ISSN 1948-5204 (online)

World Journal of *Gastrointestinal Oncology*

World J Gastrointest Oncol 2024 July 15; 16(7): 2867-3367

Published by Baishideng Publishing Group Inc

World Journal of Gastrointestinal Oncology

Contents

Monthly Volume 16 Number 7 July 15, 2024

EDITORIAL

REVIEW

MINIREVIEWS

ORIGINAL ARTICLE

Case Control Study

Retrospective Study

World Journal of Gastrointestinal Oncology

Monthly Volume 16 Number 7 July 15, 2024

Contents

World Journal of Gastrointestinal Oncology

Monthly Volume 16 Number 7 July 15, 2024

Contents

World Journal of Gastrointestinal Oncology

Monthly Volume 16 Number 7 July 15, 2024

Contents

ABOUT COVER

Editorial Board Member of *World Journal of Gastrointestinal Oncology*, Meysam Ebrahimifar, MSc, PhD, Research Assistant Professor, Department of Toxicology, Islamic Azad University, Isfahan 1477893855, Iran. ebrahimifar67@gmail.com

AIMS AND SCOPE

The primary aim of *World Journal of Gastrointestinal Oncology* (*WJGO*, *World J Gastrointest Oncol*) is to provide scholars and readers from various fields of gastrointestinal oncology with a platform to publish high-quality basic and clinical research articles and communicate their research findings online.

WJGO mainly publishes articles reporting research results and findings obtained in the field of gastrointestinal oncology and covering a wide range of topics including liver cell adenoma, gastric neoplasms, appendiceal neoplasms, biliary tract neoplasms, hepatocellular carcinoma, pancreatic carcinoma, cecal neoplasms, colonic neoplasms, colorectal neoplasms, duodenal neoplasms, esophageal neoplasms, gallbladder neoplasms, *etc*.

INDEXING/ABSTRACTING

The *WJGO* is now abstracted and indexed in PubMed, PubMed Central, Science Citation Index Expanded (SCIE, also known as SciSearch®), Journal Citation Reports/Science Edition, Scopus, Reference Citation Analysis, China Science and Technology Journal Database, and Superstar Journals Database. The 2024 edition of Journal Citation Reports® cites the 2023 journal impact factor (JIF) for *WJGO* as 2.5; JIF without journal self cites: 2.5; 5-year JIF: 2.8; JIF Rank: 71/143 in gastroenterology and hepatology; JIF Quartile: Q2; and 5-year JIF Quartile: Q2. The *WJGO*'s CiteScore for 2023 is 4.2 and Scopus CiteScore rank 2023: Gastroenterology is 80/167; Oncology is 196/404.

RESPONSIBLE EDITORS FOR THIS ISSUE

NAME OF JOURNAL
World Journal of Gastrointestinal Oncology

ISSN
ISSN 1948-5204 (online)

LAUNCH DATE
February 15, 2009

FREQUENCY
Monthly

EDITORS-IN-CHIEF
Monjur Ahmed, Florin Burada

EDITORIAL BOARD MEMBERS
https://www.wjgnet.com/1948-5204/editorialboard.htm

PUBLICATION DATE
July 15, 2024

INSTRUCTIONS TO AUTHORS
https://www.wjgnet.com/bpg/gerinfo/204

GUIDELINES FOR ETHICS DOCUMENTS
https://www.wjgnet.com/bpg/GerInfo/287

GUIDELINES FOR NON-NATIVE SPEAKERS OF ENGLISH
https://www.wjgnet.com/bpg/gerinfo/240

PUBLICATION ETHICS
https://www.wjgnet.com/bpg/GerInfo/288

PUBLICATION MISCONDUCT
https://www.wjgnet.com/bpg/gerinfo/208

ARTICLE PROCESSING CHARGE
https://www.wjgnet.com/bpg/gerinfo/242

STEPS FOR SUBMITTING MANUSCRIPTS
https://www.wjgnet.com/bpg/GerInfo/239

ONLINE SUBMISSION
https://www.f6publishing.com

World Journal of Gastrointestinal Oncology

Submit a Manuscript: https://www.f6publishing.com

World J Gastrointest Oncol 2024 July 15; 16(7): 3284-3298

DOI: 10.4251/wjgo.v16.i7.3284

ISSN 1948-5204 (online)

ORIGINAL ARTICLE

Basic Study

Canopy FGF signaling regulator 3 affects prognosis, immune infiltration, and PI3K/AKT pathway in colon adenocarcinoma

Xu-Can Gao, Biao-Huan Zhou, Zhou-Xin Ji, Qiang Li, Hui-Ning Liu

Specialty type: Oncology

Provenance and peer review: Unsolicited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's classification

Scientific Quality: Grade A, Grade A

Novelty: Grade A, Grade A

Creativity or Innovation: Grade A, Grade A

Scientific Significance: Grade A, Grade B

P-Reviewer: Basson MD, United States

Received: March 23, 2024

Revised: May 14, 2024

Accepted: June 4, 2024

Published online: July 15, 2024

Processing time: 111 Days and 4.4 Hours

Xu-Can Gao, Biao-Huan Zhou, Zhou-Xin Ji, Qiang Li, Hui-Ning Liu, Department of Anorectal Surgery, Shenzhen People's Hospital (The Second Clinical Medical College, Jinan University, The First Affiliated Hospital, Southern University of Science and Technology), Shenzhen 518020, Guangdong Province, China

Corresponding author: Xu-Can Gao, MD, Doctor, Department of Anorectal Surgery, Shenzhen People's Hospital (The Second Clinical Medical College, Jinan University, The First Affiliated Hospital, Southern University of Science and Technology), No. 1017 Dongmen North Road, Cuizhu Street, Luohu District, Shenzhen 518020, Guangdong Province, China.
logia@szhospital.com

Abstract

BACKGROUND

Colon adenocarcinoma (COAD) is a malignant tumor of the digestive system. The mechanisms underlying COAD development and progression are still largely unknown.

AIM

To identify the role of canopy FGF signaling regulator 3 (CNPY3) in the development and progression of COAD by using bioinformatic tools and functional experiments.

METHODS

Bioinformatic data were downloaded from public databases. The associations of clinicopathological features, survival, and immune function with the expression of CNPY3 were analyzed. Gene Ontology and Kyoto Encyclopedia of Genes and Genomes analyses and Gene Set Enrichment Analysis were used to explore the related pathways. Then, quantitative real-time PCR and immunohistochemistry were used for validation of CNPY3 expression in clinical samples and tumor cell lines. Cell lines with CNPY3 knockdown were constructed to further analyze gene functions. The functional experiments included proliferation, invasion, migration and apoptosis assays.

RESULTS

In both the TCGA cohort and the merged dataset, elevated CNPY3 expression was observed in tumor tissues. High CNPY3 expression correlated with adverse survival and compromised immune functions. Functional enrichment analysis suggested that the pro-oncogenic properties of CNPY3 might be linked to the

PI3K-AKT signaling pathway. CNPY3 expression was validated at both the RNA and protein levels. Functional assays indicated that cell proliferation, invasion, and migration were inhibited and cell apoptosis was promoted after CNPY3 knockdown. Additionally, Western blot results revealed the downregulation of key proteins in the PI3K/AKT pathway following CNPY3 knockdown. PI3K/AKT pathway activator reversed the decrease in proliferation, invasion, and migration and the increase in apoptosis. Notably, CNPY3 knockdown still affected the cells when the pathway was inhibited.

CONCLUSION

This study showed that CNPY3 is upregulated in COAD and might regulate COAD development and progression by the PI3K/AKT pathway. Thus, CNPY3 might be a promising therapeutic target.

Key Words: Canopy FGF signaling regulator 3; Colon cancer; Biomarker; Immune infiltration; Prognosis

Core Tip: First, this is the first study reporting that canopy FGF signaling regulator 3 (CNPY3) was up-regulated in colon adenocarcinoma and might regulate tumor development and progression. Second, CNPY3 expression was validated in clinical samples and tumor cell lines. Third, functional experiments indicated that cell proliferation, invasion, and migration were inhibited and cell apoptosis was promoted after CNPY3 knockdown.

Citation: Gao XC, Zhou BH, Ji ZX, Li Q, Liu HN. Canopy FGF signaling regulator 3 affects prognosis, immune infiltration, and PI3K/AKT pathway in colon adenocarcinoma. *World J Gastrointest Oncol* 2024; 16(7): 3284-3298
URL: https://www.wjgnet.com/1948-5204/full/v16/i7/3284.htm
DOI: https://dx.doi.org/10.4251/wjgo.v16.i7.3284

INTRODUCTION

Colon cancer is a malignant tumor affecting the digestive system[1,2]. In 2020, a total of 1.9 million cases of colon cancer and 0.9 million colon cancer-related deaths were reported worldwide[3]. This statistic highlights the pervasive nature of the disease and its major impact on global health. Furthermore, the prevalence and fatality rates of colon cancer continue to increase, presenting a major public health challenge[4]. Considerable progress has been made in increasing the survival rates, particularly in the field of immunotherapy. Immunotherapy is a promising treatment method that harnesses the body's immune functions and immune responses to effectively combat and eliminate cancer cells[5]. For example, the success of pembrolizumab and other programmed cell death 1 (PD-1) inhibitors in treating colon cancer underscores the importance of immunotherapy as a valuable addition to the treatment armamentarium for this disease[6]. However, the 5-year survival of this disease is low[7], and immune therapy does not work for some patients because of resistance to therapy and treatment intolerance[8]. Thus, exploring new treatment targets is necessary to expand colon cancer therapeutics.

The development of bioinformatic tools has shed new light on colon cancer research. Ding *et al*[9] reported that metabolism-related genes could augment the immunotherapeutic response and that CYP19A1 could promote vascular abnormalities and inhibit $CD8^+$ T-cell functions. The combined use of CYP19A1 inhibition and PD-1 blockade exhibits promising therapeutic potential. A study conducted by Bao *et al*[10] revealed an important finding regarding YTHDF1 and its association with immune-related biomarkers in colorectal cancer (CRC). Specifically, the researchers reported that targeting YTHDF1 promotes anti-PD-1 efficacy and augments antitumor immunity. Sorrentino *et al*[11] analyzed the Cancer Genome Atlas (TCGA)-CRC data and reported the immune cell context in CRCs, which provided insight into overcoming immunotherapy resistance. However, our understanding of tumor immunity, biomarkers, and the underlying mechanisms is still limited[12,13]. Thus, further studies are still needed to identify more precise biomarkers that can increase the treatment efficacy of colon cancer treatment.

This study focused on colon adenocarcinoma (COAD), which is the most prevalent and malignant form of colon cancer. Through bioinformatic analysis, we found abnormal expression of canopy FGF signaling regulator 3 (CNPY3) in COAD. The function of CNPY3 has been implicated in various cellular processes, including tumor development. Notably, this protein is localized both within the endoplasmic reticulum and extracellular space, which underscores its versatility in modulating diverse signaling pathways[14]. The pro-oncogenic nature of this protein has been noted in hepatocellular carcinoma and gastric cancer[15]. Although the tumor-promoting role of CNPY3 has been implicated in various cancers, its specific involvement in COAD remains unknown. Hence, this study aimed to elucidate the function of CNPY3 in COAD using bioinformatic tools and functional experiments, in order to provide a reference for clinical treatment.

MATERIALS AND METHODS

Data download and annotation

Bioinformatic analyses were performed based on the following databases: The Genotype Tissue Expression (GTEx), the Gene Expression Omnibus (GEO), and TCGA databases. The following datasets were used: GTEx-colon cohort (GTEx database, 308 normal samples), GSE17536 cohort (GEO database, 177 tumor samples), GSE17537 cohort (GEO database, 55 tumor samples), and TCGA-COAD cohort (TCGA database, 41 normal and 471 tumor samples). All datasets were annotated by gene symbols.

Gene expression analysis

The expression data of CNPY3 among various cancers were acquired by accessing the TIMER database[16]. To expand the number of normal samples, the sequencing data from TCGA and GTEx cohorts were matched and batch-corrected. Then, CNPY3 gene expression was detected in two datasets: The merged dataset (349 normal and 471 tumor samples) and the TCGA-COAD dataset. Furthermore, the connections between clinical features (age, sex, and tumor-node-metastasis stage) and CNPY3 expression were analyzed using the nonparametric test.

Prognostic analysis

Probabilities of survival, including overall survival (OS) and disease-specific survival (DSS), were evaluated. Receiver operating characteristic (ROC) curve analysis was performed to determine the prognostic value of CNPY3 expression. Prediction accuracy was determined by the area under curve (AUC). Possible prognostic factors were identified using univariate prognostic analysis. Then, multivariate analysis was performed to adjust confounding variables and identify independent prognostic factors.

Immune correlation analysis

Immune microenvironment-related indices were calculated with the help of the ESTIMATE algorithm[17]. The indices include stromal, immune, and ESTIMATE scores. The abundance of immune cells was calculated using the CIBERSORT algorithm[18] and the differences between groups were calculated. Correlations between CNPY3 and immune cells were calculated by Pearson correlation analysis and visualized in the Lollipop plot. Immune therapy-related factors, including 65 immune checkpoint-related factors, 43 immunostimulant-related factors, and 22 immunosuppressant-related factors, were acquired from previous studies. The relationship between CNPY3 and immune therapy-related factors was tested by Spearman's correlation analysis. Immunotherapy scores were used to assess the likelihood of a favorable therapeutic response to immunotherapy interventions. The scores were obtained from the Cancer Immunome Atlas database.

Functional enrichment analysis

The genes co-expressed with CNPY3 were identified with the "limma" package. Functional annotation was performed based on the Gene Ontology (GO) terms. Pathway enrichment analysis was conducted using terms from the Kyoto Encyclopedia of Genes and Genomes (KEGG) and Reactome (obtained from Molecular Signatures Database)[19]. Gene Set Enrichment Analysis (GSEA) was carried out to identify pathways positively and negatively associated with CNPY3 expression.

Clinical sample collection

The clinical sample collection process was performed after the approval of the local Ethics Committee. Eight normal people and thirty-two COAD patients were enrolled. All patients included in this study did not receive radiotherapy or chemotherapy before resection. The samples for quantitative real-time PCR (qRT-PCR) were immediately frozen at -80 °C, and the samples for immunohistochemistry were stored in 4% paraformaldehyde.

qRT-PCR

TRIzol was used for isolating RNA from samples. Briefly, the TRIzol method includes homogenization of the sample in TRIzol reagent to disrupt cells and release RNA, followed by chloroform extraction to separate RNA from DNA and proteins. On the 7900HT Sequence Detection System (ABI), qRT-PCR was performed. The procedure involved a denaturation program (95 °C, 10 min) and 45 amplification and quantification cycles, with each cycle lasting 15 s at 95 °C and 34 s at 60 °C. Primer sequences include: *CNPY3*: Forward, CGG AGC TGA GGA GAA CGA C and reverse, ATA GCC CGT GCC AAT CAC C; *GAPDH*: Forward, GGA CCT GAC CTG CCG TCT AG and reverse, GTA GCC CAG GAT GCC CTT GA. The *CNPY3* expression level was determined using the $2^{-\Delta\Delta CT}$ method.

Immunohistochemistry

Immunohistochemistry was performed on a tissue microarray according to standard protocols. Antibodies used include rabbit anti-CNPY3 (Proteintech, 1:200) and HRP-conjugated rabbit secondary antibodies. The tissue section was dewaxed, repaired, blocked, and subsequently incubated with the primary antibody at 4 °C. The sections were then exposed to HRP-conjugated rabbit secondary antibody for 1 h and subsequently incubated with the DAB substrate solution to obtain brown immunoreactive signals. The immunohistochemistry results were evaluated by two pathologists independently and the immunohistochemical score (IHS) was calculated for the evaluation of staining intensity and quantity.

Cell culture and transfection

The following cell lines were used: Human colonic epithelial cell lines (HCoEpiC and NCM460) and colon cancer cell lines (Caco-2, HCT-116, HT-29, SW48, SW480, and SW620). Cell lines were sourced from the American Type Culture Collection or the Chinese Academy of Science Cell Bank. All cells were cultured in Dulbecco's modified Eagle's medium with 10% fetal calf serum. CNPY3 knockdown vectors were constructed, and lentivirus was packaged by GenePharma company (Shanghai, China). The cells were grouped into CNPY3-Con and CNPY3-knockdown groups based on treatment. Cell transfections were performed as described by the manufacturer[20]. Wherever mentioned, the cells were treated with 20 μmol/L 740Y-P (PI3K/AKT pathway activator) or 20 μmol/L LY294002 (PI3K/AKT pathway inhibitor) for 24 h.

Cell proliferation assay

The proliferation abilities of HT-29 and SW620 were evaluated by CCK-8 assay. A 96-well plate was used for cell culture and 2000 cells were seeded into each well. Following cell seeding, the plate was incubated under standard cell culture conditions. The cells were allowed to adhere to the culture plate and proliferate over the specified time intervals. The CCK-8 solution was added at predetermined time points (24, 48, 72, and 96 h post-incubation) and the absorbance values were measured.

Transwell assay

Transwell device, which consists of two chambers separated by an 8 mm positron emission tomography semi-permeable membrane, was used. For the migration assay, medium without serum was added to the upper chamber, while the lower chamber was supplemented with medium with 20% serum. The cells were allowed to attach to the membrane and migrate to the lower chamber for 48 h. After the incubation period, cells migrating to the lower chamber were fixed (methyl) and stained (crystal violet). The Matrigel-coated membrane was used in the invasion assay to mimic the natural environment that cells encounter in tissues.

Cell apoptosis assay

Apoptosis analysis was conducted by flow cytometry. The cells were centrifuged, rinsed with phosphate buffered saline for 3 times, and labeled with the FITC-labeled Annexin V and propidium iodide solution. The FACSCalibur flow cytometry system from BD Biosciences was used to identify apoptotic cells.

Western blot analysis

Antibodies used are as follows: Anti-CNPY3 (Proteintech, 1:2000), anti-PI3K (Affinity Biosciences, 1:1000), anti-p-PI3K (Affinity Biosciences, 1:1000), anti-AKT (Cell Signaling Technology, 1:1000), anti-p-AKT (Cell Signaling Technology, 1:000), anti-GAPDH (Cell Signaling Technology, 1:2000), and HRP-conjugated secondary antibodies (Cell Signaling Technology, 1:2000). The experiment was performed following routine experimental steps[21]. Briefly, proteins from tissue samples and cells were released using a lysis buffer. After centrifugation, the BCA method (KeyGEN BioTECH) was used to measure the protein concentration. Equal proteins were separated using 10% SDS-PAGE and a nitrocellulose membrane was used for protein transfer. Then, the membrane was blocked with milk and treated with primary and secondary antibodies. The immunoblots were developed using New Super ECL.

Statistical analysis

R (4.2.1) and SPSS (26.0) were used for all bioinformatic and statistical analyses. The Student's *t*-test was used to evaluate differences in continuous data, and the χ^2 test was used to test differences in count data. *P*-values less than 0.05 were considered significant.

RESULTS

Gene expression analysis

CNPY3 exhibited significant up-regulation in several digestive system cancers, including COAD, cholangiocarcinoma, esophageal carcinoma, liver hepatocellular carcinoma, rectum adenocarcinoma, and stomach adenocarcinoma (Figure 1A). The expression of CNPY3 in COAD is shown in Figure 1B and C. CNPY3 was highly expressed in COAD based on the TCGA and merged datasets. A heatmap of clinical relevance is shown in Figure 1D. There was no difference in CNPY3 expression regarding age (Supplementary Figure 1A) and gender (Supplementary Figure 1B). Furthermore, higher CNPY3 expression was correlated with increased T stage (Figure 1E), increased M stage (Figure 1F), and increased N stage (Figure 1G).

Prognostic analysis

Kaplan-Meier curve analysis revealed that elevated CNPY3 expression correlated with decreased OS (Figure 2A) and DSS (Figure 2B). The AUC of CNPY3 expression for predicting the prognosis was 0.755 in TCGA-COAD (Figure 2C). Validation using the GSE17536 dataset yielded similar results (Figure 2D and E); the AUC was 0.687 (Figure 2F). Similar results were also acquired using GSE17537 (Figure 2G and H), and the AUC was 0.736 (Figure 2I), indicating the good predictive ability of CNPY3 expression for prognosis of COAD. Univariate regression analysis (Figure 2J) identified CNPY3, age, M stage, N stage, and T stage as significant prognostic factors for COAD and they were subsequently

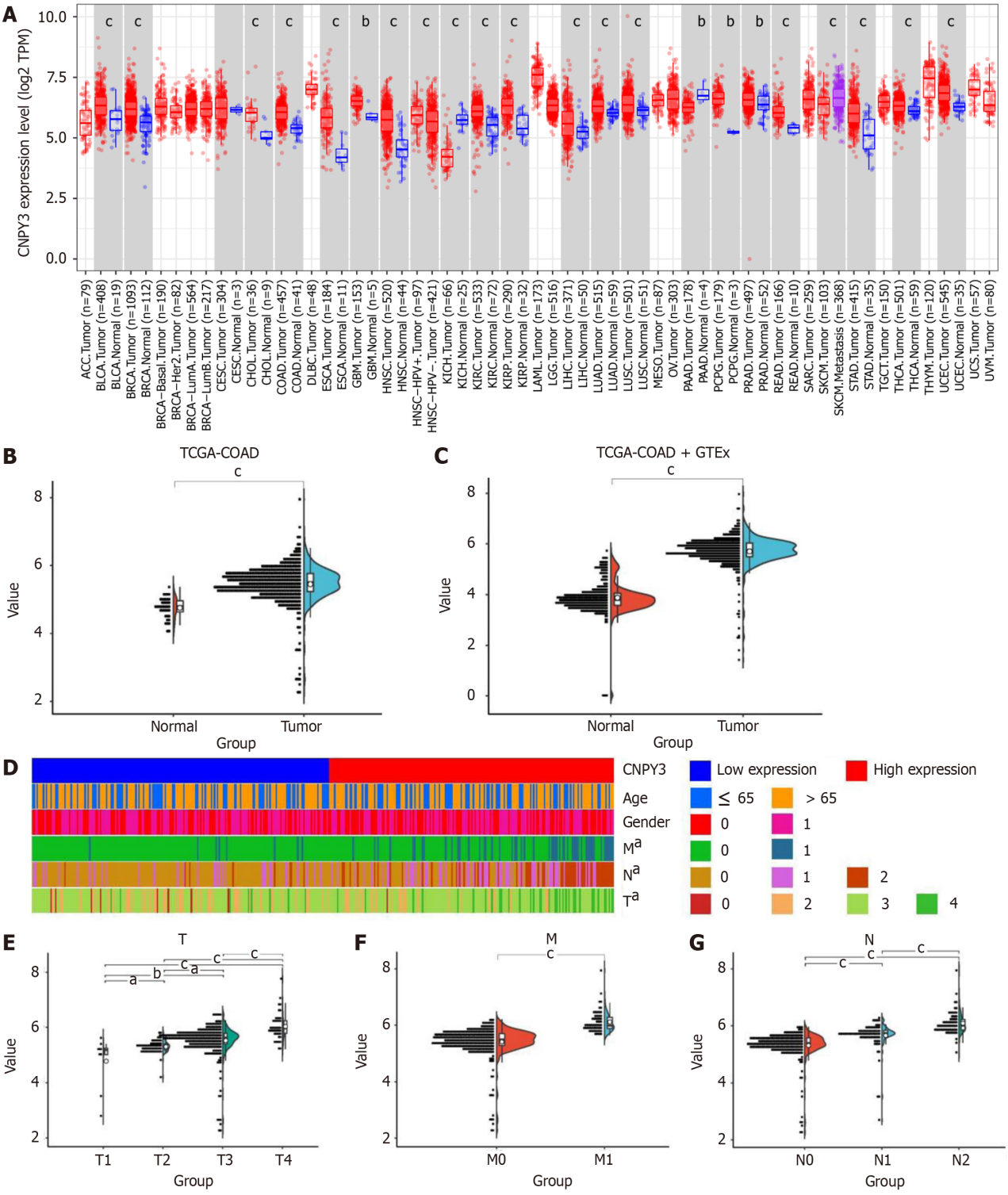


Figure 1 Expression levels of canopy FGF signaling regulator 3 in pan-cancer and Cancer Genome Atlas colon adenocarcinoma cohort. A: Canopy FGF signaling regulator 3 (CNPY3) was highly expressed in several digestive system cancers; B: CNPY3 was highly expressed in Cancer Genome Atlas (TCGA)-colon adenocarcinoma cohort; C: CNPY3 was highly expressed in merged dataset (Genotype Tissue Expression normal, TCGA normal, and TCGA cancer); D: Heatmap of associations between CNPY3 expression and clinicopathological features; E: Correlation between CNPY3 expression and T stage. F: Correlation between CNPY3 expression and M stage; G: Correlation between CNPY3 expression and N stage. $^{a}P < 0.05$; $^{b}P < 0.01$, $^{c}P < 0.001$. CNPY3: Canopy FGF signaling regulator 3; TCGA: The Cancer Genome Atlas; COAD: Colon adenocarcinoma; GTEx: Genotype Tissue Expression.

included in the multivariate regression analysis, which demonstrated that CNPY3 ($P < 0.05$) was an independent prognostic factor in COAD (Figure 2K).

Immune correlation analysis

Immune microenvironment-related indexes were calculated using the EXTIMATE algorithm. Figure 3A indicates that the groups with high CNPY3 expression tended to have lower stomal, immune, and ESTIMATE scores. The difference in

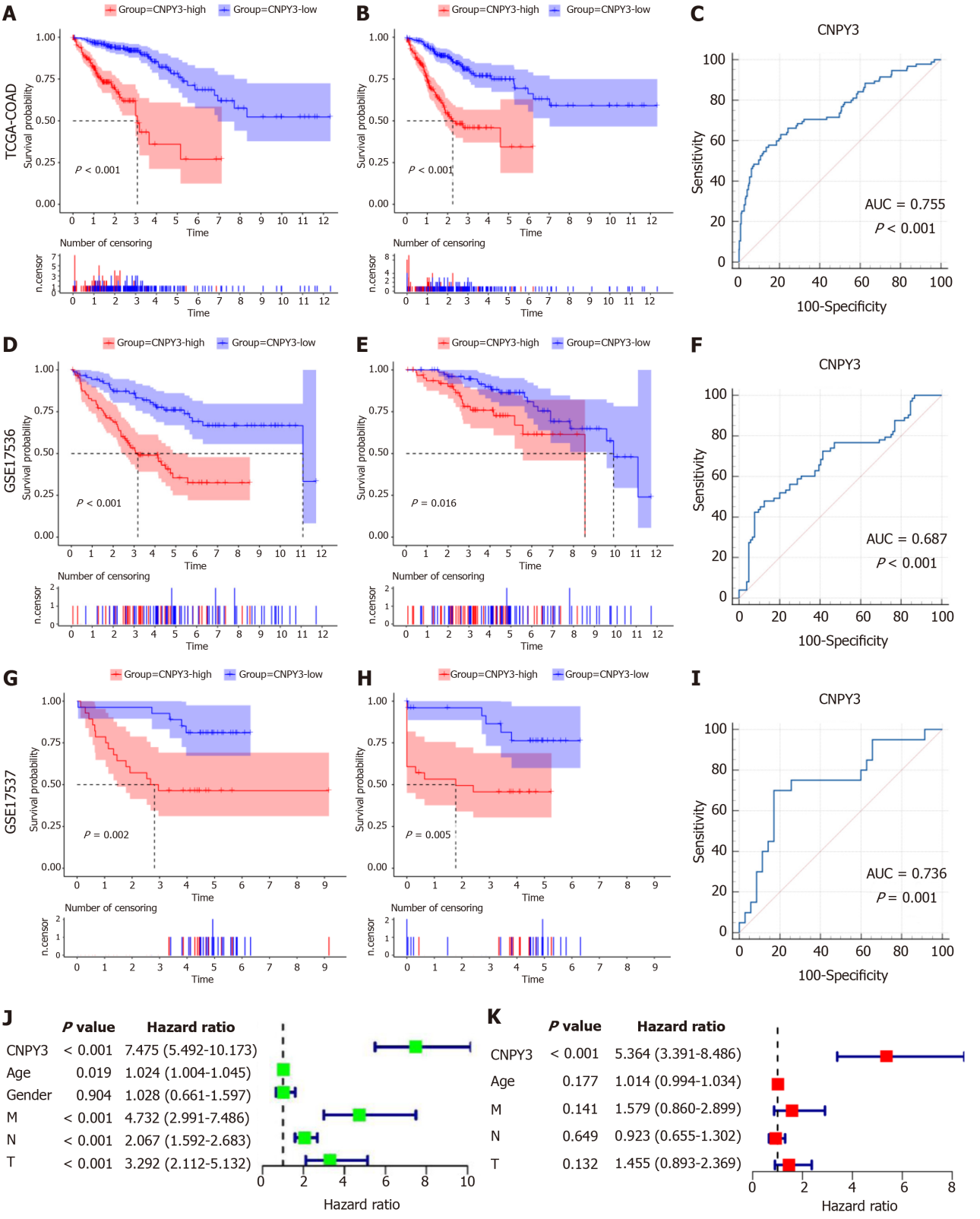
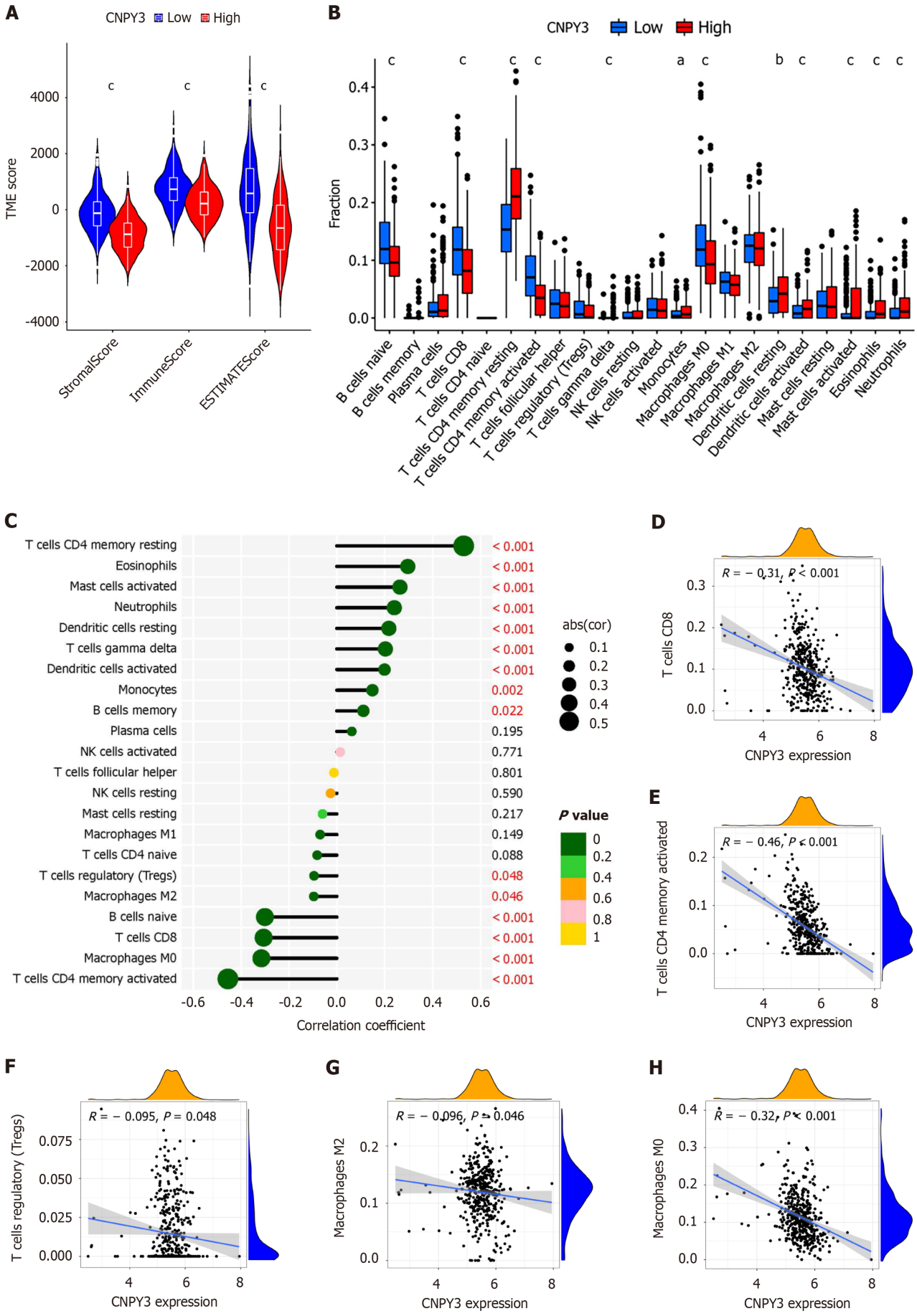


Figure 2 Prognostic analysis of canopy FGF signaling regulator 3. A: Overall survival (OS) curve of Cancer Genome Atlas (TCGA)-colon adenocarcinoma (COAD) cohort; B: Progression-free survival (PFS) curve of TCGA-COAD cohort; C: Receiver operating characteristic (ROC) curve of TCGA-COAD cohort [area under curve (AUC) = 0.755]; D: OS curve of GSE17536 cohort; E: PFS curve of GSE17536 cohort; F: ROC curve of GSE17536 cohort (AUC = 0.687); G: OS curve of GSE17537 cohort; H: PFS curve of GSE17537 cohort; I: ROC curve of GSE17537 cohort (AUC = 0.736); J: Univariate regression analysis; K: Multivariate regression analysis. CNPY3: Canopy FGF signaling regulator 3; TCGA: The Cancer Genome Atlas; COAD: Colon adenocarcinoma; GTEx: Genotype Tissue Expression.


A
CNPY3 Low High
TME score
StromalScore
ImmuneScore
ESTIMATEScore
B
CNPY3 Low High
Fraction
B cells naive
B cells memory
Plasma cells
T cells CD8
T cells CD4 naive
T cells CD4 memory resting
T cells CD4 memory activated
T cells follicular helper
T cells regulatory (Tregs)
T cells gamma delta
NK cells resting
NK cells activated
Monocytes
Macrophages M0
Macrophages M1
Macrophages M2
Dendritic cells resting
Dendritic cells activated
Mast cells resting
Mast cells activated
Eosinophils
Neutrophils
C
T cells CD4 memory resting < 0.001
Eosinophils < 0.001
Mast cells activated < 0.001
Neutrophils < 0.001
Dendritic cells resting < 0.001
T cells gamma delta < 0.001
Dendritic cells activated < 0.001
Monocytes 0.002
B cells memory 0.022
Plasma cells 0.195
NK cells activated 0.771
T cells follicular helper 0.801
NK cells resting 0.590
Mast cells resting 0.217
Macrophages M1 0.149
T cells CD4 naive 0.088
T cells regulatory (Tregs) 0.048
Macrophages M2 0.046
B cells naive < 0.001
T cells CD8 < 0.001
Macrophages M0 < 0.001
T cells CD4 memory activated < 0.001
Correlation coefficient
abs(cor)
P value
D
R = − 0.31, P < 0.001
T cells CD8
CNPY3 expression
E
R = − 0.46, P < 0.001
T cells CD4 memory activated
CNPY3 expression
F
R = − 0.095, P = 0.048
T cells regulatory (Tregs)
CNPY3 expression
G
R = − 0.096, P = 0.046
Macrophages M2
CNPY3 expression
H
R = − 0.32, P < 0.001
Macrophages M0
CNPY3 expression

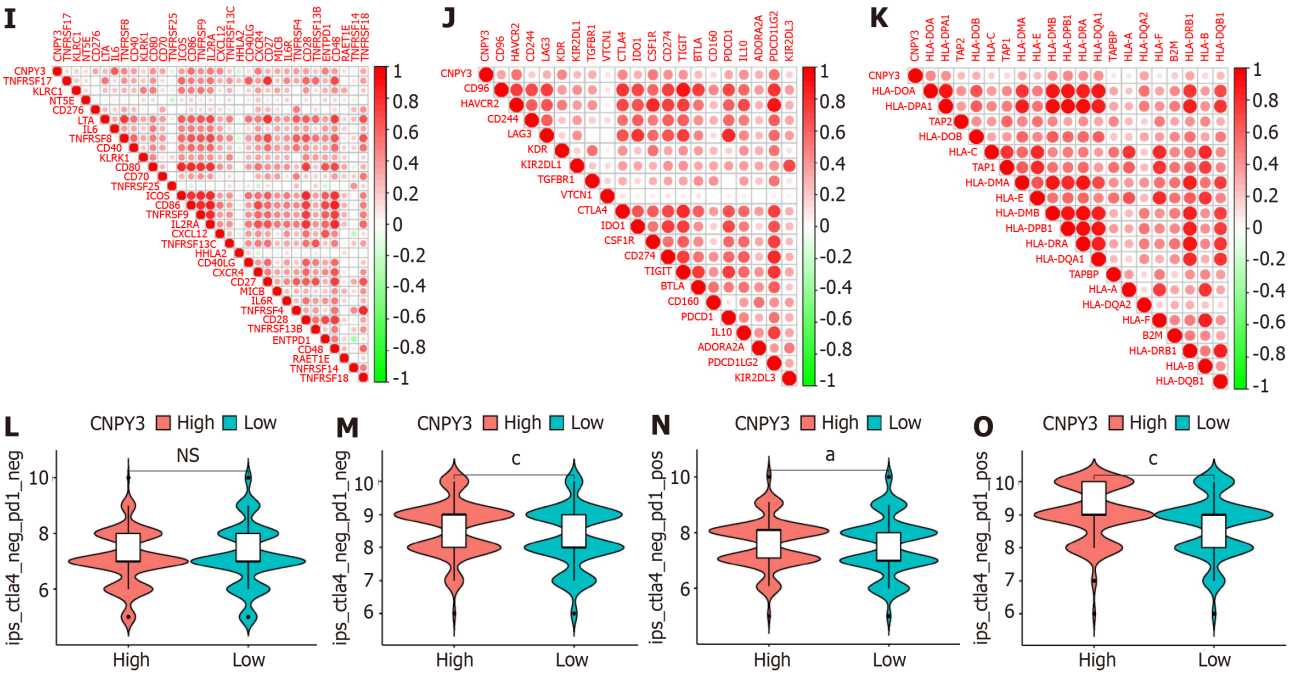


Figure 3 Immune correlation analysis. A: Differences in immune microenvironment-related indexes (stomal, immune, and ESTIMATE scores); B: Differences in immune cell infiltration; C: Correlations between canopy FGF signaling regulator 3 (CNPY3) expression and immune cell infiltration; D-H: Correlations between CNPY3 expression and activated CD8$^+$ T cells (D), CD4$^+$ T cells (E), regulatory T cells (F), M2 macrophages (G), and M0 macrophages (H); I-K: Correlations between CNPY3 expression and expression of immunostimulatory factors (I), immune inhibitors (J), and MHC molecules (K); L-O: Patient response to immune checkpoint inhibitors. $^aP < 0.05$; $^bP < 0.01$, $^cP < 0.001$, NS: Not significant; CNPY3: Canopy FGF signaling regulator 3.

immune cells is shown in Figure 3B. Increased expression of CNPY3 decreased the proportion of naive B cells, activated CD4$^+$ T cells, CD8$^+$ T cells, and M0 macrophages. Figure 3C shows the results of the correlation analysis. Specifically, several immune therapy-related cell types were negatively related to CNPY3 expression, including CD8$^+$ T cells (Figure 3D), CD4$^+$ T cells (Figure 3E), regulatory T cells (Figure 3F), M2 macrophages (Figure 3G), and M0 macrophages (Figure 3H).

Next, the correlations between CNPY3 expression and immune checkpoints were analyzed. Within the TCGA-COAD cohort, CNPY3 expression was significantly correlated with 32 out of 43 immunostimulatory factors (Figure 3I), 20 out of 22 immunoinhibitors (Figure 3J), and 20 out of 21 MHC molecules (Figure 3K). Notably, CNPY3 was positively correlated with PD-1, CTLA4, and CD274, which are vital factors influencing immunotherapy efficacy. Then, the immunotherapy scores were compared. Patients in the high CNPY3 expression group might benefit more from immunotherapy when either programmed death ligand 1 (PD-L1) or CTLA4 was positively expressed (Figure 3L-O).

Functional enrichment analysis

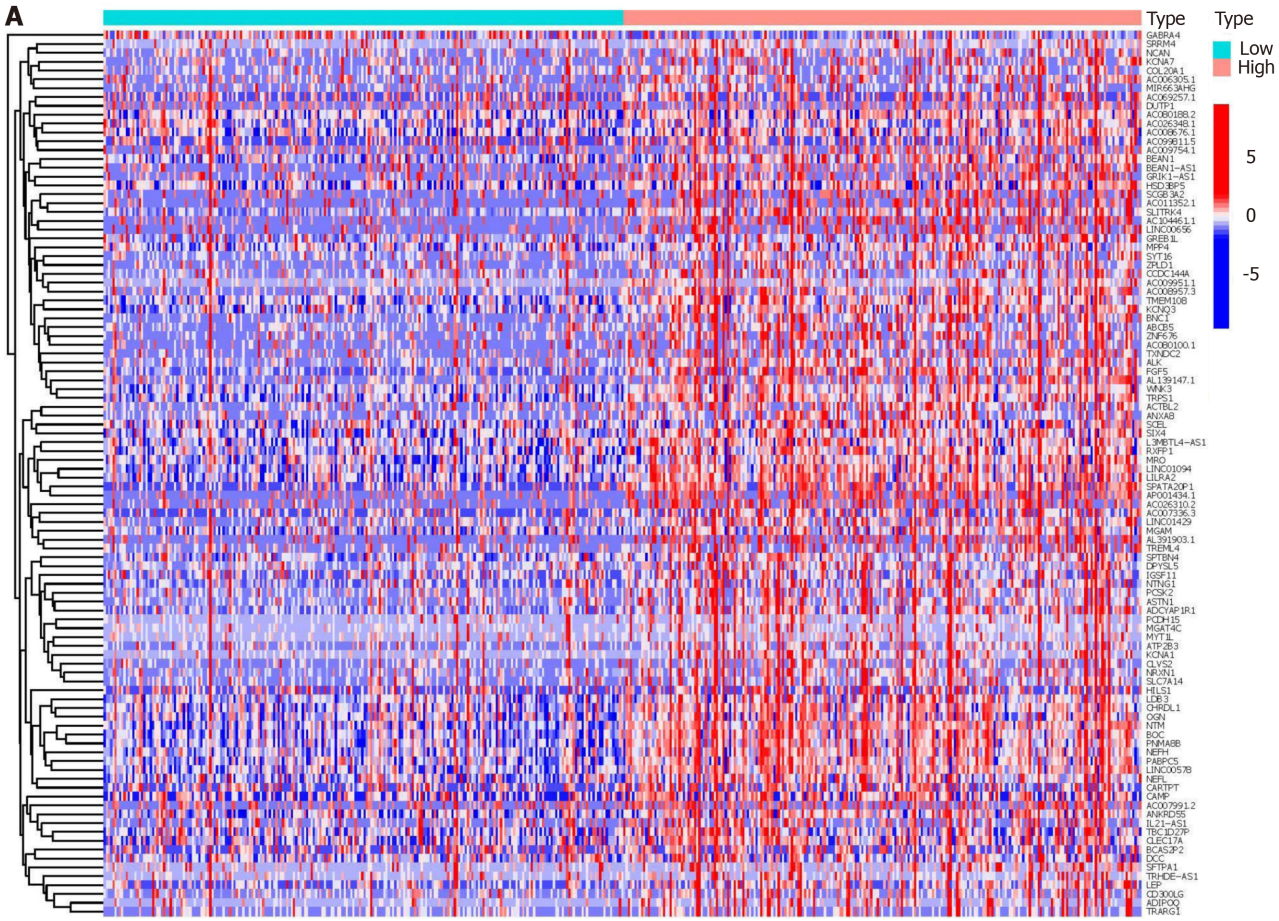
Based on the threshold (log |fold change| > 1 and *P* value < 0.05), 464 DEGs were identified. The top 50 DEGs are shown in Figure 4A. GO analysis (Figure 4B) showed that the top 3 biological process terms are external encapsulating structure, external structure, and extracellular matrix organization. The top 3 molecular function terms are extracellular matrix structural constituents, glycosaminoglycan binding, and actin binding. The top 3 cellular component terms are collagen-containing extracellular matrix, endoplasmic reticulum lumen, and cell-substrate junction. In the KEGG pathway analysis, the PI3K-AKT signaling pathway was the most enriched. Using the GSEA method, CNPY3 was found to be related to multiple cancer-related pathways, such as aggrephagy, TP53 regulating metabolic genes, and the mTOR signaling pathway (Figure 4C).

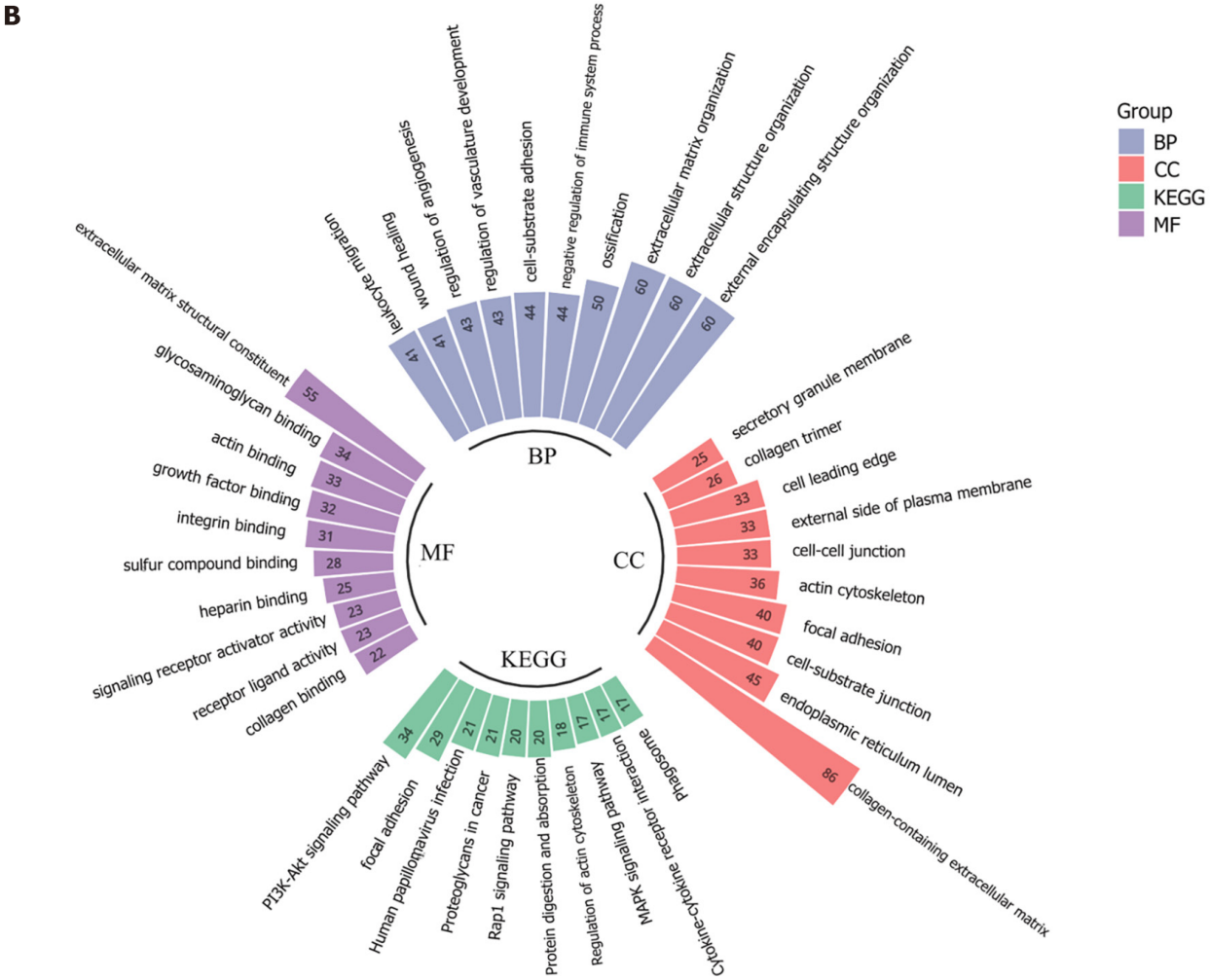
Validation of high CNPY3 expression in COAD

qRT-PCR was performed using colon cancer cell lines and normal tissue samples. As illustrated in Figure 5A, compared with that in normal cell lines, CNPY3 was more highly expressed in six cancer cell lines. CNPY3 was also more highly expressed in the tumor samples (Figure 5B). It is worth noticing that compared with normal tissues, six cancer cell lines expressed higher levels of CNPY3. However, no significant differences were found among the cancer cells and tumor samples. Then, immunohistochemistry was performed to evaluate the protein level, and the IHS was calculated. As illustrated in Figure 5C, the IHS of the COAD group (mean: 7.87 ± 2.38) was greater than that of the normal control (mean: 1.75 ± 1.03). Representative images of immunohistochemical staining are presented in Figure 5D and E. These findings collectively suggest that CNPY3 is highly expressed in COAD samples and cancer cell lines.

Functional experiment and pathway validation

HT-29 and SW620 cell lines with CNPY3 knockdown were successfully established, as confirmed by PCR and Western blot experiments. Among the shRNA groups, shRNA1 exhibited superior silencing efficacy. Thus, it was selected for


A
Type
Type
Low
High
5
0
-5


B
Group
BP
CC
KEGG
MF
BP
CC
KEGG
MF
leukocyte migration
wound healing
regulation of angiogenesis
regulation of vasculature development
cell-substrate adhesion
negative regulation of immune system process
ossification
extracellular matrix organization
extracellular structure organization
external encapsulating structure organization
secretory granule membrane
collagen trimer
cell leading edge
external side of plasma membrane
cell-cell junction
actin cytoskeleton
focal adhesion
cell-substrate junction
endoplasmic reticulum lumen
collagen-containing extracellular matrix
PI3K-Akt signaling pathway
focal adhesion
Human papillomavirus infection
Proteoglycans in cancer
Rap1 signaling pathway
Protein digestion and absorption
Regulation of actin cytoskeleton
MAPK signaling pathway
Cytokine-cytokine receptor interaction
Phagosome
extracellular matrix structural constituent
glycosaminoglycan binding
actin binding
growth factor binding
integrin binding
sulfur compound binding
heparin binding
signaling receptor activator activity
receptor ligand activity
collagen binding

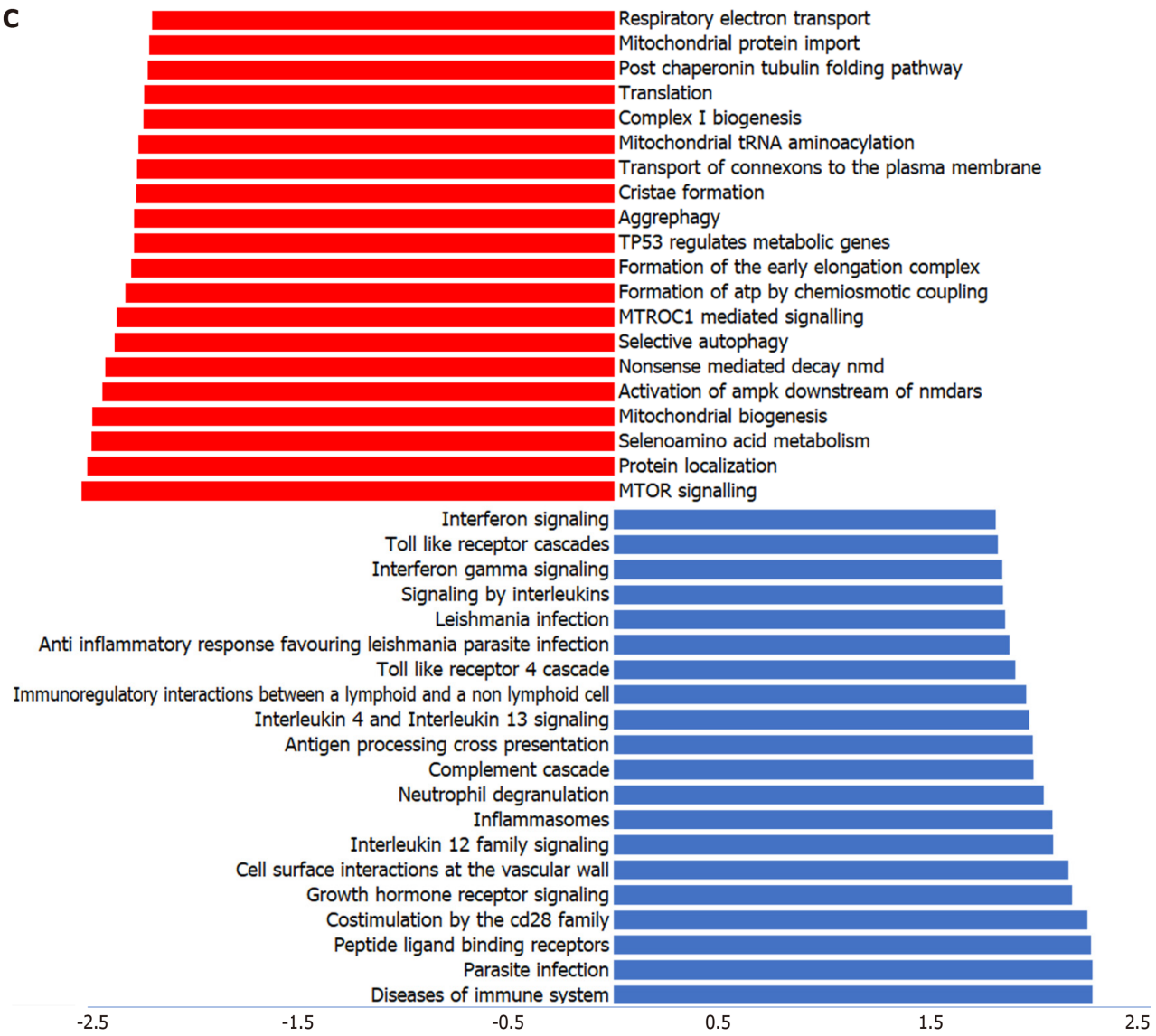


Figure 4 Functional enrichment analysis. A: Top 50 differently expressed genes (DEGs); B: Gene Ontology and Kyoto Encyclopedia of Genes and Genomes analysis of DEGs; C: Gene Set Enrichment Analysis enrichment analysis. BP: Biological process; CC: Cellular component; MF: Molecular function; KEGG: Kyoto Encyclopedia of Genes and Genomes.

subsequent experiments (Figure 6A). CNPY3 knockdown hindered cell proliferation 24 h post-incubation (Figure 6B). Transwell assays demonstrated that CNPY3 knockdown suppressed HT-29 and SW620 cell migration (Figure 6C) and invasion (Figure 6D). In addition, flow cytometry revealed greater levels of apoptosis in CNPY3-KD cells than in control cells (Figure 6E).

As indicated by the bioinformatics analysis, CNPY3 might be correlated with the PI3K/AKT pathway. Thus, the changes of key proteins of this pathway were tested. It was found that downregulation of CNPY3 reduced the p-PI3K and p-AKT protein levels. However, the total PI3K and AKT levels were not significantly affected (Figure 6F). To further validate this finding, the pathway activator (740Y-P) and inhibitor (LY294002) were used to treat the CNPY3-KD cell line. The functional experiment was performed as described above. The results indicated that the pathway activator reversed the decreased cell proliferation (Figure 6A), migration (Figure 6C), and invasion (Figure 6D) and increased apoptosis (Figure 6E). Notably, CNPY3 knockdown still affected the cells when the pathway was inhibited. These results further prove that CNPY3 might regulate colon cancer progression through the PI3K/AKT pathway.

DISCUSSION

In this study, elevated CNPY3 expression was observed in COAD tissues. As indicated by the bioinformatic analysis, CNPY3 was correlated with tumor-node-metastasis stage, survival rate, tumor immune functions, and immunotherapeutic efficacy. The CNPY3's pro-oncogenic nature appears to be linked to the PI3K/AKT signaling pathway, aggrephagy, and the mTOR signaling pathway. Furthermore, COAD tissues and cancer cell lines were used for CNPY3

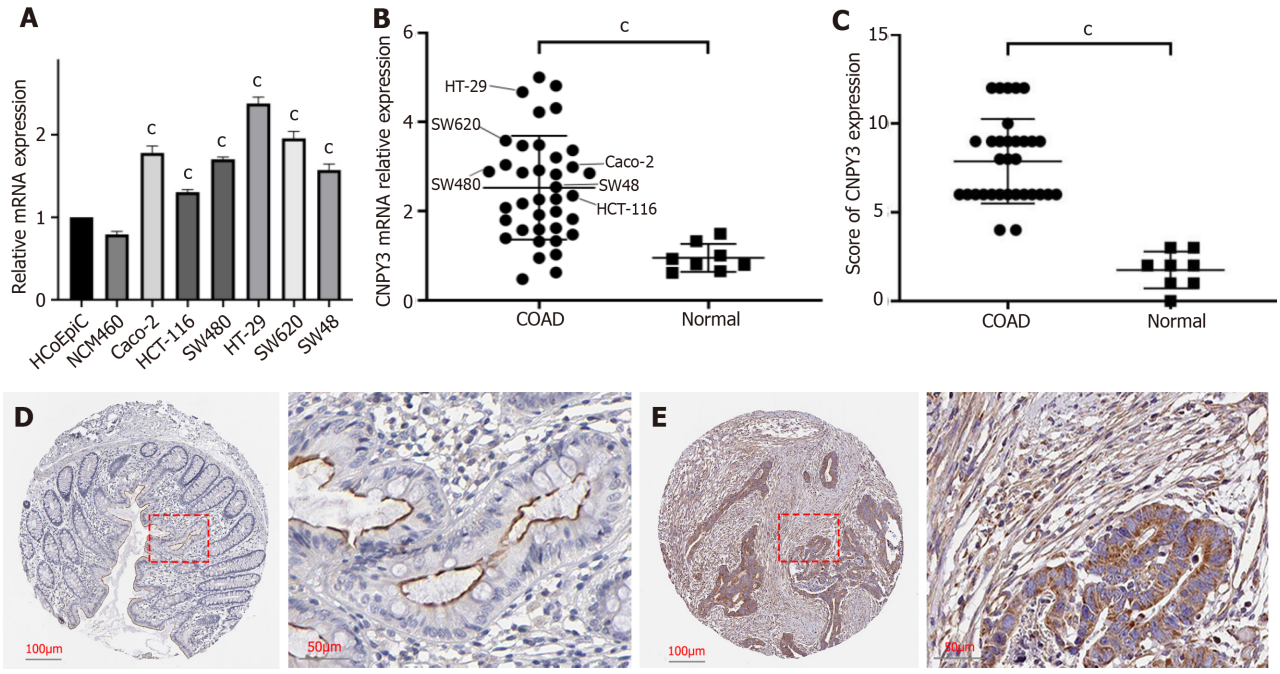


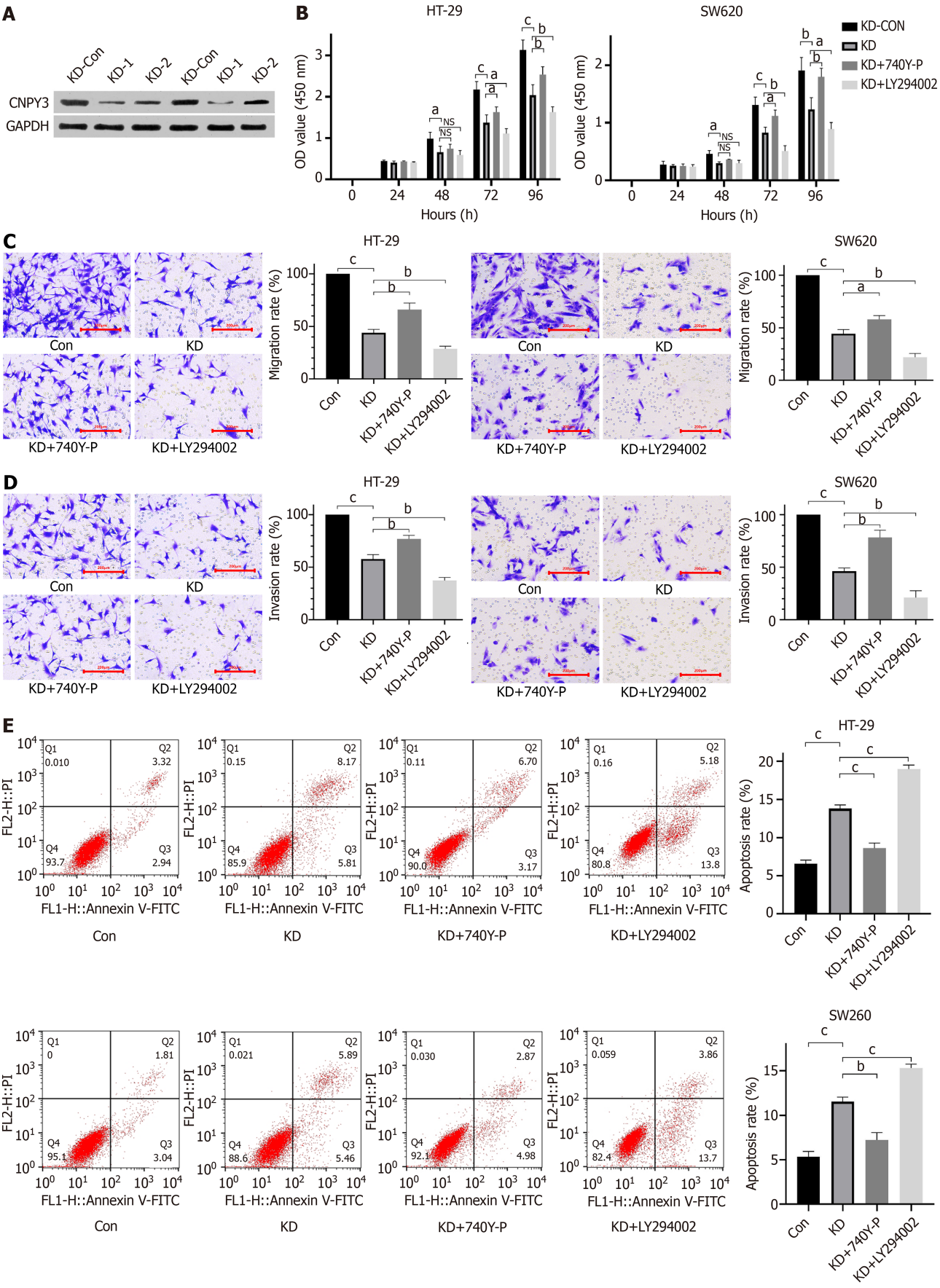
Figure 5 Validation of high canopy FGF signaling regulator 3 expression. A: Relative mRNA expression levels of canopy FGF signaling regulator 3 (*CNPY3*) in normal (HCoEpiC and NCM460) and cancer cell lines (Caco-2, HCT-116, HT-29, SW48, SW480, and SW620); B: Relative mRNA expression levels of *CNPY3* in normal tissue, tumor tissue, and tumor cell lines; C: Differences in immunohistochemical scores; D: Representative immunohistochemical staining images of normal tissue; E: Representative immunohistochemical staining images of tumor tissue. [a]*P* < 0.05; [b]*P* < 0.01, [c]*P* < 0.001. CNPY3: Canopy FGF signaling regulator 3; COAD: Colon adenocarcinoma.

expression validation. Functional experiments indicated that inhibition of CNPY3 inhibited COAD cell proliferation, invasion, and migration while inducing apoptosis-like cell behavior. Moreover, Western blot analysis revealed reduced levels of key proteins of the PI3K/AKT pathway. The pathway activator reversed the decreases in proliferation, invasion, and migration and the increase in apoptosis. Notably, CNPY3 knockdown still affected the cells when the PI3K/AKT pathway was inhibited. The findings of this study suggest that CNPY3 may function as a regulator of COAD development.

CNPY3 coordinates Toll-like receptor trafficking[22]. This protein is involved in endoplasmic reticulum functions *via* a signal peptide and a putative ER retention sequence[23,24]. Thus, CNPY3 is closely linked to immune responses, and its abnormal expression is associated with various diseases[25]. For instance, Zhou *et al*[15] reported that SLIT- and NTRK-like family member 4 could enhance the TrkB-related signaling pathway through interaction with CNPY3. In addition, CNPY3 could contribute to the formation of premetastatic niches, which could facilitate the cancer cell proliferation and metastasis. Liu *et al*[14] analyzed the internal reference of hepatocellular carcinoma cell lines and reported that CNPY3 was the stable reference gene of the cell line MHCC-97L. Zhang *et al*[26] identified CNPY3 as an important cellular target for the induction of pyroptosis, which may have therapeutic implications in prostate cancer. In addition to tumors, CNPY3 was also the hub gene of Alzheimer's disease[27] and end-stage renal disease[28].

The immune microenvironment is crucial for COAD development and progression[29,30]. Ruan *et al*[8] noted that immunosurveillance gradually decreased during malignant transformation. This phase was characterized by decreased infiltration of cytotoxic T lymphocytes, Th1 $CD4^+$ T lymphocytes, and natural killer cells and increased infiltration of immunosuppressive regulatory T cells[31]. Zhou *et al*[32] found that immune cell infiltration was an indicator for COAD prognosis. In this study, CNPY3 was found to be correlated with the proportion of immune cells and might regulate immune cell infiltration. Notably, CNPY3 expression was negatively correlated with the proportion of several types of T cells, especially $CD8^+$ T cells. $CD8^+$ T cells are capable of selectively detecting and eliminating cancer cells, and the exhaustion of $CD8^+$ T cells has been found in multiple cancers[33]. In addition, tumor cells gradually become poor targets for immune attack due to the loss of tumor antigens and the increased production of immunosuppressive-related molecules, such as PD-L1, PD-L2, and CTLA4[34,35]. In this study, we found that CNPY3 was correlated with several immune regulators. The differences in immunotherapy scores also revealed that targeting CNPY3 might increase immunotherapeutic efficacy[36].

The functional enrichment and Western blot analyses revealed that CNPY3 might be related to the PI3K-AKT signaling pathway. The relationship between the PI3K/AKT pathway and COAD have been well illustrated in many studies. Narayanankutty[37] reported that PI3K/AKT/mTOR signaling plays an important role in COAD and concluded that inhibiting the pathway was effective in COAD treatment. Song *et al*[38] noted that TIMP1 was overexpressed in COAD and that knockdown of TIMP1 could reverse COAD tumorigenesis and metastasis through the PI3K/AKT pathway. Similar results were reported by Li *et al*[39], Lin *et al*[40], and Liu *et al*[41]. In our study, we utilized bioinformatic tools to explore the correlation of CNPY3 with the PI3K/AKT pathway, and these findings were subsequently validated through Western blot analysis and functional experiments. The connection was further validated by the finding that a PI3K/AKT


A
KD-Con
KD-1
KD-2
KD-Con
KD-1
KD-2
CNPY3
GAPDH
B
HT-29
SW620
KD-CON
KD
KD+740Y-P
KD+LY294002
OD value (450 nm)
Hours (h)
C
HT-29
SW620
Con
KD
KD+740Y-P
KD+LY294002
Migration rate (%)
D
HT-29
SW620
Invasion rate (%)
E
HT-29
SW260
FL2-H::PI
FL1-H::Annexin V-FITC
Q1
Q2
Q3
Q4
0.010
3.32
93.7
2.94
0.15
8.17
85.9
5.81
0.11
6.70
90.0
3.17
0.16
5.18
80.8
13.8
0
1.81
95.1
3.04
0.021
5.89
88.6
5.46
0.030
2.87
92.1
4.98
0.059
3.86
82.4
13.7
Apoptosis rate (%)

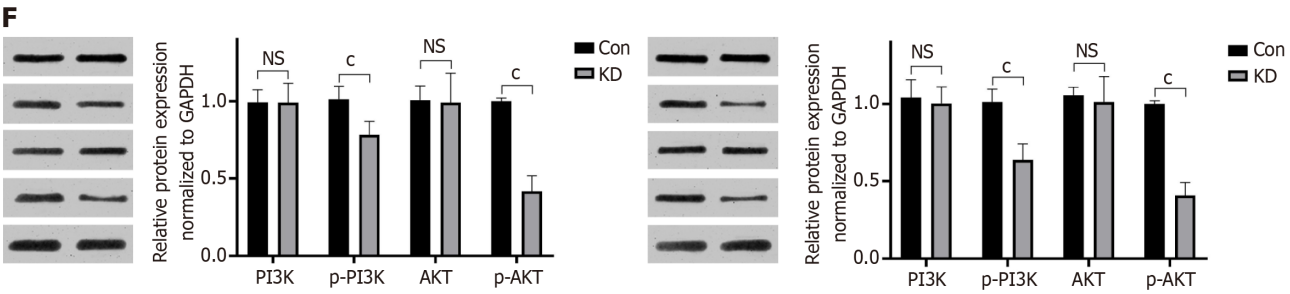


Figure 6 Functional experiments. A: Results of Western blot analysis after transfection; B: Results of CCK-8 assay; C: Representative images of migration; D: Representative images of invasion; E: Results of apoptosis assay; F: Expression of key proteins in PI3K/AKT pathway. [a]*P* < 0.05; [b]*P* < 0.01, [c]*P* < 0.001. CNPY3: Canopy FGF signaling regulator 3; NS: Not significant.

pathway activator rescued the functional loss caused by CNPY3 knockdown. Another interesting finding was that CNPY3 knockdown still affected the cells when the pathway was inhibited. The results could be explained as follows. First, knockdown of CNPY3 affected the pathway to some extent, and the pathway was not fully blocked. Thus, treatment methods targeting CNPY3 might achieve better therapeutic results when combined with PI3K/AKT pathway inhibitors. Second, CNPY3 can affect tumorigenesis through other mechanisms and pathways. The underlying mechanisms are worthy of further exploration. Taken together, these findings support our conclusion regarding the association between CNPY3 knockdown and the suppression of PI3K/AKT pathway, underscoring the potential importance of CNPY3 as a player in COAD progression and offering insights into therapeutic avenues targeting this pathway.

This study has several limitations. First, the study was based on bioinformatic tools and functional experiments, and more high-quality studies are still needed for further validation of our findings. Notably, due to the limitations of bioinformatics data, a few cofounders were included in the univariate and multivariate analyses. Second, the underlying mechanism needs to be further explored by basic experiments. While this study has limitations, it is the first study reporting the prognostic value of CNPY3 and revealing its cancer-promoting role in COAD.

CONCLUSION

This study found that CNPY3 was a prognostic factor for COAD patients and higher CNPY3 was associated with poorer immune functions. The tumor-promoting role of CNPY3 might be correlated with the PI3K-AKT signaling pathway. Moreover, functional experiments revealed that knockdown of CNPY3 promoted HT-29 and SW620 apoptosis while suppressing cell invasion, migration, and proliferation. Finally, the expression of CNPY3 was validated in cancer cell lines and tumor samples, indicating that the findings of this study are reliable and worthy of further investigation.

FOOTNOTES

Author contributions: Gao XC, Zhou BH, Ji ZX, Li Q, and Liu HN designed the research study; Gao XC, Zhou BH, and Ji ZX performed the research; Li Q and Liu HN analyzed the data; Gao XC and Zhou BH wrote the manuscript; all authors have read and approved the final manuscript.

Institutional review board statement: The clinical sample collection process was performed after the approval of the local Ethics Committee. The study was reviewed and approved by the Shenzhen People's Hospital Institutional Review Board (No. LL-KY-2021816).

Conflict-of-interest statement: The authors have no relevant financial or non-financial interests to disclose.

Data sharing statement: No additional data are available.

Country of origin: China

ORCID number: Xu-Can Gao 0000-0002-8173-8925.

S-Editor: Li L
L-Editor: Wang TQ
P-Editor: Che XX

REFERENCES

1 **Fidelle M**, Yonekura S, Picard M, Cogdill A, Hollebecque A, Roberti MP, Zitvogel L. Resolving the Paradox of Colon Cancer Through the Integration of Genetics, Immunology, and the Microbiota. *Front Immunol* 2020; **11**: 600886 [PMID: 33381121 DOI: 10.3389/fimmu.2020.600886]

2 **Verkuijl SJ**, Jonker JE, Trzpis M, Burgerhof JGM, Broens PMA, Furnée EJB. Functional outcomes of surgery for colon cancer: A systematic review and meta-analysis. *Eur J Surg Oncol* 2021; **47**: 960-969 [PMID: 33277056 DOI: 10.1016/j.ejso.2020.11.136]

3 **Sung H**, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, Bray F. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA Cancer J Clin* 2021; **71**: 209-249 [PMID: 33538338 DOI: 10.3322/caac.21660]

4 **Benson AB**, Venook AP, Al-Hawary MM, Arain MA, Chen YJ, Ciombor KK, Cohen S, Cooper HS, Deming D, Farkas L, Garrido-Laguna I, Grem JL, Gunn A, Hecht JR, Hoffe S, Hubbard J, Hunt S, Johung KL, Kirilcuk N, Krishnamurthi S, Messersmith WA, Meyerhardt J, Miller ED, Mulcahy MF, Nurkin S, Overman MJ, Parikh A, Patel H, Pedersen K, Saltz L, Schneider C, Shibata D, Skibber JM, Sofocleous CT, Stoffel EM, Stotsky-Himelfarb E, Willett CG, Gregory KM, Gurski LA. Colon Cancer, Version 2.2021, NCCN Clinical Practice Guidelines in Oncology. *J Natl Compr Canc Netw* 2021; **19**: 329-359 [PMID: 33724754 DOI: 10.6004/jnccn.2021.0012]

5 **Almquist DR**, Ahn DH, Bekaii-Saab TS. The Role of Immune Checkpoint Inhibitors in Colorectal Adenocarcinoma. *BioDrugs* 2020; **34**: 349-362 [PMID: 32246441 DOI: 10.1007/s40259-020-00420-3]

6 **Ganesh K**, Stadler ZK, Cercek A, Mendelsohn RB, Shia J, Segal NH, Diaz LA Jr. Immunotherapy in colorectal cancer: rationale, challenges and potential. *Nat Rev Gastroenterol Hepatol* 2019; **16**: 361-375 [PMID: 30886395 DOI: 10.1038/s41575-019-0126-x]

7 **Angell HK**, Bruni D, Barrett JC, Herbst R, Galon J. The Immunoscore: Colon Cancer and Beyond. *Clin Cancer Res* 2020; **26**: 332-339 [PMID: 31413009 DOI: 10.1158/1078-0432.CCR-18-1851]

8 **Ruan H**, Leibowitz BJ, Zhang L, Yu J. Immunogenic cell death in colon cancer prevention and therapy. *Mol Carcinog* 2020; **59**: 783-793 [PMID: 32215970 DOI: 10.1002/mc.23183]

9 **Ding X**, Liu H, Yuan Y, Zhong Q, Zhong X. Roles of GFPT2 Expression Levels on the Prognosis and Tumor Microenvironment of Colon Cancer. *Front Oncol* 2022; **12**: 811559 [PMID: 35330716 DOI: 10.3389/fonc.2022.811559]

10 **Bao Y**, Zhai J, Chen H, Wong CC, Liang C, Ding Y, Huang D, Gou H, Chen D, Pan Y, Kang W, To KF, Yu J. Targeting m(6)A reader YTHDF1 augments antitumour immunity and boosts anti-PD-1 efficacy in colorectal cancer. *Gut* 2023; **72**: 1497-1509 [PMID: 36717220 DOI: 10.1136/gutjnl-2022-328845]

11 **Sorrentino C**, D'Antonio L, Fieni C, Ciummo SL, Di Carlo E. Colorectal Cancer-Associated Immune Exhaustion Involves T and B Lymphocytes and Conventional NK Cells and Correlates With a Shorter Overall Survival. *Front Immunol* 2021; **12**: 778329 [PMID: 34975867 DOI: 10.3389/fimmu.2021.778329]

12 **Li X**, Wen D, Li X, Yao C, Chong W, Chen H. Identification of an Immune Signature Predicting Prognosis Risk and Lymphocyte Infiltration in Colon Cancer. *Front Immunol* 2020; **11**: 1678 [PMID: 33013820 DOI: 10.3389/fimmu.2020.01678]

13 **Tesniere A**, Schlemmer F, Boige V, Kepp O, Martins I, Ghiringhelli F, Aymeric L, Michaud M, Apetoh L, Barault L, Mendiboure J, Pignon JP, Jooste V, van Endert P, Ducreux M, Zitvogel L, Piard F, Kroemer G. Immunogenic death of colon cancer cells treated with oxaliplatin. *Oncogene* 2010; **29**: 482-491 [PMID: 19881547 DOI: 10.1038/onc.2009.356]

14 **Liu Y**, Qin Z, Cai L, Zou L, Zhao J, Zhong F. Selection of internal references for qRT-PCR assays of human hepatocellular carcinoma cell lines. *Biosci Rep* 2017; **37** [PMID: 29180379 DOI: 10.1042/bsr20171281]

15 **Zhou YQ**, Bao TS, Xie JX, Yao LL, Yu ST, Li Q, Huang PQ, Zhou WZ, Wang YY, Chen SY, Wang XQ, Zhang XL, Jiang SH, Yi SQ, Zhang ZG, Ma MZ, Hu LP, Xu J, Li J. The SLITRK4-CNPY3 axis promotes liver metastasis of gastric cancer by enhancing the endocytosis and recycling of TrkB in tumour cells. *Cell Oncol (Dordr)* 2023; **46**: 1049-1067 [PMID: 37012514 DOI: 10.1007/s13402-023-00795-9]

16 **Li T**, Fan J, Wang B, Traugh N, Chen Q, Liu JS, Li B, Liu XS. TIMER: A Web Server for Comprehensive Analysis of Tumor-Infiltrating Immune Cells. *Cancer Res* 2017; **77**: e108-e110 [PMID: 29092952 DOI: 10.1158/0008-5472.CAN-17-0307]

17 **Wang X**, Zhang Y, Song N, Li K, Lei S, Wang J, Wang Z, Zhang W. CILP2: A prognostic biomarker associated with immune infiltration in colorectal cancer. *Heliyon* 2023; **9**: e15535 [PMID: 37144183 DOI: 10.1016/j.heliyon.2023.e15535]

18 **Chen B**, Khodadoust MS, Liu CL, Newman AM, Alizadeh AA. Profiling Tumor Infiltrating Immune Cells with CIBERSORT. *Methods Mol Biol* 2018; **1711**: 243-259 [PMID: 29344893 DOI: 10.1007/978-1-4939-7493-1_12]

19 **Croft D**, Mundo AF, Haw R, Milacic M, Weiser J, Wu G, Caudy M, Garapati P, Gillespie M, Kamdar MR, Jassal B, Jupe S, Matthews L, May B, Palatnik S, Rothfels K, Shamovsky V, Song H, Williams M, Birney E, Hermjakob H, Stein L, D'Eustachio P. The Reactome pathway knowledgebase. *Nucleic Acids Res* 2014; **42**: D472-D477 [PMID: 24243840 DOI: 10.1093/nar/gkt1102]

20 **Kang T**, Wei Y, Honaker Y, Yamaguchi H, Appella E, Hung MC, Piwnica-Worms H. GSK-3 beta targets Cdc25A for ubiquitin-mediated proteolysis, and GSK-3 beta inactivation correlates with Cdc25A overproduction in human cancers. *Cancer Cell* 2008; **13**: 36-47 [PMID: 18167338 DOI: 10.1016/j.ccr.2007.12.002]

21 **Troschel FM**, Palenta H, Borrmann K, Heshe K, Hua SH, Yip GW, Kiesel L, Eich HT, Götte M, Greve B. Knockdown of the prognostic cancer stem cell marker Musashi-1 decreases radio-resistance while enhancing apoptosis in hormone receptor-positive breast cancer cells *via* p21(WAF1/CIP1). *J Cancer Res Clin Oncol* 2021; **147**: 3299-3312 [PMID: 34291358 DOI: 10.1007/s00432-021-03743-y]

22 **Tabeta K**, Hoebe K, Janssen EM, Du X, Georgel P, Crozat K, Mudd S, Mann N, Sovath S, Goode J, Shamel L, Herskovits AA, Portnoy DA, Cooke M, Tarantino LM, Wiltshire T, Steinberg BE, Grinstein S, Beutler B. The Unc93b1 mutation 3d disrupts exogenous antigen presentation and signaling *via* Toll-like receptors 3, 7 and 9. *Nat Immunol* 2006; **7**: 156-164 [PMID: 16415873 DOI: 10.1038/ni1297]

23 **Morales C**, Li Z. Drosophila canopy b is a cochaperone of glycoprotein 93. *J Biol Chem* 2017; **292**: 6657-6666 [PMID: 28275054 DOI: 10.1074/jbc.M116.755538]

24 **Hirate Y**, Okamoto H. Canopy1, a novel regulator of FGF signaling around the midbrain-hindbrain boundary in zebrafish. *Curr Biol* 2006; **16**: 421-427 [PMID: 16488878 DOI: 10.1016/j.cub.2006.01.055]

25 **Ghait M**, Husain RA, Duduskar SN, Haack TB, Rooney M, Göhrig B, Bauer M, Rubio I, Deshmukh SD. The TLR-chaperone CNPY3 is a critical regulator of NLRP3-inflammasome activation. *Eur J Immunol* 2022; **52**: 907-923 [PMID: 35334124 DOI: 10.1002/eji.202149612]

26 **Zhang XW**, Li L, Liao M, Liu D, Rehman A, Liu Y, Liu ZP, Tu PF, Zeng KW. Thermal Proteome Profiling Strategy Identifies CNPY3 as a Cellular Target of Gambogic Acid for Inducing Prostate Cancer Pyroptosis. *J Med Chem* 2024 [PMID: 38511243 DOI: 10.1021/acs.jmedchem.4c00140]

27 **Madar IH**, Sultan G, Tayubi IA, Hasan AN, Pahi B, Rai A, Sivanandan PK, Loganathan T, Begum M, Rai S. Identification of marker genes in Alzheimer's disease using a machine-learning model. *Bioinformation* 2021; **17**: 348-355 [PMID: 34234395 DOI: 10.6026/97320630017348]

28 **Dai H**, Zhou J, Zhu B. Gene co-expression network analysis identifies the hub genes associated with immune functions for nocturnal hemodialysis in patients with end-stage renal disease. *Medicine (Baltimore)* 2018; **97**: e12018 [PMID: 30212930 DOI: 10.1097/MD.0000000000012018]

29 **Chong W**, Shang L, Liu J, Fang Z, Du F, Wu H, Liu Y, Wang Z, Chen Y, Jia S, Chen L, Li L, Chen H. m(6)A regulator-based methylation modification patterns characterized by distinct tumor microenvironment immune profiles in colon cancer. *Theranostics* 2021; **11**: 2201-2217 [PMID: 33500720 DOI: 10.7150/thno.52717]

30 **Wang Z**, Song J, Azami NLB, Sun M. Identification of a Novel Immune Landscape Signature for Predicting Prognosis and Response of Colon Cancer to Immunotherapy. *Front Immunol* 2022; **13**: 802665 [PMID: 35572595 DOI: 10.3389/fimmu.2022.802665]

31 **Finn OJ**. Cancer immunology. *N Engl J Med* 2008; **358**: 2704-2715 [PMID: 18565863 DOI: 10.1056/NEJMra072739]

32 **Zhou R**, Zhang J, Zeng D, Sun H, Rong X, Shi M, Bin J, Liao Y, Liao W. Immune cell infiltration as a biomarker for the diagnosis and prognosis of stage I-III colon cancer. *Cancer Immunol Immunother* 2019; **68**: 433-442 [PMID: 30564892 DOI: 10.1007/s00262-018-2289-7]

33 **Philip M**, Schietinger A. CD8(+) T cell differentiation and dysfunction in cancer. *Nat Rev Immunol* 2022; **22**: 209-223 [PMID: 34253904 DOI: 10.1038/s41577-021-00574-3]

34 **Sharma P**, Allison JP. The future of immune checkpoint therapy. *Science* 2015; **348**: 56-61 [PMID: 25838373 DOI: 10.1126/science.aaa8172]

35 **Topalian SL**, Drake CG, Pardoll DM. Immune checkpoint blockade: a common denominator approach to cancer therapy. *Cancer Cell* 2015; **27**: 450-461 [PMID: 25858804 DOI: 10.1016/j.ccell.2015.03.001]

36 **Shi Y**, Fu Y, Zhang X, Zhao G, Yao Y, Guo Y, Ma G, Bai S, Li H. Romidepsin (FK228) regulates the expression of the immune checkpoint ligand PD-L1 and suppresses cellular immune functions in colon cancer. *Cancer Immunol Immunother* 2021; **70**: 61-73 [PMID: 32632663 DOI: 10.1007/s00262-020-02653-1]

37 **Narayanankutty A**. PI3K/ Akt/ mTOR Pathway as a Therapeutic Target for Colorectal Cancer: A Review of Preclinical and Clinical Evidence. *Curr Drug Targets* 2019; **20**: 1217-1226 [PMID: 31215384 DOI: 10.2174/1389450120666190618123846]

38 **Song G**, Xu S, Zhang H, Wang Y, Xiao C, Jiang T, Wu L, Zhang T, Sun X, Zhong L, Zhou C, Wang Z, Peng Z, Chen J, Wang X. TIMP1 is a prognostic marker for the progression and metastasis of colon cancer through FAK-PI3K/AKT and MAPK pathway. *J Exp Clin Cancer Res* 2016; **35**: 148 [PMID: 27644693 DOI: 10.1186/s13046-016-0427-7]

39 **Li G**, Zhang C, Liang W, Zhang Y, Shen Y, Tian X. Berberine regulates the Notch1/PTEN/PI3K/AKT/mTOR pathway and acts synergistically with 17-AAG and SAHA in SW480 colon cancer cells. *Pharm Biol* 2021; **59**: 21-30 [PMID: 33417512 DOI: 10.1080/13880209.2020.1865407]

40 **Lin F**, Zhang G, Yang X, Wang M, Wang R, Wan M, Wang J, Wu B, Yan T, Jia Y. A network pharmacology approach and experimental validation to investigate the anticancer mechanism and potential active targets of ethanol extract of Wei-Tong-Xin against colorectal cancer through induction of apoptosis *via* PI3K/AKT signaling pathway. *J Ethnopharmacol* 2023; **303**: 115933 [PMID: 36403742 DOI: 10.1016/j.jep.2022.115933]

41 **Liu F**, Liang Y, Sun R, Yang W, Liang Z, Gu J, Zhao F, Tang D. Astragalus mongholicus Bunge and Curcuma aromatica Salisb. inhibits liver metastasis of colon cancer by regulating EMT *via* the CXCL8/CXCR2 axis and PI3K/AKT/mTOR signaling pathway. *Chin Med* 2022; **17**: 91 [PMID: 35922850 DOI: 10.1186/s13020-022-00641-4]

Published by **Baishideng Publishing Group Inc**
7041 Koll Center Parkway, Suite 160, Pleasanton, CA 94566, USA
Telephone: +1-925-3991568
E-mail: office@baishideng.com
Help Desk: https://www.f6publishing.com/helpdesk
https://www.wjgnet.com