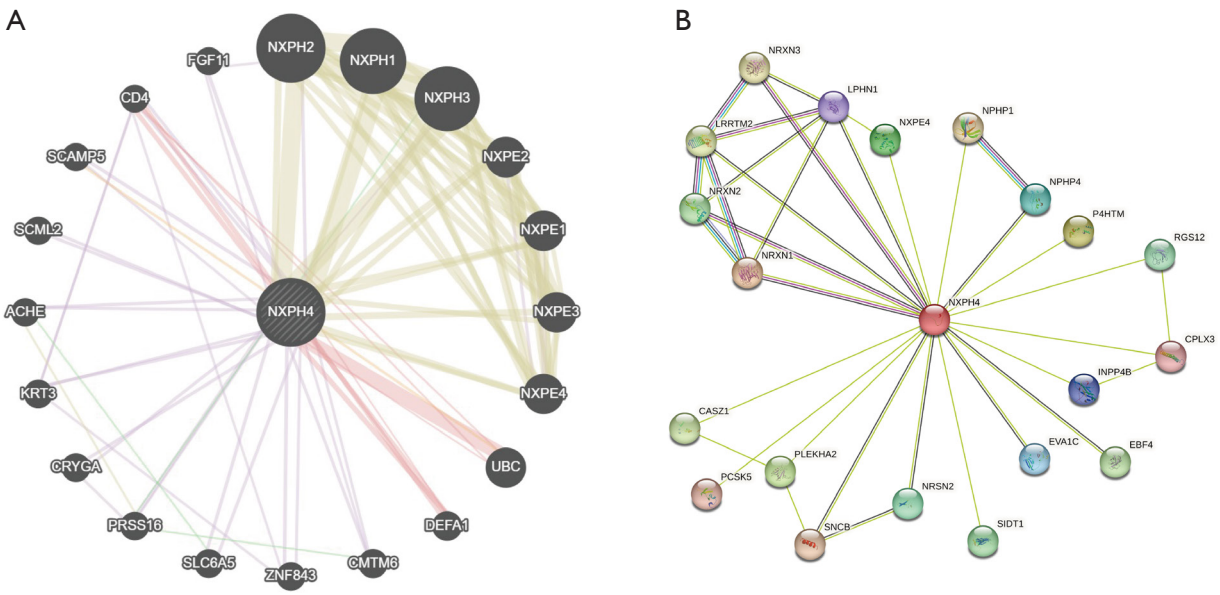


Figure 6 PPI analysis of NXPH4. The PPI network constructed on (A) GeneMANIA database and (B) STRING database. NXPH4, neurexophilin 4; PPI, protein-protein interaction; GeneMANIA, Gene Multiple Association Network Integration Algorithm; STRING, Search Tool for the Retrieval of Interacting Genes.

GSEA of genes related to NXPH4 in COAD

To gain a broader perspective on the BPs and pathways regulated by NXPH4 in COAD, we further performed GSEA analysis to determine the biological functions regulated by NXPH4 between high and low NXPH4 expression groups based on the normalized enrichment score (NES) and FDR q-value. GSEA assesses coordinate changes in groups of related genes, providing insight beyond individual DEGs. Intriguingly, NXPH4-associated genes were enriched in several immune-related pathways, including interferon gamma response, allograft rejection, inflammatory response, IL6 Jak STAT3 signaling, and complement signaling (*Figure 7A-7E*). This points to a potential link between NXPH4 and immune evasion or modulation in the tumor microenvironment (TME). We also observed enrichment for glycolysis, KRAS signaling, hypoxia response, and coagulation (*Figure 7F-7I*), suggesting involvement in metabolic and environmental adaptation pathways important for cancer progression. These GSEA results implicate NXPH4 in regulating diverse BPs relevant to COAD pathogenesis, which provides a more comprehensive perspective on the complex molecular networks influenced by NXPH4 in colon cancer.

Correlation between NXPH4 expression and genes related to glycolysis and hypoxia in COAD

Given the GESA analysis implicating NXPH4 in glycolysis and hypoxia pathway, we further evaluated the correlation between NXPH4 and 10 glycolysis- and 10 hypoxia-related genes in TCGA-COAD. The results showed that NXPH4 expression was remarkably positively associated with genes related to glycolysis, including SLC16A3, DDIT4, PGAM1, HSPA5, MIF, B3GALT6, GMPPA, EGLN3, PYGL, and FKBP4 (*Figure 8A*). NXPH4 expression was also positively associated with hypoxia-response genes like ENO2, GAPDH, TPI1, ENO1, MT2A, MT1E, PFKP, GPI, ANGPTL4, and P4HA1 (*Figure 8B*).

We next examined the expression of glycolysis- and hypoxia-related genes in TCGA-COAD database. The results showed that three genes, B3GALT6, ENO2, and P4HA1, were positively correlated with NXPH4 (*Figure 8C*) and exhibited higher expression in tumor tissues compared to normal tissues (*Figure 8D*). Furthermore, higher expression of these three genes associated with poorer OS in COAD patients (*Figure 8E*). These findings strengthen the link between NXPH4 and critical glycolytic and hypoxic response pathways in COAD. The coordinated

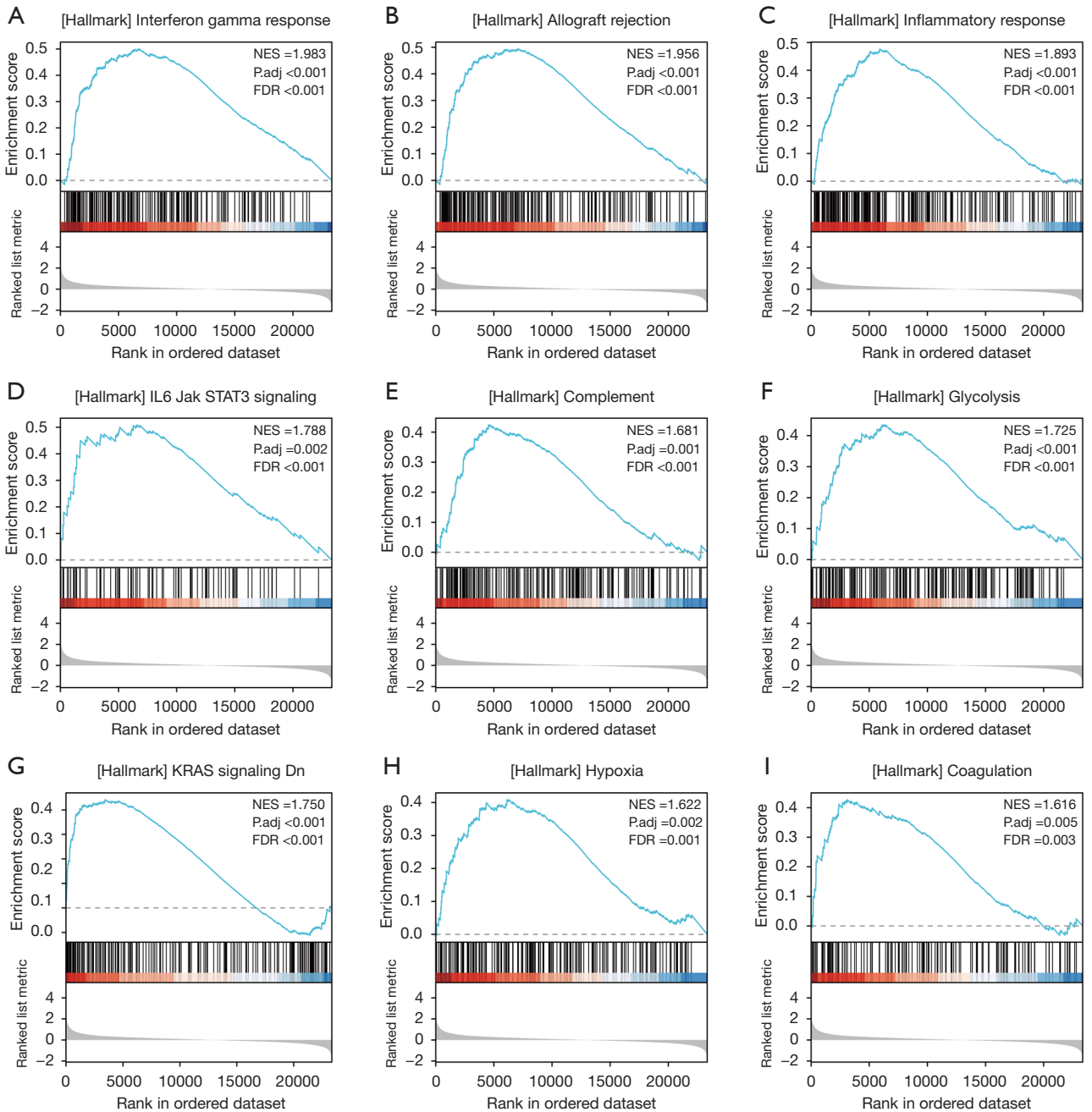
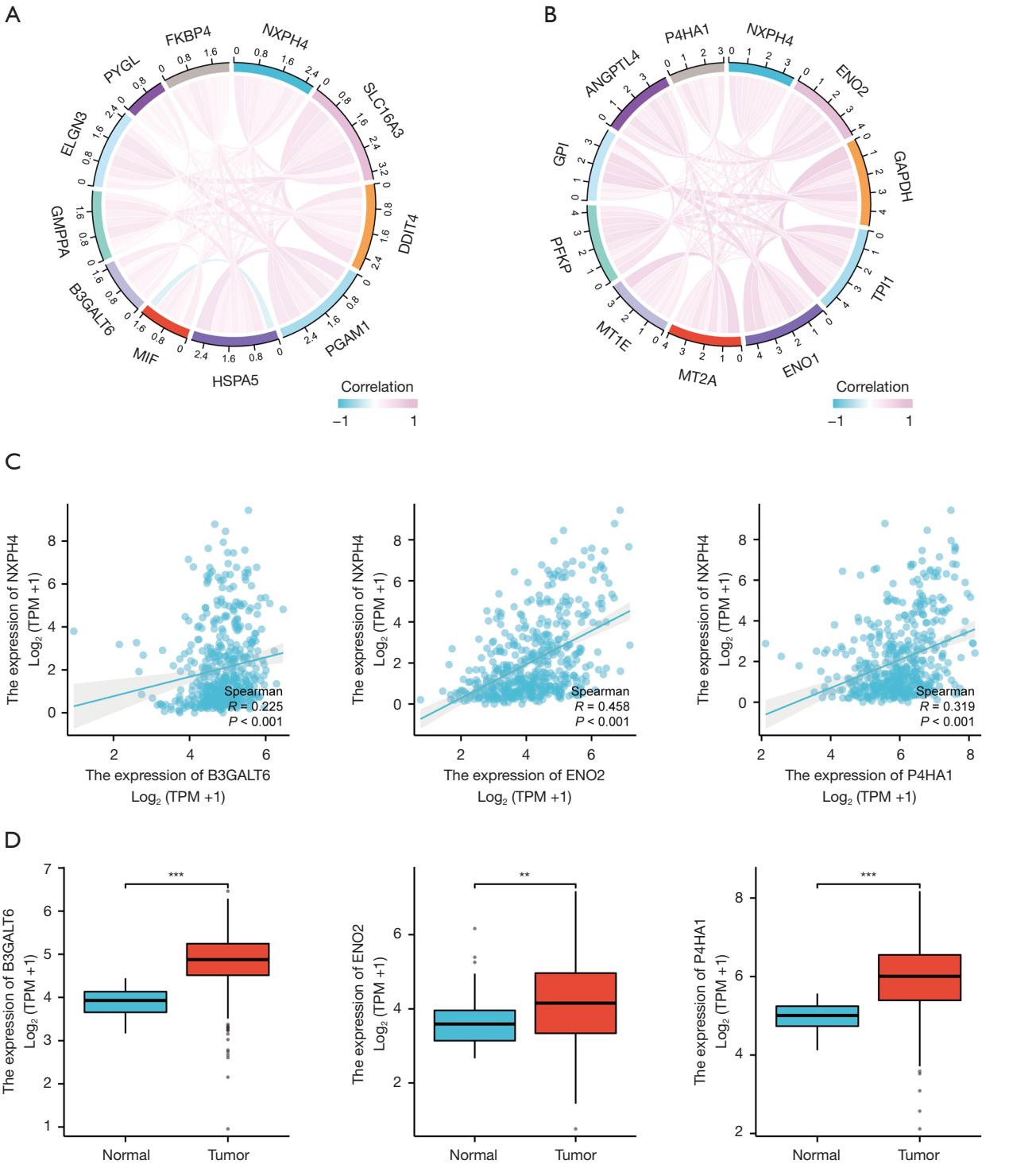


Figure 7 Enrichment plots from the GSEA. Genes related to NXPH4 in COAD were enriched in (A) interferon gamma response, (B) allograft rejection, (C) inflammatory response, (D) IL6 Jak STAT3 signaling, (E) complement, (F) glycolysis, (G) KRAS signaling Dn (genes down-regulated by KRAS activation), (H) hypoxia, and (I) coagulation. NES, normalized enrichment score; FDR, false discovery rate; GSEA, gene set enrichment analysis; NXPH4, neurexophilin 4; COAD, colon adenocarcinoma.



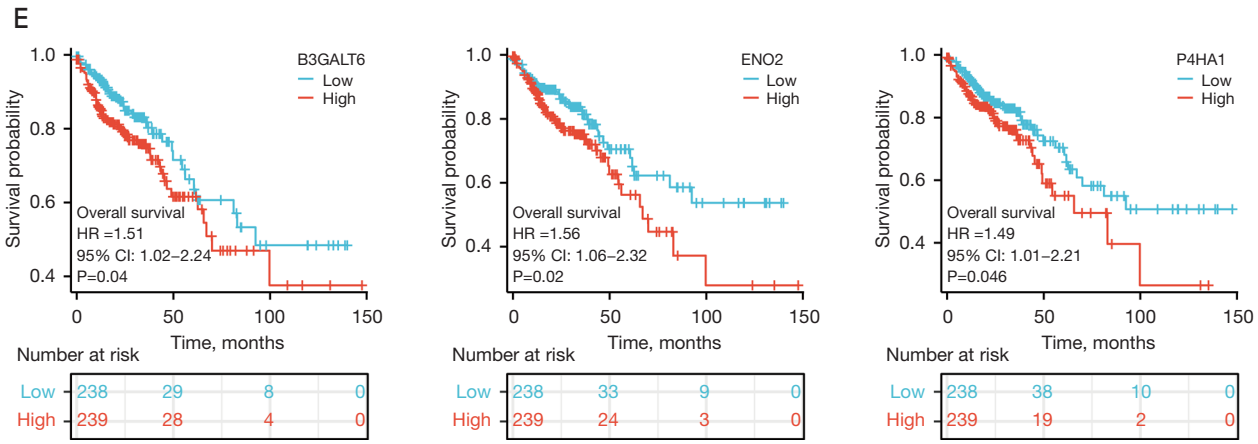


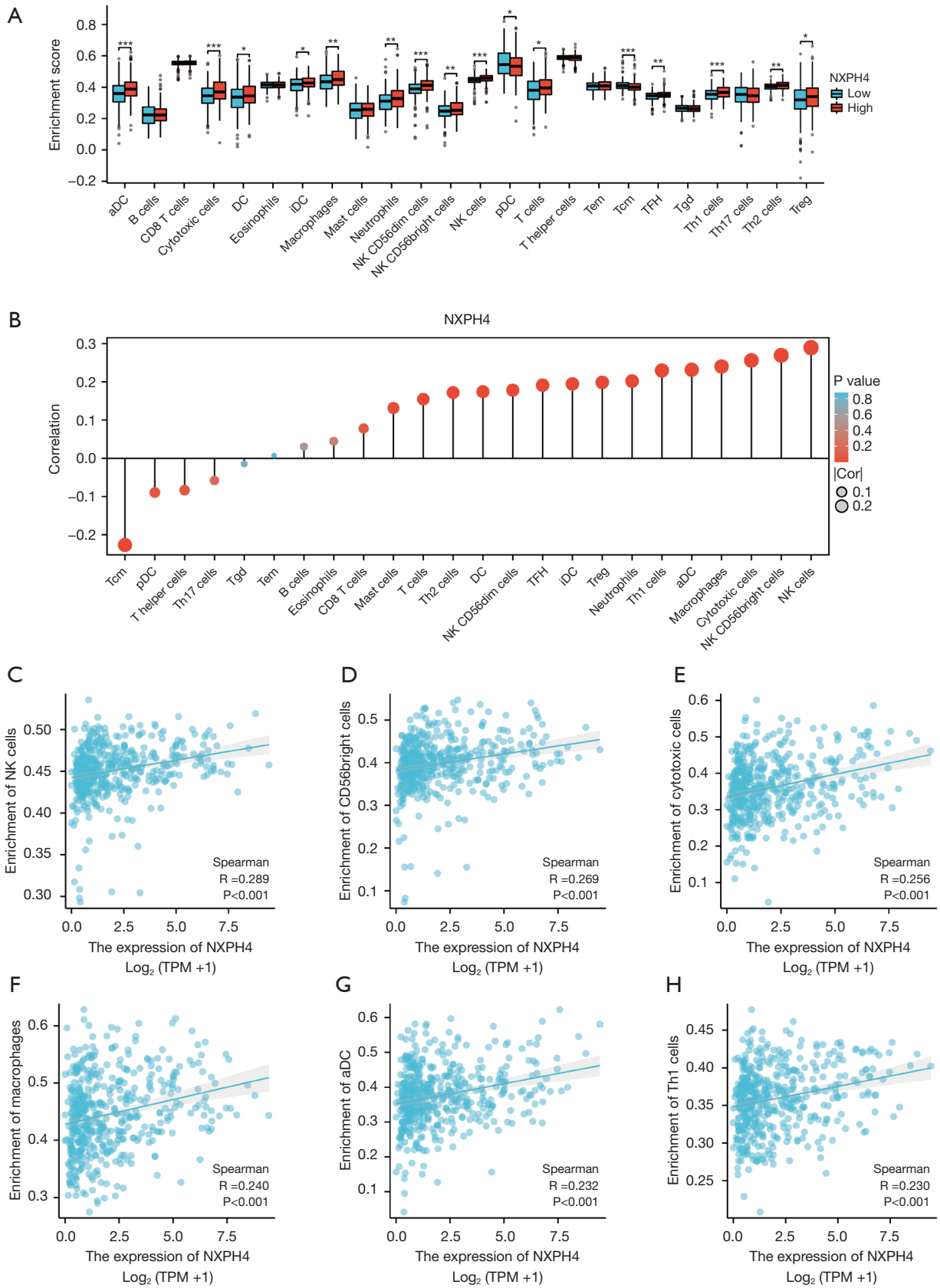
Figure 8 Analysis of genes co-expressed with NXPH4 and related to glycolysis and hypoxia in COAD. (A,B) Chord diagrams of the relationships between the expression of NXPH4 and 10 glycolysis-related genes and 10 hypoxia-related genes in TCGA-COAD. (C) Scatter plots of the correlation between the expression of NXPH4 and B3GALT6, ENO2, and P4HA1. (D) Analysis of B3GALT6, ENO2, and P4HA1 expression in tumor tissues and adjacent normal tissues in TCGA-COAD. (E) Association between B3GALT6, ENO2, and P4HA1 expression and OS in COAD patients. **, P<0.01; ***, P<0.001. NXPH4, neurexophilin 4; TPM, transcripts per million; B3GALT6, β-1,3galactosyltransferase 6; ENO2, enolase 2; P4HA1, prolyl 4-hydroxylase subunit α1; HR, hazard ratio; COAD, colon adenocarcinoma; TCGA, The Cancer Genome Atlas; OS, overall survival.

overexpression of NXPH4 with key metabolic/hypoxia genes in tumors versus normal tissue implies functional cooperation in promoting colon cancer progression. Therapeutically targeting these NXPH4-associated pathways may potentially disrupt adaptive mechanisms supporting tumor growth and metastasis.

Correlation between NXPH4 expression and immune infiltration

The GSEA results pointed to a potential link between NXPH4 and immune pathways, so we evaluated associations with immune cell abundance in COAD. Comparing tumors with high versus low NXPH4 revealed increased infiltration of 24 immune cell subtypes in COAD as determined by ssGSEA (*Figure 9*). The proportion of immune cell subtypes in different NXPH4 expression groups displayed that high expression of NXPH4 was linked to a significant increase in the abundance of activated dendritic cells (aDC) cells, cytotoxic cells, dendritic cells (DC) cells, immature dendritic cells (iDC) cells, macrophages, neutrophils, natural killer (NK) CD56bright cells, NK CD56dim cells, NK cells, T cells, follicular helper T (TFH) cells, T helper 1 (Th1) cells, and regulatory T (Treg) cells; meanwhile, the abundance of plasmacytoid dendritic cells (pDC) cells and central memory T (Tcm) cells were significantly decreased in high NXPH4 group (*Figure 9A*). Furthermore, we observed that NXPH4 expression was positively correlated with infiltration abundance of NK cells, NK CD56bright cells, cytotoxic cells, macrophages, aDC cells, Th1 cells, neutrophils, Treg cells, and negatively associated with abundance of Tcm cells in COAD (*Figure 9B-9K*).

We next analyzed the correlation between NXPH4 and specific immunosuppressive or stimulatory proteins using TISIDB database. The results showed that NXPH4 expression was positively correlated with well-known inhibitory signal molecules (*Figure 10A*), including PVRL2 (R=0.332, P=3.68e−13), TGFB1 (R=0.3, P=6.8e−11), PDCD1 (R=0.229, P=7.57e−07), LAG3 (R=0.256, P=2.88e−08), and CTLA4 (R=0.159, P=6.21e−04) (Figure S3A-S3E). It was also positively correlated with several stimulatory proteins like CD276 (R=0.28, P=1.22e−09), CD70 (R=0.221, P=1.86e−06), and CXCR4 (R=0.248, P=8.06e−08) (*Figure 10B* and Figure S3F-S3H). NXPH4 also associated with various chemokines and their receptors including positive relation with CCL3 (R=0.249, P=7.42e−08), CCL4 (R=0.224, P=1.32e−06), CCL18 (R=0.202, P=1.34e−05), CCL23 (R=0.211, P=5.4e−06), CCL25 (R=0.214, P=3.99e−06), CXCL16 (R=0.253, P=4.61e−08), and CXCR4 (R=0.248, P=8.06e−08), as well as negatively correlated with the expression of CXCL14 (R=−0.296, P=1.12e−10) (*Figure 10C,10D*, and



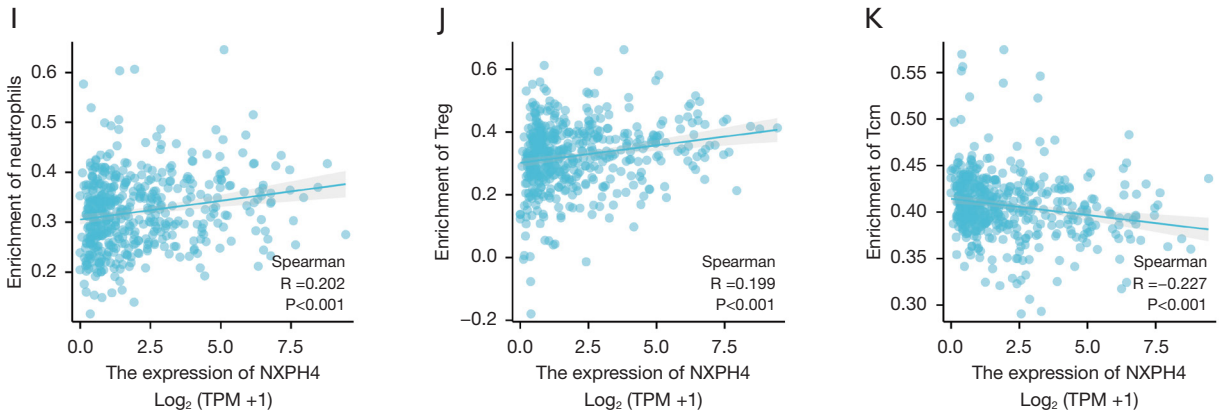


Figure 9 Correlation analysis of NXPH4 expression and immune infiltration in COAD. (A) The infiltrating levels of 24 subtypes of immune cells in high and low NXPH4 expression groups in COAD. (B) The correlation between NXPH4 expression and 24 tumor-infiltrating immune cells in COAD. (C-K) The correlation of NXPH4 expression with immune infiltration levels of NK cells, NK CD56bright cells, cytotoxic cells, macrophages, aDC cells, Th1 cells, neutrophils, Treg cells, and Tcm cells. *, P<0.05; **, P<0.01; ***, P<0.001. NXPH4, neurexophilin 4; aDC, activated dendritic cells; DC, dendritic cells; iDC, immature dendritic cells; NK, natural killer; pDC, plasmacytoid dendritic cells; Tem, effector memory T; Tcm, central memory T; TFH, follicular helper T; Tgd, gamma-delta T; Th1, T helper 1; Treg, regulatory T; TPM, transcripts per million; COAD, colon adenocarcinoma.

Figure S4A-S4H). These results imply NXPH4 may promote immune evasion in COAD by enhancing infiltration of suppressive cell types and upregulating inhibitory molecules while downregulating immune-stimulating signals. The correlations with chemokines suggest NXPH4 may regulate immune cell recruitment into the TME. Targeting the NXPH4-mediated immunosuppressive phenotype could potentially restore anti-tumor immunity.

Construction of ceRNA network of NXPH4 in COAD

We next investigated whether NXPH4 participates in a ceRNA network in COAD. ceRNA crosstalk, where RNAs competitively bind shared miRNAs, enables complex post-transcriptional regulation (34). To build a NXPH4-centered ceRNA network, we first predicted its targeting miRNAs using ENCORI and TargetScan as shown in the Venn diagram (*Figure 11A* and Table S1). Correlation analysis showed that seven miRNAs were negatively correlated with NXPH4 expression in COAD, including hsa-miR-204-5p, hsa-miR-485-5p, hsa-miR-497-5p, hsa-miR-320c, hsa-miR-24-3p, hsa-miR-195-5p, and hsa-miR-541-3p (*Figure 11B* and Figure S5A). Four of these miRNAs were downregulated in tumor tissues versus adjacent normal tissues, including hsa-miR-204-5p, hsa-miR-485-5p, hsa-miR-497-5p, and hsa-miR-320c (*Figure 11C* and Figure S5B).

Moreover, we examined the correlation between the expression levels of NXPH4 and co-expressed lncRNAs in the TCGA-COAD dataset using Spearman's correlation coefficients (r>0 and P<0.05 were set as threshold for identifying positive co-expressed lncRNAs). We then predicted the targeted lncRNAs of hsa-miR-204-5p, hsa-miR-485-5p, hsa-miR-497-5p, and hsa-miR-320c using ENCORI, as well as by taking the intersection of lncRNAs co-expressed with NXPH4 and the above predicted lncRNAs (Table S2).

Based on the ceRNA hypothesis, potential lncRNAs should have negative correlations with miRNAs and positive correlations with NXPH4 in COAD. Intriguingly, MELTF-AS1, RPARP-AS1, SNHG7, and SNHG10 were negatively correlated with hsa-miR-485-5p, AC129492.1 and AGAP2-AS1 were negatively correlated with hsa-miR-497-5p, and TYMSOS was negatively correlated with hsa-miR-320c (*Figure 11D*). Further analysis demonstrated that these seven lncRNAs positively correlated with the expression of NXPH4 (*Figure 11E*), and were significantly upregulated in tumor tissue compared with normal tissues in COAD (*Figure 11F*). By integrating miRNA, lncRNA, and mRNA expression analysis with target prediction, we constructed a NXPH4-centered ceRNA network in COAD ceRNA network in COAD (*Figure 11G*). This network provides a roadmap to explore the functional and clinical

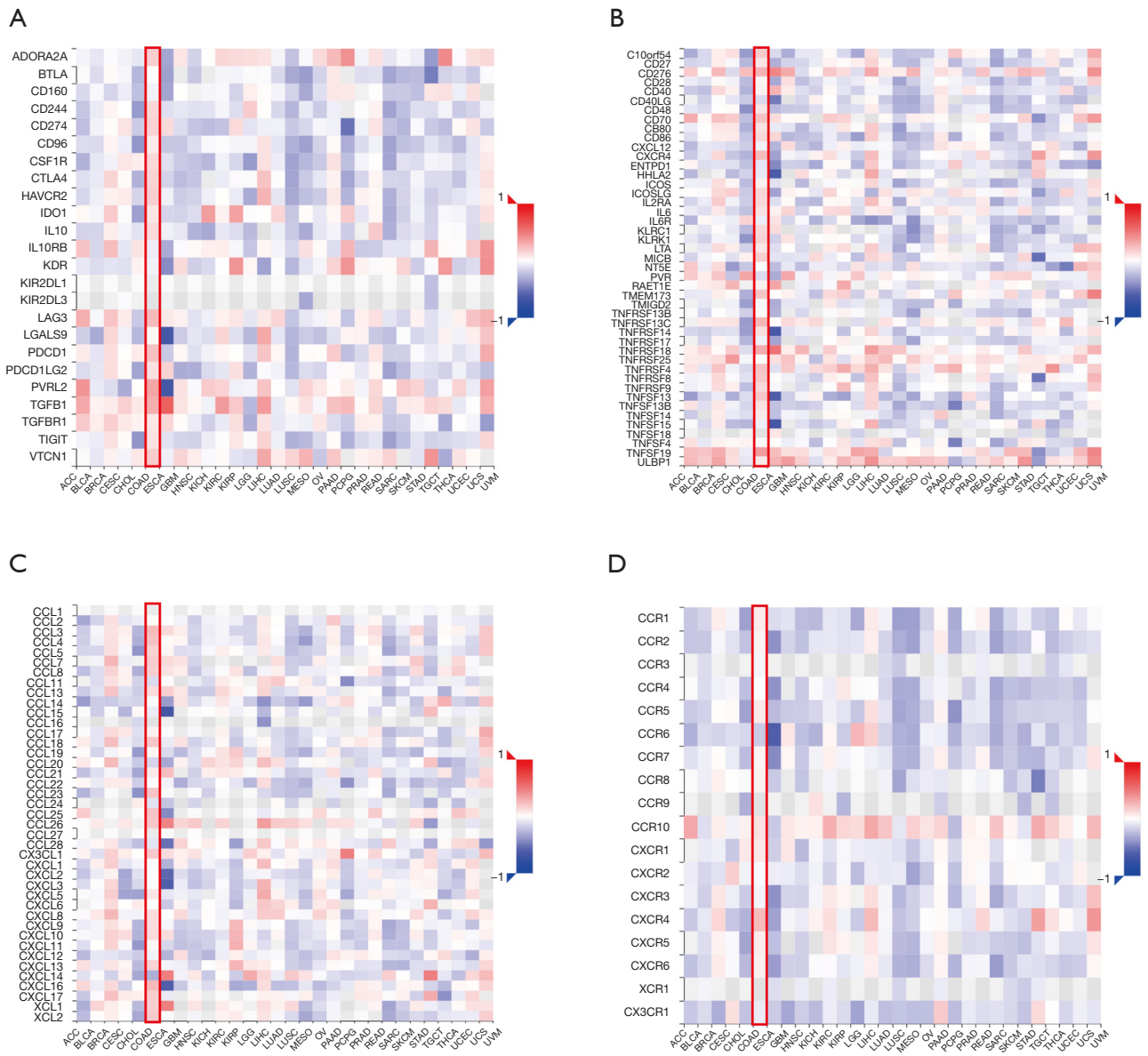
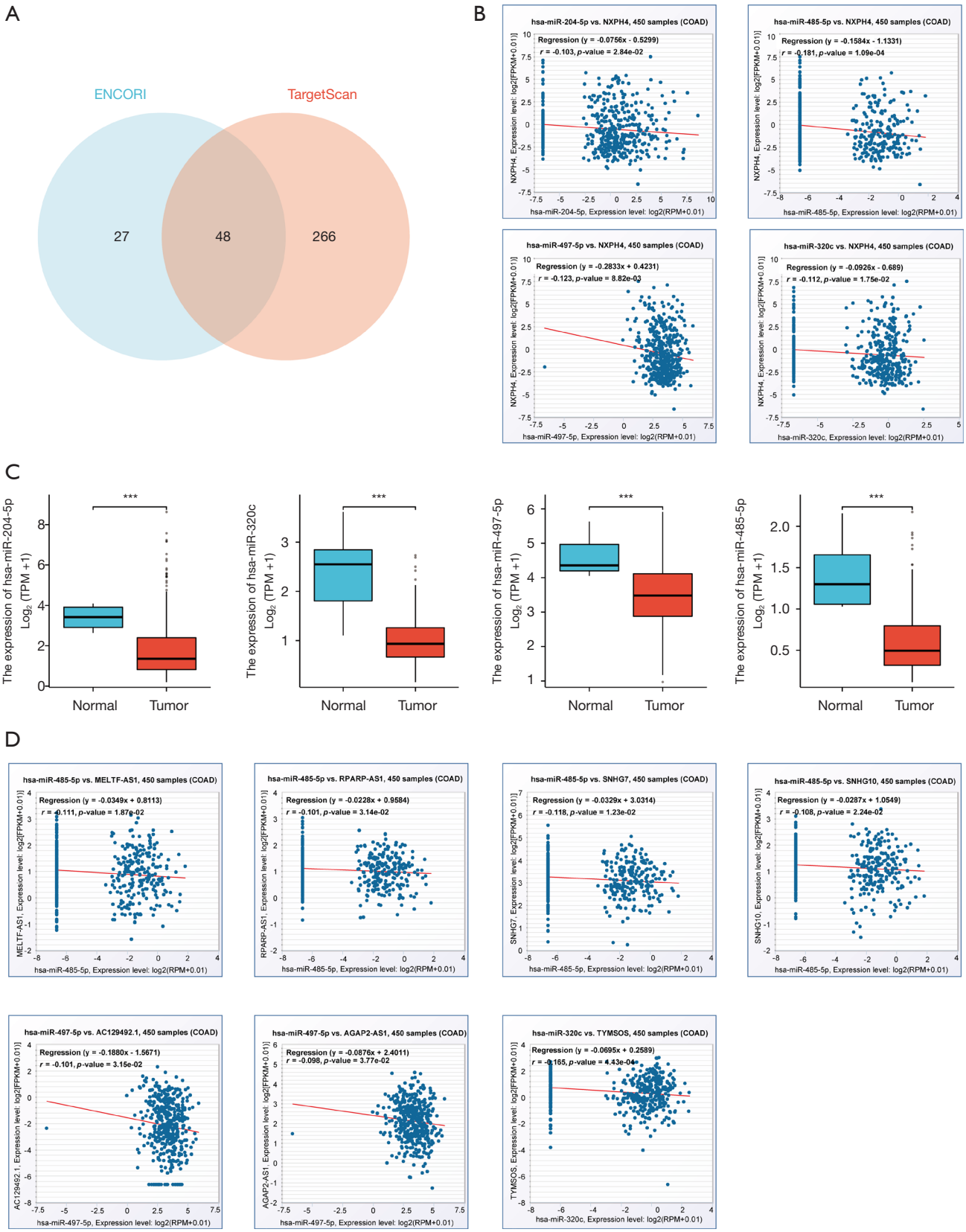


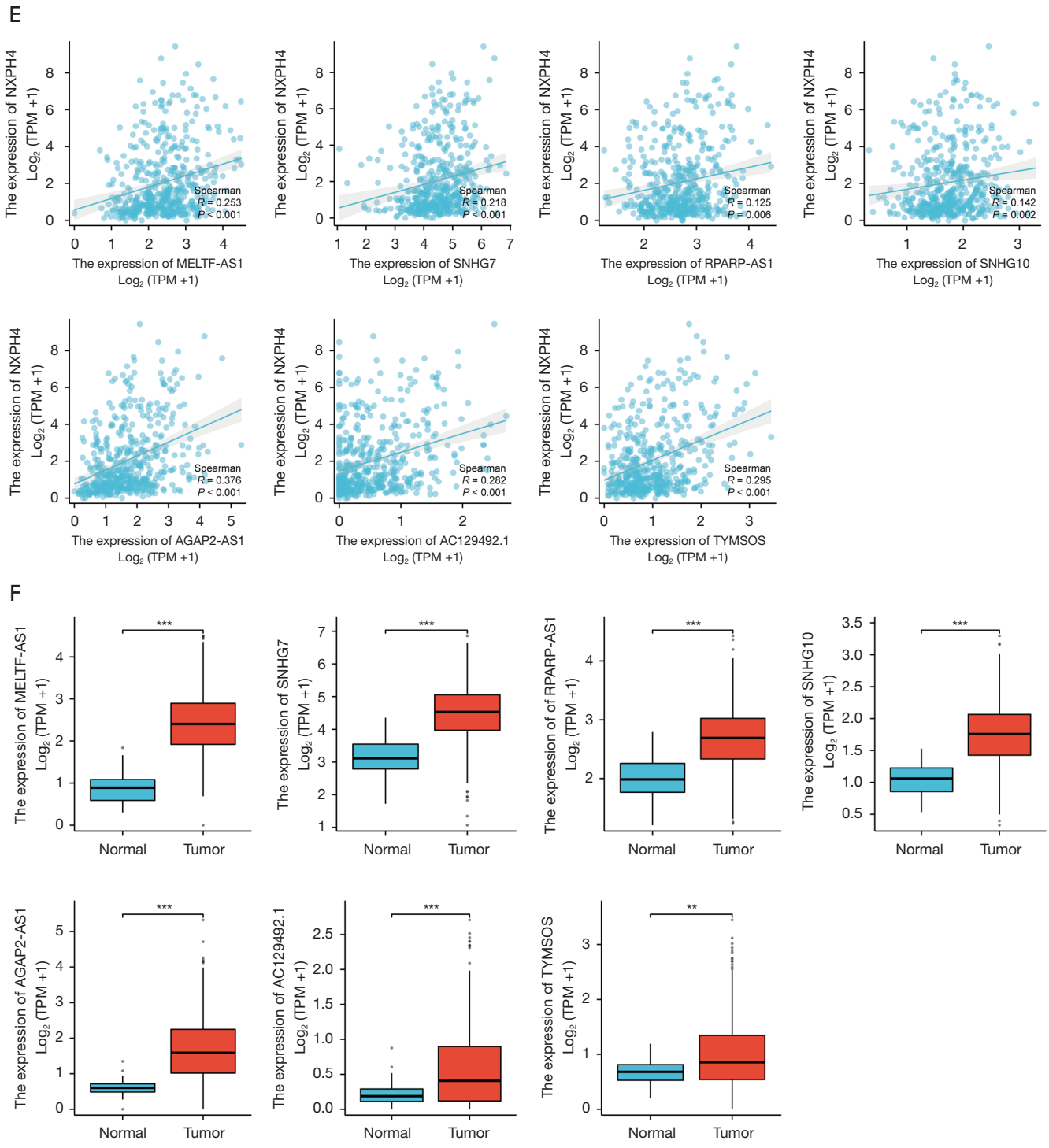
Figure 10 Correlation analysis of NXPH4 expression with immunomodulators, chemokines, and chemokine receptors. (A-D) Heatmap analysis of the correlation between NXPH4 and immunoinhibitors (A), immunostimulators (B), chemokines (C), and chemokine receptors (D) in COAD. NXPH4, neurexophilin 4; COAD, colon adenocarcinoma.

impact of crosstalk between NXPH4 and ncRNA partners. Therapeutically disrupting these ceRNA interactions could modulate oncogenic signaling.

Discussion

In this study, we performed a comprehensive *in silico* analysis of NXPH4 in COAD using multi-omics data. Our results reveal NXPH4 is significantly overexpressed in COAD compared to normal colon tissue. High NXPH4 expression associates with advanced tumor stage, unfavorable clinical features, and poorer survival outcomes. Furthermore, NXPH4 demonstrates excellent performance as both a prognostic biomarker and diagnostic indicator for COAD. Integrative analysis of NXPH4-associated genes and pathways implicates NXPH4 in regulating key processes





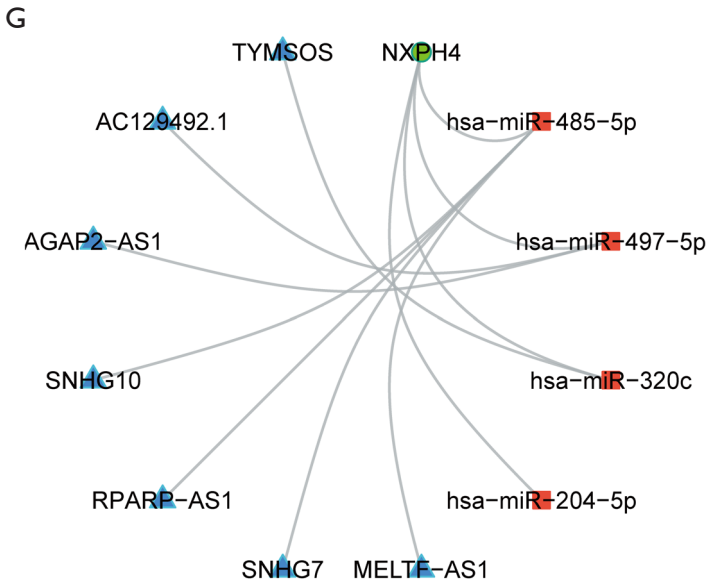


Figure 11 The ceRNA network of NXPH4 was predicted and constructed in COAD. (A) The Venn diagram displays the target miRNAs of NXPH4 predicted by ENCORI and TargetScan databases. (B) Correlation analysis between NXPH4 expression and hsa-miR-204-5p, hsa-miR-485-5p, hsa-miR-497-5p, and hsa-miR-320c. (C) Analysis of hsa-miR-204-5p, hsa-miR-485-5p, hsa-miR-497-5p, and hsa-miR-320c in tumor tissues and adjacent normal tissues in TCGA-COAD. (D) Correlation analysis between miRNAs (hsa-miR-485-5p, hsa-miR-497-5p, and hsa-miR-320c) and lncRNAs (MELTF-AS1, RPARP-AS1, SNHG7, SNHG10, AC129492.1, AGAP2-AS1, and TYMSOS) in COAD. (E) Correlation analysis between NXPH4 expression and MELTF-AS1, RPARP-AS1, SNHG7, SNHG10, AC129492.1, AGAP2-AS1, and TYMSOS in COAD. (F) Analysis of MELTF-AS1, RPARP-AS1, SNHG7, SNHG10, AC129492.1, AGAP2-AS1, and TYMSOS expression in tumor tissues and adjacent normal tissues in TCGA-COAD. (G) A ceRNA network based on NXPH4. **, P<0.01; ***, P<0.001. NXPH4, neurexophilin 4; COAD, colon adenocarcinoma; RPM, reads per million; FPKM, fragments per kilobase per million; TPM, transcripts per million; ceRNA, competing endogenous RNA; TCGA, The Cancer Genome Atlas; miRNAs, microRNAs; lncRNAs, long noncoding RNAs.

involved in colon cancer progression, including glycolysis, hypoxia response, immune evasion, and ceRNA crosstalk.

Our pan-cancer analysis of TCGA data with 33 cancers revealed that NXPH4 is broadly overexpressed across multiple tumor types including COAD, and associates with poor prognosis—providing initial evidence that it may have oncogenic functions. We validated that NXPH4 is significantly upregulated in COAD tumors compared to normal colon samples using both TCGA and independent GEO datasets. This tumor-specific overexpression implies NXPH4 upregulation is selected for during colon carcinogenesis and progression. Notably, high expression of NXPH4 was shown to be correlated with advanced T stage, lymphatic invasion, and certain aggressive histological subtypes, linking NXPH4 to clinically relevant disease features. Survival analyses further establish NXPH4 as an independent prognostic biomarker in COAD. Increased NXPH4 was shown to be associated with reduced OS, DSS, and PFI. Moreover, ROC curve analysis confirmed that NXPH4 can accurately discriminate between malignant and normal colon tissue. Together, these consistent associations between NXPH4 overexpression and tumor progression, survival outcomes, and diagnostic performance strongly support its clinical utility as a promising prognostic indicator and diagnostic biomarker for COAD.

To translate our findings into a clinical tool, we developed a prognostic nomogram by combining NXPH4 expression with conventional clinical parameters to predict 1-, 3- and 5-year OS in COAD patients. This multivariate model demonstrated improved accuracy compared to using

clinical features alone. The C-index and calibration curves confirm the nomogram has good discriminative ability and alignment between predicted and observed outcomes. Time-dependent ROC analysis also showed favorable performance for predicting survival probability at multiple time points post-diagnosis. Overall, integrating molecular data with clinical factors holds promise for better risk stratification and individualized decision making. Patients with high NXPH4 levels classified as high-risk by the nomogram may benefit from more aggressive therapy or increased surveillance to monitor for recurrence. With additional validation, this nomogram could assist clinicians in tailoring management plans based on the underlying molecular landscape of each patient's tumor.

Analysis of the top co-expressed genes with NXPH4 provides clues into its functional roles in COAD. For instance, NADH dehydrogenase 1 alpha subcomplex 4-like 2 (NDUFA4L2) was among the most positively correlated genes. This mitochondrial complex I inhibitor was identified to be overexpressed in colorectal cancer (CRC) tissues and that is associated with tumor progression and poor prognosis (35). A prior study showed that NXPH4 can enhance cancer cell proliferation and invasion by modulating NDUFA4L2 in bladder cancer (13). To the other co-expressed genes, the hydroxymethyltransferase 2 (SHMT2) was reported as key regulators in the serine metabolism pathway and is involved in colon cancer proliferation (36). Slow skeletal muscle troponin T (TNNT1) was reported to be a novel prognostic indicator in COAD, and can promote tumor progression by mediating epithelial-mesenchymal transition (EMT) process (37). Low expression of flavin mono-oxygenase enzyme 4 (FMO4) was reported to be an adverse biomarker for hepatocellular carcinoma (38). NHL repeat containing 3 (NHLRC3) was shown to inhibit the progression of colon cancer and can be regulated by DNMBP-AS1-miR-93-5p/17-5p (39). The coordinated overexpression of these genes with NXPH4 implies that they may be part of shared oncogenic programs driving tumor progression. In future studies, validating these expression correlations and experimentally perturbing the identified genes could help elucidate the key functional partners and signaling networks through which NXPH4 exerts its oncogenic activities.

Mapping the PPI network for NXPH4 revealed close connections with other NXPH family numbers, including NXPH1, NXPH2, and NXPH3. Intriguingly, NXPH1 and NXPH2 are highly expressed in pancreatic ductal adenocarcinoma and linked to higher carcinoma development (40). NXPH1 has also been identified as a novel biomarker for the prognosis of neuroblastoma (41), and for the diagnosis of low-grade breast cancer (42). Other NXPH4-related genes, such as NRXN1, NRXN2, NRXN3, LPHN1, and NXPE4 have been found to be involved in various human malignancies (43-47). The coordinated overexpression and physical interactions between NXPH4 and these binding partners imply that they may cooperate to drive oncogenic signaling. Dysregulation of neurexin-mediated cell adhesion and neuropeptide signaling is likely contributing to the tumor-promoting functions of NXPH4.

GO, KEGG, and GSEA analyses implied that NXPH4-associated DEGs play important roles including intracellular signaling transduction, inflammatory response, glycolysis, and hypoxia. Metabolic reprogramming is a hallmark of cancer, providing energy and macromolecules to support rapid proliferation (48). Increased aerobic glycolysis and adaptations to hypoxic stress are most common metabolic reprogramming events that facilitate glucose uptake and energy production (49). In this study, we discovered that NXPH4 was positively correlated with the expression of glycolysis- and hypoxia-related genes including B3GALT6, ENO2, and P4HA1. Hypoxia shapes the landscape of the TME that contributes to tumor plasticity and heterogeneity and promotes highly aggressive and metastatic phenotypes (50). A previous study also showed that NXPH4 expression can promote reactive oxygen species (ROS) and glycolysis activation through modulating NDUFA4L2 to enhance gemcitabine resistance in bladder cancer cells (13). Another recent study reported that knockdown of NXPH4 was shown to inhibit cell proliferation, migration, and glycolysis in CRC cells (51). While the specific mechanisms in COAD remain unclear, NXPH4 likely cooperates with these metabolic pathways to facilitate energy generation and oxygen adaptation. These results are consistent with previously reported studies (52-54), and targeting NXPH4-associated glycolysis and hypoxia response genes could offer new therapeutic approaches to disrupt the bioenergetics and stress tolerance pathways critical for colon cancer progression.

Emerging evidence highlights the role of immune cell infiltration in the development, metastasis, and antitumor therapies of COAD (55). NXPH4 has been reported to play a role for the immune infiltration in bladder cancer (11) and hepatocellular carcinoma (14). Our analysis revealed that upregulated NXPH4 expression associates with increased infiltration of various immune cell types including NK cells, NK CD56bright cells, cytotoxic cells, macrophages,

aDC cells, Th1 cells, neutrophils, and Treg cells in COAD. NXPH4 overexpression also correlates with heightened expression of immune checkpoint genes including PDCD1, LAG3, and CTLA4. This immunoprofile implies that NXPH4 may promote an immunosuppressive microenvironment by recruiting pro-tumoral immune cells while upregulating signals that impair anti-tumor immunity. Therefore, targeting the NXPH4-mediated effects on the tumor immune landscape could potentially shift the balance from an immune-evasive to an immune-active phenotype, highlighting the nexus between NXPH4 and tumor immunology as a promising area for future exploration.

Chemokines and their receptors are critical for orchestrating immune cell infiltration and modulating the TME in COAD (56). We found that NXPH4 expression correlates with levels of various pro-tumoral chemokines like CCL3, CCL4, CCL18, CCL23, as well as key receptors such as CXCR4. CCL3 is involved in tumor immunity through its role in recruitment of T-/B-lymphocytes, dendritic cells and natural killer cells (57). A recent study also demonstrated that CCL3-CCR5 axis promotes cell migration and invasion of COAD via Akt signaling pathway (58). CCL4 was reported to be upregulated in CRC tissues compared with in normal tissue, correlated with disease stage (59). CCL18 can act as an independent prognostic biomarker (60), and promote Treg recruitment (61) in CRC. High CCL23 expression induces T-cell exhaustion in high-grade serous ovarian cancer (HGSC) patients (62). Colon cancer cells with high expression of CXCR4 exhibit metastatic potential and predict poor prognosis (63). The coordinated overexpression of these immune cell trafficking modulators with NXPH4 implies that they may cooperate to shape an immunosuppressive, pro-metastatic TME.

Noncoding RNAs (ncRNAs) including miRNAs and lncRNAs play important regulatory roles in cancer through diverse mechanisms (64,65). Here, we identified miRNAs (hsa-miR-204-5p, hsa-miR-485-5p, hsa-miR-497-5p, and hsa-miR-320c) and lncRNAs (MELTF-AS1, RPARP-AS1, SNHG7, SNHG10, AC129492.1, AGAP2-AS1, and TYMSOS) as candidate upstream regulators of NXPH4 in COAD. These findings are consistent with earlier studies that these specific ncRNAs contribute to the ceRNA regulatory networks in different tumors (66-76). By integrating expression correlation, target prediction, and functional annotation, we constructed a candidate NXPH4-centered ceRNA network in COAD. These results provide a roadmap to explore whether directly targeting these ncRNA-NXPH4 interactions could provide a new approach to disrupt oncogenic signaling. Therapeutically restoring tumor suppressive miRNAs or inhibiting oncogenic lncRNAs may achieve similar outcomes as directly suppressing NXPH4. Overall, our ceRNA analysis offers fresh insight into the post-transcriptional regulatory landscape enabling NXPH4 overexpression in colon cancer. Nevertheless, future studies are required to validate the potential molecular mechanisms and upstream ncRNA network of NXPH4 in COAD.

Our present study has revealed new findings about NXPH4 in COAD. However, certain limitations should be noted. Firstly, though retrospective data from multiple databases has been utilized, it is necessary to carry out prospective validation studies in order to determine the clinical significance of NXPH4 in COAD. Furthermore, the limited availability of detailed treatment data hindered the evaluation of the correlation between NXPH4 expression and the therapeutic effects of chemotherapy and radiation therapy. Finally, in order to obtain a comprehensive understanding of the biological function and potential molecular mechanisms of NXPH4 in COAD, it is crucial to conduct additional fundamental experiments both *in vitro* and *in vivo*.

Conclusions

In summary, our study establishes NXPH4 as a candidate diagnostic and prognostic biomarker for COAD and mediator of aggressive tumor phenotypes. This is the first comprehensive investigation of the relation between NXPH4 expression and glycolysis, hypoxia, immune infiltration, and ceRNA regulatory network in COAD.

Acknowledgments

Funding: This work was supported by the National Key R&D Program of China (No. 2018YFA0901702); the National Science Foundation of Shandong (No. ZR2022MC057); the Foundation of Hubei Province Supporting Enterprise Technology Innovation Development (No. 2021BAB126); and Wuhan East Lake High-tech Zone "JieBangGuaShuai" Project (No. 2022KJB113).

Footnote

Reporting Checklist: The authors have completed the TRIPOD reporting checklist. Available at https://jgo.

amegroups.com/article/view/10.21037/jgo-23-956/rc

Peer Review File: Available at https://jgo.amegroups.com/article/view/10.21037/jgo-23-956/prf

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://jgo.amegroups.com/article/view/10.21037/jgo-23-956/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

References

1. Siegel RL, Miller KD, Wagle NS, et al. Cancer statistics, 2023. CA Cancer J Clin 2023;73:17-48.
2. Siegel RL, Wagle NS, Cercek A, et al. Colorectal cancer statistics, 2023. CA Cancer J Clin 2023;73:233-54.
3. Bando H, Ohtsu A, Yoshino T. Therapeutic landscape and future direction of metastatic colorectal cancer. Nat Rev Gastroenterol Hepatol 2023;20:306-22.
4. Sveen A, Kopetz S, Lothe RA. Biomarker-guided therapy for colorectal cancer: strength in complexity. Nat Rev Clin Oncol 2020;17:11-32.
5. Xie YH, Chen YX, Fang JY. Comprehensive review of targeted therapy for colorectal cancer. Signal Transduct Target Ther 2020;5:22.
6. Sun J, Zhao J, Jiang F, et al. Identification of novel protein biomarkers and drug targets for colorectal cancer by integrating human plasma proteome with genome. Genome Med 2023;15:75.
7. Missler M, Südhof TC. Neurexophilins form a conserved family of neuropeptide-like glycoproteins. J Neurosci 1998;18:3630-8.
8. Petrenko AG, Ullrich B, Missler M, et al. Structure and evolution of neurexophilin. J Neurosci 1996;16:4360-9.
9. Wilson SC, White KI, Zhou Q, et al. Structures of neurexophilin-neurexin complexes reveal a regulatory mechanism of alternative splicing. EMBO J 2019;38:e101603.
10. Meng X, McGraw CM, Wang W, et al. Neurexophilin4 is a selectively expressed α-neurexin ligand that modulates specific cerebellar synapses and motor functions. Elife 2019;8:e46773.
11. Sun X, Xin S, Jin L, et al. Neurexophilin 4 is a prognostic biomarker correlated with immune infiltration in bladder cancer. Bioengineered 2022;13:13986-99.
12. Gui Z, Ying X, Liu C. NXPH4 Used as a New Prognostic and Immunotherapeutic Marker for Muscle-Invasive Bladder Cancer. J Oncol 2022;2022:4271409.
13. Wang D, Zhang P, Liu Z, et al. NXPH4 Promotes Gemcitabine Resistance in Bladder Cancer by Enhancing Reactive Oxygen Species and Glycolysis Activation through Modulating NDUFA4L2. Cancers (Basel) 2022;14:3782.
14. Tang Q, Chen YM, Shen MM, et al. Increased Expression of NXPH4 Correlates with Immune Cell Infiltration and Unfavorable Prognosis in Hepatocellular Carcinoma. J Oncol 2022;2022:5005747.
15. Eun JW, Jang JW, Yang HD, et al. Serum Proteins, HMMR, NXPH4, PITX1 and THBS4; A Panel of Biomarkers for Early Diagnosis of Hepatocellular Carcinoma. J Clin Med 2022;11:2128.
16. Yang Z, Wei B, Qiao A, et al. A novel EZH2/NXPH4/CDKN2A axis is involved in regulating the proliferation and migration of non-small cell lung cancer cells. Biosci Biotechnol Biochem 2022;86:340-50.
17. Tomczak K, Czerwińska P, Wiznerowicz M. The Cancer Genome Atlas (TCGA): an immeasurable source of knowledge. Contemp Oncol (Pozn) 2015;19:A68-77.
18. An N, Shi X, Zhang Y, et al. Discovery of a Novel Immune Gene Signature with Profound Prognostic Value in Colorectal Cancer: A Model of Cooperativity Disorientation Created in the Process from Development to Cancer. PLoS One 2015;10:e0137171.
19. Solé X, Crous-Bou M, Cordero D, et al. Discovery and validation of new potential biomarkers for early detection of colon cancer. PLoS One 2014;9:e106748.
20. Barrett T, Wilhite SE, Ledoux P, et al. NCBI GEO: archive for functional genomics data sets--update. Nucleic Acids Res 2013;41:D991-5.

21. Győrffy B. Discovery and ranking of the most robust prognostic biomarkers in serous ovarian cancer. Geroscience 2023;45:1889-98.
22. Liu J, Lichtenberg T, Hoadley KA, et al. An Integrated TCGA Pan-Cancer Clinical Data Resource to Drive High-Quality Survival Outcome Analytics. Cell 2018;173:400-416.e11.
23. Blanche P, Dartigues JF, Jacqmin-Gadda H. Estimating and comparing time-dependent areas under receiver operating characteristic curves for censored event times with competing risks. Stat Med 2013;32:5381-97.
24. Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol 2014;15:550.
25. Yu G, Wang LG, Han Y, et al. clusterProfiler: an R package for comparing biological themes among gene clusters. OMICS 2012;16:284-7.
26. Warde-Farley D, Donaldson SL, Comes O, et al. The GeneMANIA prediction server: biological network integration for gene prioritization and predicting gene function. Nucleic Acids Res 2010;38:W214-20.
27. Szklarczyk D, Kirsch R, Koutrouli M, et al. The STRING database in 2023: protein-protein association networks and functional enrichment analyses for any sequenced genome of interest. Nucleic Acids Res 2023;51:D638-46.
28. Hänzelmann S, Castelo R, Guinney J. GSVA: gene set variation analysis for microarray and RNA-seq data. BMC Bioinformatics 2013;14:7.
29. Bindea G, Mlecnik B, Tosolini M, et al. Spatiotemporal dynamics of intratumoral immune cells reveal the immune landscape in human cancer. Immunity 2013;39:782-95.
30. Ru B, Wong CN, Tong Y, et al. TISIDB: an integrated repository portal for tumor-immune system interactions. Bioinformatics 2019;35:4200-2.
31. Li JH, Liu S, Zhou H, et al. starBase v2.0: decoding miRNA-ceRNA, miRNA-ncRNA and protein-RNA interaction networks from large-scale CLIP-Seq data. Nucleic Acids Res 2014;42:D92-7.
32. McGeary SE, Lin KS, Shi CY, et al. The biochemical basis of microRNA targeting efficacy. Science 2019;366:eaav1741.
33. Gomez AM, Traunmüller L, Scheiffele P. Neurexins: molecular codes for shaping neuronal synapses. Nat Rev Neurosci 2021;22:137-51.
34. Tay Y, Rinn J, Pandolfi PP. The multilayered complexity of ceRNA crosstalk and competition. Nature 2014;505:344-52.
35. Lv Y, Nie SL, Zhou JM, et al. Overexpression of NDUFA4L2 is associated with poor prognosis in patients with colorectal cancer. ANZ J Surg 2017;87:E251-5.
36. Lin C, Zhang Y, Chen Y, et al. Long noncoding RNA LINC01234 promotes serine hydroxymethyltransferase 2 expression and proliferation by competitively binding miR-642a-5p in colon cancer. Cell Death Dis 2019;10:137.
37. Hao YH, Yu SY, Tu RS, et al. TNNT1, a prognostic indicator in colon adenocarcinoma, regulates cell behaviors and mediates EMT process. Biosci Biotechnol Biochem 2020;84:111-7.
38. Luo Y, Chen AN, Fu JT, et al. FMO4 shapes immuno-metabolic reconfiguration in hepatocellular carcinoma. Clin Transl Med 2022;12:e740.
39. Yang L, Yang T, Wang H, et al. DNMBP-AS1 Regulates NHLRC3 Expression by Sponging miR-93-5p/17-5p to Inhibit Colon Cancer Progression. Front Oncol 2022;12:765163.
40. Jin JS, Tsai WC. The detection of tumor location and lymph node metastasis by aberrant NXPH1 and NXPH2 expressions in pancreatic ductal adenocarcinomas. Chin J Physiol 2016;59:348-54.
41. Xia Y, Li X, Tian X, et al. Identification of a Five-Gene Signature Derived From MYCN Amplification and Establishment of a Nomogram for Predicting the Prognosis of Neuroblastoma. Front Mol Biosci 2021;8:769661.
42. Faryna M, Konermann C, Aulmann S, et al. Genome-wide methylation screen in low-grade breast cancer identifies novel epigenetically altered genes as potential biomarkers for tumor diagnosis. FASEB J 2012;26:4937-50.
43. Alkhathami AG, Verma AK, Alfaifi M, et al. Role of miRNA-495 and NRXN-1 and CNTN-1 mRNA Expression and Its Prognostic Importance in Breast Cancer Patients. J Oncol 2021;2021:9657071.
44. Zhu S, Lin F, Chen Z, et al. Identification of a Twelve-Gene Signature and Establishment of a Prognostic Nomogram Predicting Overall Survival for Medulloblastoma. Front Genet 2020;11:563882.
45. Zhao Y, Hong XH, Li K, et al. ZNF582 hypermethylation promotes metastasis of nasopharyngeal carcinoma by regulating the transcription of adhesion molecules Nectin-3 and NRXN3. Cancer Commun (Lond) 2020;40:721-37.
46. Kocibalova Z, Guzyova M, Borovska I, et al. Development of Multidrug Resistance in Acute Myeloid Leukemia Is Associated with Alterations of the LPHN1/GAL-9/TIM-3 Signaling Pathway. Cancers (Basel) 2021;13:3629.
47. Liu YR, Hu Y, Zeng Y, et al. Neurexophilin and PC-

esterase domain family member 4 (NXPE4) and prostate androgen-regulated mucin-like protein 1 (PARM1) as prognostic biomarkers for colorectal cancer. J Cell Biochem 2019;120:18041-52.
48. Yang J, Shay C, Saba NF, et al. Cancer metabolism and carcinogenesis. Exp Hematol Oncol 2024;13:10.
49. Chelakkot C, Chelakkot VS, Shin Y, et al. Modulating Glycolysis to Improve Cancer Therapy. Int J Mol Sci 2023;24:2606.
50. Liao C, Liu X, Zhang C, et al. Tumor hypoxia: From basic knowledge to therapeutic implications. Semin Cancer Biol 2023;88:172-86.
51. Fan Q, He W, Shang Y. Forkhead box protein K1 regulated neurexophilin 4 promotes proliferation, metastasis and glycolysis in colorectal cancer. Exp Ther Med 2023;26:434.
52. Wu ZY, He YQ, Wang TM, et al. Glycogenes in Oncofetal Chondroitin Sulfate Biosynthesis are Differently Expressed and Correlated With Immune Response in Placenta and Colorectal Cancer. Front Cell Dev Biol 2021;9:763875.
53. Pan X, Wu H, Chen G, et al. Prognostic Value of Enolase Gene Family in Colon Cancer. Med Sci Monit 2020;26:e922980.
54. Chen Z, Chen M, Xue Z, et al. Comprehensive Analysis of Gene Expression Profiles Identifies a P4HA1-Related Gene Panel as a Prognostic Model in Colorectal Cancer Patients. Cancer Biother Radiopharm 2021;36:693-704.
55. Chandra R, Karalis JD, Liu C, et al. The Colorectal Cancer Tumor Microenvironment and Its Impact on Liver and Lung Metastasis. Cancers (Basel) 2021;13:6206.
56. Jia SN, Han YB, Yang R, et al. Chemokines in colon cancer progression. Semin Cancer Biol 2022;86:400-7.
57. Allen F, Rauhe P, Askew D, et al. CCL3 Enhances Antitumor Immune Priming in the Lymph Node via IFNγ with Dependency on Natural Killer Cells. Front Immunol 2017;8:1390.
58. Guan B, Li H, Yao J, et al. CCL3-CCR5 axis promotes cell migration and invasion of colon adenocarcinoma via Akt signaling pathway. Environ Toxicol 2023;38:172-84.
59. Shamoun L, Landerholm K, Balboa Ramilo A, et al. Association of gene and protein expression and genetic polymorphism of CC chemokine ligand 4 in colorectal cancer. World J Gastroenterol 2021;27:5076-87.
60. Yuan R, Chen Y, He X, et al. CCL18 as an independent favorable prognostic biomarker in patients with colorectal cancer. J Surg Res 2013;183:163-9.
61. Sun Z, Du C, Xu P, et al. Surgical trauma-induced CCL18 promotes recruitment of regulatory T cells and colon cancer progression. J Cell Physiol 2019;234:4608-16.
62. Kamat K, Krishnan V, Dorigo O. Macrophage-derived CCL23 upregulates expression of T-cell exhaustion markers in ovarian cancer. Br J Cancer 2022;127:1026-33.
63. Zhang SS, Han ZP, Jing YY, et al. CD133(+)CXCR4(+) colon cancer cells exhibit metastatic potential and predict poor prognosis of patients. BMC Med 2012;10:85.
64. Eptaminitaki GC, Stellas D, Bonavida B, et al. Long non-coding RNAs (lncRNAs) signaling in cancer chemoresistance: From prediction to druggability. Drug Resist Updat 2022;65:100866.
65. Wang H, Meng Q, Qian J, et al. Review: RNA-based diagnostic markers discovery and therapeutic targets development in cancer. Pharmacol Ther 2022;234:108123.
66. Gao W, Wu Y, He X, et al. MicroRNA-204-5p inhibits invasion and metastasis of laryngeal squamous cell carcinoma by suppressing forkhead box C1. J Cancer 2017;8:2356-68.
67. Peng Y, Leng W, Duan S, et al. Long noncoding RNA BLACAT1 is overexpressed in hepatocellular carcinoma and its downregulation suppressed cancer cell development through endogenously competing against hsa-miR-485-5p. Biomed Pharmacother 2019;116:109027.
68. Tian LL, Qian B, Jiang XH, et al. MicroRNA-497-5p Is Downregulated in Hepatocellular Carcinoma and Associated with Tumorigenesis and Poor Prognosis in Patients. Int J Genomics 2021;2021:6670390.
69. Chen H, Shi X, Ren L, et al. Identification of the miRNA-mRNA regulatory network associated with radiosensitivity in esophageal cancer based on integrative analysis of the TCGA and GEO data. BMC Med Genomics 2022;15:249.
70. Lu X, Wang J, Wang W, et al. Copy number amplification and SP1-activated lncRNA MELTF-AS1 regulates tumorigenesis by driving phase separation of YBX1 to activate ANXA8 in non-small cell lung cancer. Oncogene 2022;41:3222-38.
71. Ren Y, Zhao C, He Y, et al. RPARP-AS1/miR125a-5p Axis Promotes Cell Proliferation, Migration and Invasion in Colon Cancer. Onco Targets Ther 2021;14:5035-43.
72. Cheng D, Fan J, Ma Y, et al. LncRNA SNHG7 promotes pancreatic cancer proliferation through ID4 by sponging miR-342-3p. Cell Biosci 2019;9:28.
73. Zhang H, Fang Z, Guo Y, et al. Long noncoding RNA SNHG10 promotes colorectal cancer cells malignant progression by targeting miR-3690. Bioengineered 2021;12:6010-20.

74. Chen W, Deng J, Zhou Y. The construction of a novel ferroptosis-related lncRNA model to predict prognosis in colorectal cancer patients. Medicine (Baltimore) 2023;102:e33114.
75. Hui B, Ji H, Xu Y, et al. RREB1-induced upregulation of the lncRNA AGAP2-AS1 regulates the proliferation and migration of pancreatic cancer partly through suppressing ANKRD1 and ANGPTL4. Cell Death Dis 2019;10:207.
76. Yuan Y, Jiang X, Tang L, et al. FOXM1/lncRNA TYMSOS/miR-214-3p-Mediated High Expression of NCAPG Correlates With Poor Prognosis and Cell Proliferation in Non-Small Cell Lung Carcinoma. Front Mol Biosci 2022;8:785767.

Cite this article as: Sun Z, Wang H, Xu Y, Liu Y, Wang L, Zhou R, Zhou R, Ma W, Zhang T. High expression of NXPH4 correlates with poor prognosis, metabolic reprogramming, and immune infiltration in colon adenocarcinoma. J Gastrointest Oncol 2024;15(2):641-667. doi: 10.21037/jgo-23-956

