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# The phospholipase PNPLA7 functions as a positive indicator in human colorectal and gastric cancers

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**Abstract**

Early diagnosis of gastrointestinal tumors remains a clinical challenge due to their insidious onset. Patatin-like phospholipase domain containing protein 7 (PNPLA7) has been shown to be associated with the occurrence and development of hepatocellular carcinoma. However, the expressions of PNPLA7 in colorectal and gastric cancers remain unclear. The online gene expression profiling interactive analysis and Kaplan–Meier Plotter databases were used for the analysis of the expression of PNPLA7 and the survival curve, respectively. The tumor tissues and their corresponding normal noncancerous tissues from colorectal cancer or gastric cancer patients were collected and quantitative real-time polymerase chain reaction assay was performed to evaluate the expression of related genes. PNPLA7 was significantly down-regulated in gastric and colorectal cancer tumor tissues compared to adjacent normal tissues. Receiver operating characteristic analysis showed that PNPLA7 could be used as a diagnostic marker for gastric and colorectal tumors. The overall survival of patients with high expression of PNPLA7 was also significantly higher than that of patients with low expression in stomach and rectum adenocarcinoma. Phospholipase PNPLA7 can be used as a positive diagnostic indicator for colorectal and gastric cancers.

**Abbreviations:** AUC = area under the curve, COAD = colon adenocarcinoma, GEPIA = gene expression profiling interactive analysis, PNPLA7 = patatin-like phospholipase domain containing protein 7, READ = rectum adenocarcinoma, ROC = receiver operating characteristic, STAD = stomach adenocarcinoma.

**Keywords:** biomarker, colorectal cancer, gastric cancer, PNPLA7

## 1. Introduction

Colorectal and gastric cancers seriously threaten human life and health.[1] Gastric cancer ranks 4th among malignant tumors in the world, and its etiology may be related to dietary factors, *Helicobacter pylori* infection, genetic factors, and geographical environment.[2] The 5-year survival rate of 27% of newly diagnosed gastric cancer patients is 30.4%, which remains stable over the past 30 to 40 years, and the 5-year overall survival rate of patients is < 5%.[3,4] The incidence and mortality of colorectal cancer are on the rise.[5] 25% to 30% of colorectal tumors are advanced at the time of diagnosis and cannot be surgically removed.[6] Approximately 50% of patients undergoing surgery (including radical surgery, palliative surgery) develop recurrent or metastatic colorectal tumors within 5 years of surgery.[7,8]

Patatin-like phospholipase domain containing protein 7 (PNPLA7) is a pyrophosphokinase localized to the endoplasmic reticulum membrane.[9,10] PNPLA7 is highly expressed in tissues with active energy metabolism and lipid turnover, and its expression is regulated by nutritional status.[11] PNPLA7 regulates the secretion of very low-density lipoprotein by interacting with apolipoprotein E to regulate the accumulation of fat in the liver.[12] Cyclic nucleotides can regulate the binding of PNPLA7 to lipid droplets in fat storage organelles.[13] It has been reported that the alteration of metabolic patterns between tumor cells and normal cells lead to genes that affect energy metabolism that may be potential targets for tumor therapy or diagnosis.[14] Consistently, PNPLA7 has also been shown to play a role in tumorigenesis, and a lower expression state of PNPLA7 has been detected in liver cancer cells.[11] However, the expression of PNPLA7 and its regulatory role in colorectal and gastric cancers remain unclear.

In this study, we hypothesized that PNPLA7 could serve as a diagnostic marker for colorectal and gastric cancers. We used a database to analyze the expression of PNPLA7 in colorectal and gastric cancers and its relationship with patient survival, and tested the hypothesis in patients samples.

*The authors have no funding and conflicts of interest to disclose.*

*The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.*

*All experiments in this study have been reviewed and approved by the Research Ethics Committee of the 904th Hospital of Joint Logistic Support Force of PLA.*

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## 2. Methods

### 2.1. Gene expression profiling interactive analysis (GEPIA)

The online GEPIA database (http://gepia2.cancer-pku.cn) was used for the analysis of the expression of PNPLA7 in stomach adenocarcinoma (STAD), colon adenocarcinoma (COAD), rectum adenocarcinoma (READ), and the adjacent normal tissues, respectively. All options were selected by default during the data analysis.

### 2.2. Kaplan–Meier plotter analysis

The overall survival of patients with stomach adenocarcinoma and rectum adenocarcinoma was analyzed by Kaplan–Meier Plotter online (http://kmplot.com). All options were selected by default during the data analysis.

### 2.3. Tissue samples

Tumor tissues and their corresponding normal noncancerous tissues were collected from 62 patients diagnosed with colorectal cancer and 68 patients diagnosed with human gastric adenocarcinoma. All tissue-derived patients had signed written informed consent. The patients were pathologically and clinically diagnosed as colorectal cancer or gastric cancer according to Chinese tumor diagnostic criteria. The patient did not receive radiotherapy or chemotherapy before removal of the tumor tissues. All fresh tissue samples were stored at −80°C immediately before being used for further experiments. All experiments in this study have been reviewed and approved by the Research Ethics Committee of the 904th Hospital of Joint Logistic Support Force of PLA (#20220801).

### 2.4. Quantitative real-time polymerase chain reaction

Trizol was used to extract 10 μg of total RNA from tumor tissue and normal tissue, respectively, and the experimental operation was performed according to the instructions of the RT-PCR kit (SuperScript IV 1-Step RT-PCR System, Thermo Fisher). The primer sequences used in this study are shown in Table 1.

### 2.5. Statistical analysis

Student *t* test was performed for data analysis between 2 groups using SPSS 26.0 software (SPSS, Chicago, IL). $P < .05$ indicates a significant difference.

## 3. Results

### 3.1. Expression pattern of PNPLA7 in colorectal and gastric cancers

In order to investigate the potential association between PNPLA7 and the pathogenesis of colorectal and gastric cancers, we conducted an analysis of PNPLA7's expression profile using the publicly available cancer database GEPIA. Our findings indicate a significant down-regulation of PNPLA7 in STAD (Fig. 1A), COAD (Fig. 1B), and READ (Fig. 1C), suggesting a potential pivotal role of PNPLA7 in the initiation and progression of colorectal and gastric cancers. To substantiate these observations, we proceeded to validate the expression of PNPLA7 in tumor tissues of colorectal and gastric cancers, as well as the corresponding adjacent tissues from patients. Employing real-time PCR, we observed a considerable decrease in the expression of PNPLA7 in both colorectal cancer (Fig. 2A) and gastric cancer tumor tissues (Fig. 2B) compared to normal paracancerous tissues. In conclusion, our dataset underscores a substantial down-regulation of PNPLA7 in colorectal and gastric cancers.

**Table 1**

**Primers used for real-time PCR.**

| Gene name | | Sequences |
|---|---|---|
| PNPLA7-human | Forward | 5'- GGAAAAGCGTGATGGTTGC-3' |
| | Reverse | 5'- GAGCAGGTCCTTCTTGGCA-3' |
| CCNA2-human | Forward | 5'-ACTGCTGCTATGCTGTTA-3' |
| | Reverse | 5'-TGGTGTAGGTATCATCTGTAAT-3' |
| CCNB1-human | Forward | 5'- GCAGCAGGAGCTTTTTGCTT -3' |
| | Reverse | 5'-CCAGGTGCTGCATAACTGGA-3' |
| GAPDH-human | Forward | 5'-ACAGCCTCAAGATCATCAGC-3' |
| | Reverse | 5'-GGTCATGAGTCCTTCCACGAT-3' |

PNPLA7 = Patatin-like phospholipase domain containing protein 7.

### 3.2. Diagnostic value of PNPLA7 in colorectal and gastric cancers

Subsequently, we performed receiver operating characteristic (ROC) analysis on the outcomes presented in Figure 2 to ascertain the diagnostic potential of PNPLA7 in colorectal and gastric cancers. The results of our analysis revealed that the Area Under the Curve (AUC) for colorectal cancer and gastric cancer were 0.9277 (Fig. 3A) and 0.9702 (Fig. 3B), respectively. These AUC values surpass 0.5, implying a diagnostic utility of PNPLA7 in both colorectal and gastric cancers.

### 3.3. Correlation analysis between the expression level of PNPLA7 and cell cycle-related proteins

Elevated expression of cell cycle-related proteins is widely recognized as a contributing factor in the development of colorectal and gastric cancers. The publicly accessible tumor database GEPIA predicted a negative correlation between PNPLA7 and CCNA2 as well as CCNB1 in COAD, READ, and STAD (Figure S1 to S3, Supplemental Digital Content, http://links.lww.com/MD/K185). Building upon this prediction, we quantified the expressions of CCNA2 and CCNB1 in colorectal cancer and gastric cancer tissues via real-time PCR. Our results demonstrated a significant up-regulation of both CCNA2 and CCNB1 in colorectal cancer (Fig. 4A and B) and gastric cancer tumor tissues (Fig. 4C and D) relative to normal paracancerous tissues. Subsequently, we scrutinized the relationship between the expression levels of PNPLA7 and CCNA2/CCNB1 in tumor tissues from colorectal and gastric cancers. Our data uncovered a noteworthy negative correlation between PNPLA7 and CCNA2 or CCNB1 in both colorectal cancer (Fig. 5A and B) and gastric cancer tissues (Fig. 5C and D).

### 3.4. Survival analysis of PNPLA7 in colorectal and gastric cancers

To further substantiate the potential of PNPLA7 as a positive prognostic marker for patients afflicted with colorectal and gastric cancers, we executed survival analyses using the KM Plotter database. Our investigation unveiled a markedly higher overall survival rate in patients with elevated PNPLA7 expression suffering from gastric (Fig. 6A, $P = .033$) and rectal (Fig. 6B, $P = .0336$) cancers in contrast to patients with lower expression levels. Collectively, our findings suggest that PNPLA7 could serve as a valuable adjunct indicator for prognosticating the outcomes of colorectal and gastric cancers.

## 4. Discussion

Colorectal and gastric cancers are malignant tumors of the digestive tract and ancillary digestive organs, including esophageal cancer, gastric cancer, colorectal cancer, pancreatic cancer, and

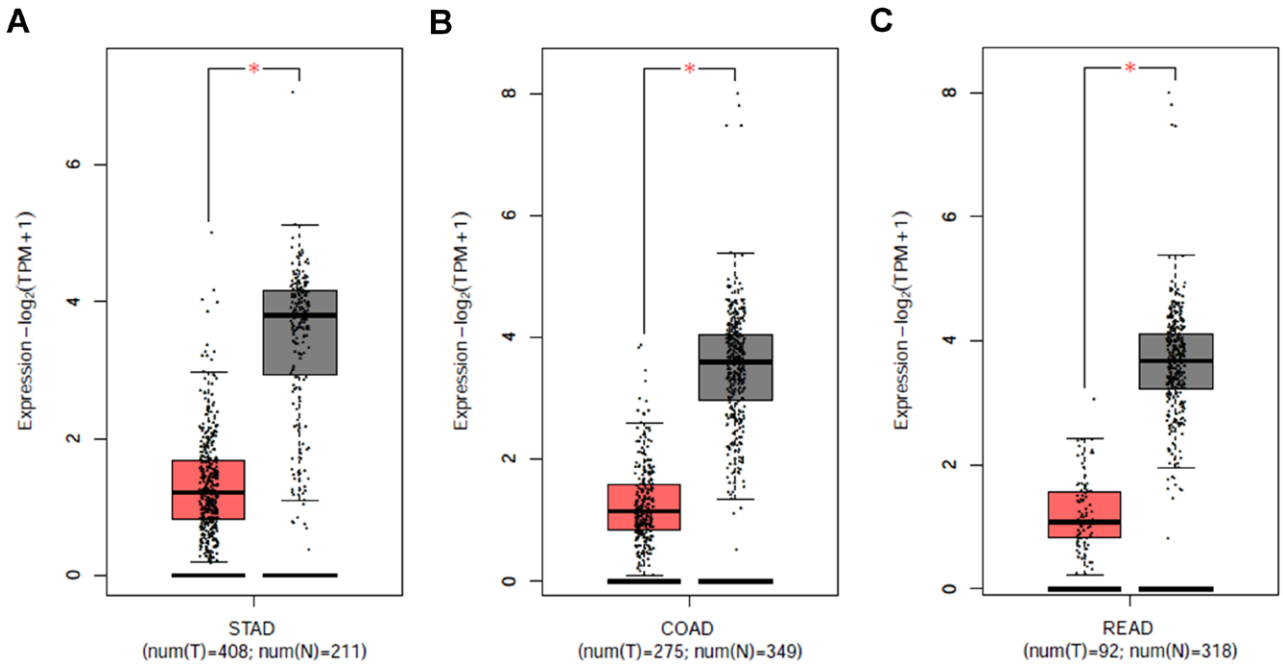


**Figure 1.** PNPLA7 is predicted down-regulated in colorectal and gastric cancers by the public tumor database. (A–C) The public tumor database GEPIA2 was used to predict the expression of PNPLA7 in STAD (A), COAD (B) and READ (C). *$P < .01$. COAD = colon adenocarcinoma, GEPIA = gene expression profiling interactive analysis, N = normal, PNPLA7 = patatin-like phospholipase domain containing protein 7, READ = rectum adenocarcinoma, STAD = stomach adenocarcinoma, T = tumor.

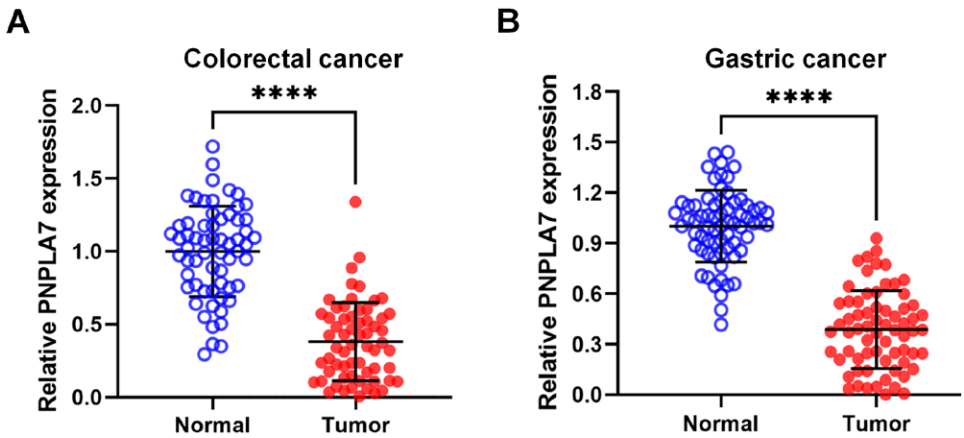


**Figure 2.** Real-time PCR shows that PNPLA7 is elevated in colorectal and gastric cancers. (A) The tumor tissues and paracancerous normal tissues from colorectal cancer were used for real-time PCR analysis against PNPLA7. N = 62. B. The tumor tissues and paracancerous normal tissues from gastric adenocarcinoma were used for real-time PCR analysis against PNPLA7. N = 68. ****$P < .0001$. PNPLA7 = patatin-like phospholipase domain containing protein 7.

liver cancer.[15] Colorectal and gastric cancers account for more than 50% of cancer-related morbidity and mortality worldwide. In recent years, the morbidity and mortality of colorectal cancer, gastric cancer, and hepatocellular carcinoma have been increasing due to changes in dietary habits and environment.[16] According to the global cancer statistics in 2020, the incidence of colorectal cancer is second only to breast cancer and lung cancer, and the mortality rate of colorectal cancer, liver cancer and gastric cancer is second only to lung cancer.[17] Although we have made great progress in the diagnosis and treatment of digestive system tumors, most patients are already in the middle and late stages of cancer when they are diagnosed due to the difficulty of early diagnosis, and their treatment and prognosis are very poor.[18] Therefore, the molecular mechanism and early prediction of the occurrence and development of cancers are currently hot issues in research.

In this study, we firstly used data from the public cancer database GEPIA to find that PNPLA7 was significantly under-expressed in colorectal and gastric cancers. Then, we collected relevant tumor tissues from patients in our hospital for further confirmation, and found that PNPLA7 was significantly low-expressed in both colorectal cancer and gastric cancer tumor tissues compared with the adjacent normal tissues. Meanwhile, we performed ROC analysis to explore the diagnostic value of PNPLA7 in colorectal and gastric cancers. The results showed that the AUC of the ROC of PNPLA7 was > 0.5 in both colorectal cancer and gastric cancer, indicating the diagnostic effect of PNPLA7 on these 2 tumors.

The PNPLA protein family plays important roles in mammalian lipid metabolism and signal transduction.[19] The PNPLA protein family contains 9 proteins divided into 3 subgroups.[20] A second subgroup of the PNPLA protein family includes

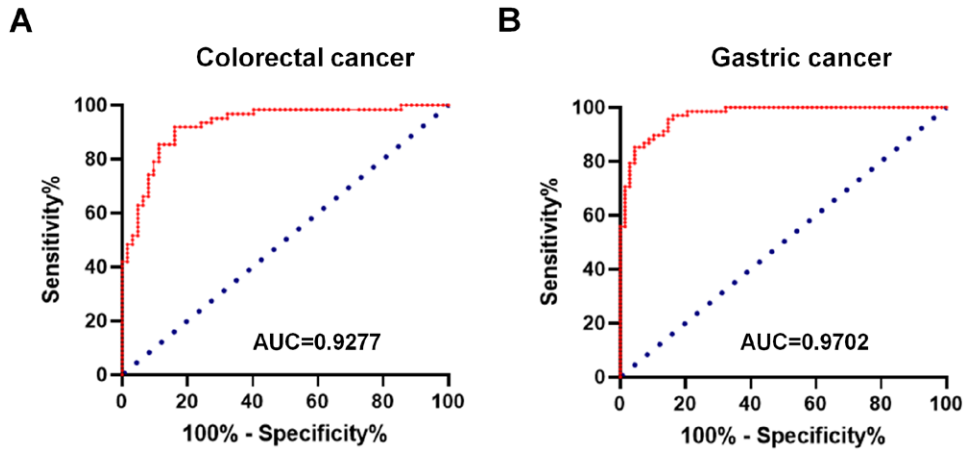


**Figure 3.** ROC curve for PNPLA7 level in tumor tissues compared with controls. (A) ROC curve was used to investigate the value of PNPLA7 in colorectal cancer diagnosis. (B) ROC curve was used to investigate the value of PNPLA7 in gastric cancer diagnosis. PNPLA7 = patatin-like phospholipase domain containing protein 7, ROC = receiver operating characteristic.

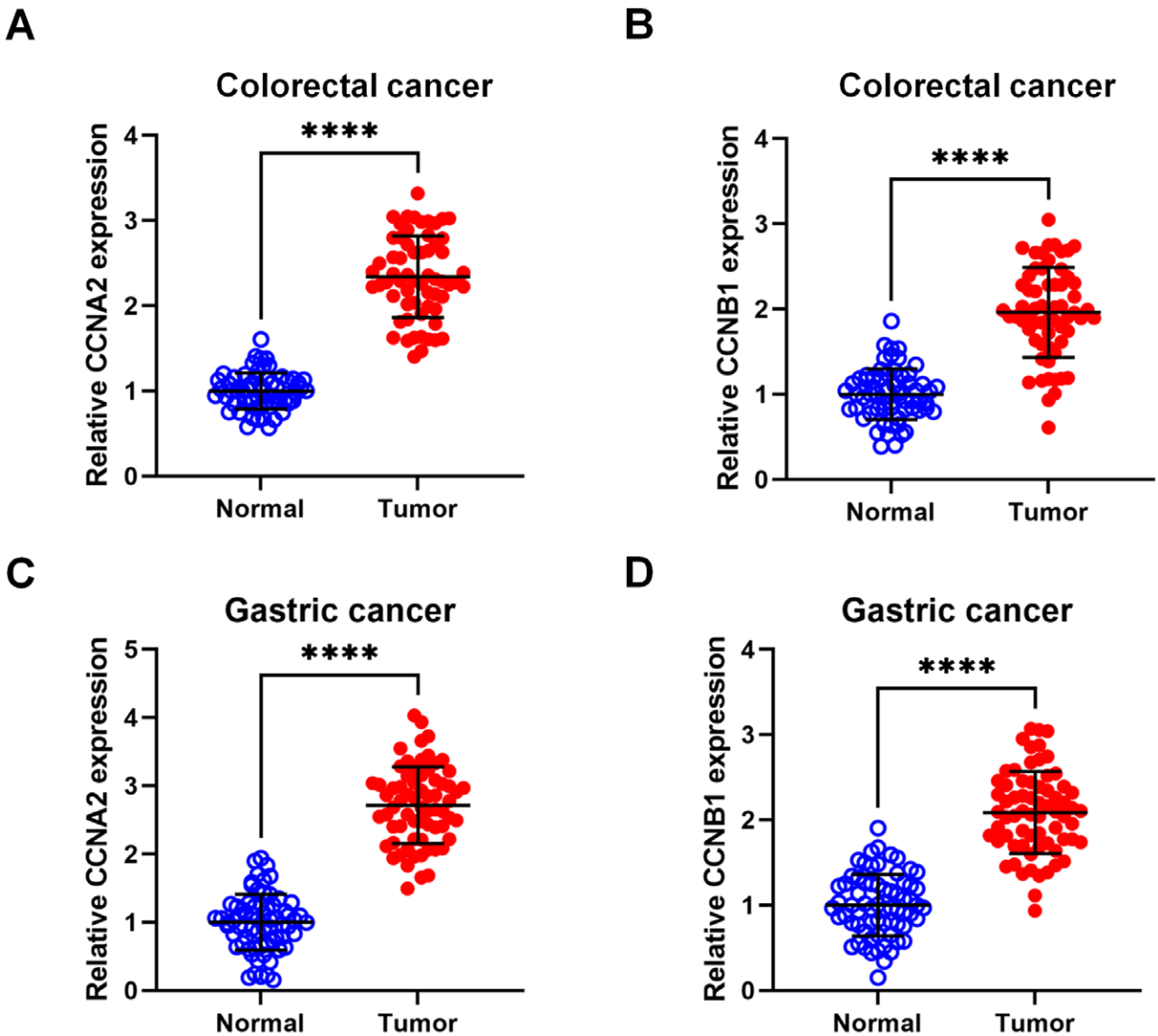


**Figure 4.** CCNA2 and CCNB1 are elevated in colorectal and gastric cancers. (A and B) The tumor tissues and paracancerous normal tissues from colorectal cancer were used for real-time PCR analysis against CCNA2 (A) and CCNB1 (B). N = 62. (C and D). The tumor tissues and paracancerous normal tissues from gastric cancer were used for real-time PCR analysis against CCNA2 (C) and CCNB1 (D). N = 68. ****$P < .0001$.

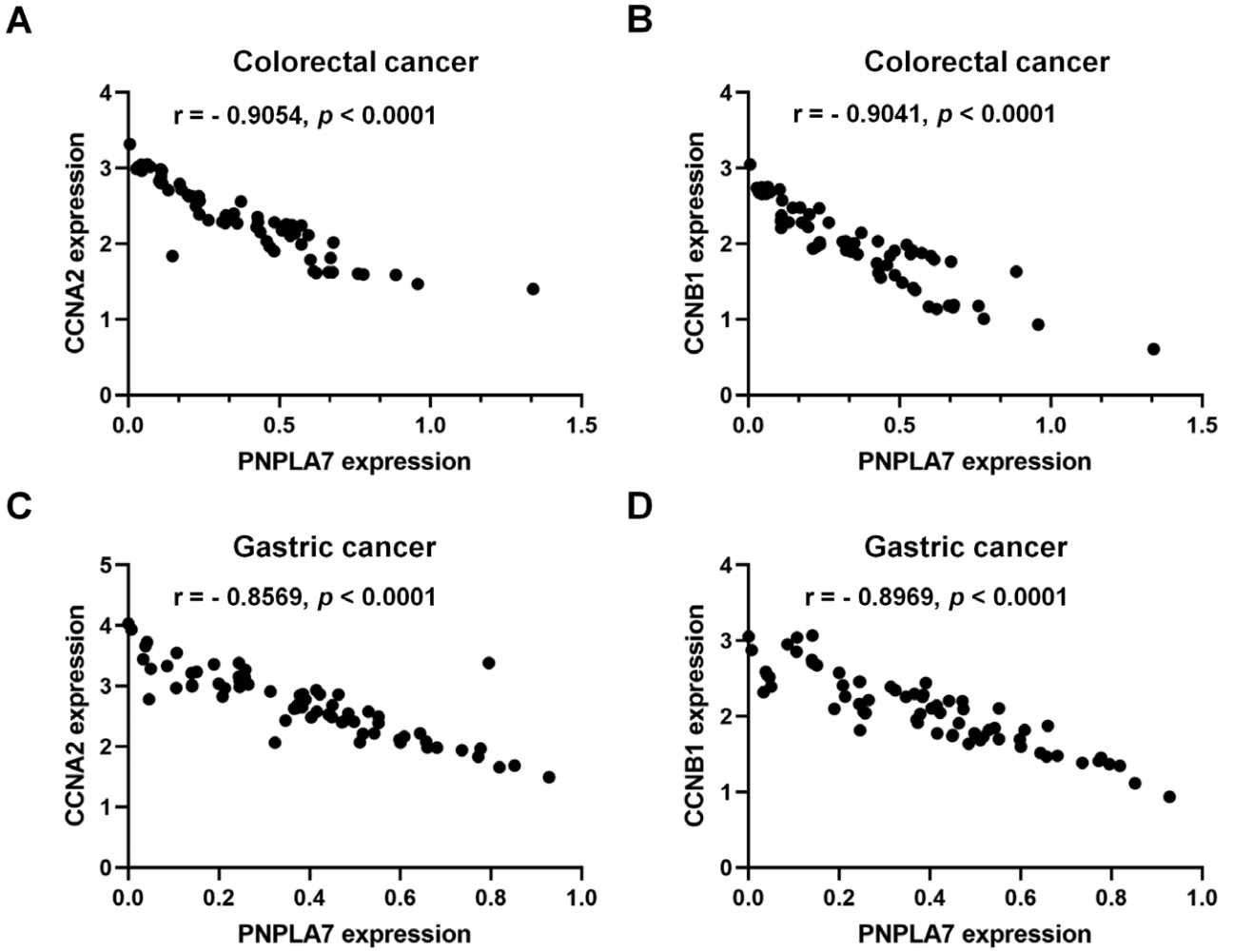


**Figure 5.** PNPLA7 expression level is negatively correlated with CCNA2 and CCNB1 expression in colorectal and gastric cancers. (A and B) Correlation analysis between PNPLA7 expression and CCNA2 (A) or CCNB1 expression (B) in tumor tissues from colorectal cancer. N = 62. (C and D). Correlation analysis between PNPLA7 expression and CCNA2 (C) or CCNB1 expression (D) in tumor tissues from gastric cancer. N = 68. PNPLA7 = Patatin-like phospholipase domain containing protein 7.

PNPLA6 and PNPLA7.[21] PNPLA6 and PNPLA7 are commonly referred to as neuropathic target esterase and neuropathic target esterase-related esterase (NRE), respectively.[22] Compared with other subgroup members of PNPLA, PNPLA7 has distinct transmembrane and cyclic nucleotide binding domains in addition to the patatin-like phospholipase domain.[23] PNPLA7 is mainly highly expressed in tissues with active fat metabolism and energy conversion, such as adipose tissue, cardiac muscle, and skeletal muscle, and its expression is regulated by nutritional status.[24] The tissue distribution, catalytic activity, cellular localization, and expression characteristics of PNPLA7 suggest that it may play a role in lipid metabolism and energy regulation.[25]

In recent years, it has been reported that PNPLA7 is also associated with tumorigenesis.[25,26] Significant differences in metabolic patterns between tumor cells and normal cells lead to genes that affect energy metabolism that may be potential targets for tumor therapy or diagnosis.[27,28] A study showed that the promoter of PNPLA7 was highly methylated in liver cancer cells, which resulted in a lower expression state of PNPLA7 in liver cancer cells.[26] However, the expression of PNPLA7 in other tumor cells and its relevance to another tumorigenesis remain unclear. In this study, we mainly demonstrate that PNPLA7 is also down-regulated in colorectal and gastric cancer tumor tissues compared with adjacent normal tissues. Previous studies have shown that abnormally high expression of cell cycle-related proteins is closely related to the occurrence of colorectal and gastric cancers. We have also found that PNPLA7, CCNA2, and CCNB1 were all significantly negatively correlated in the COAD\READ\STAD aix through database analysis. PNPLA7 has a significant negative correlation with CCNA2 and CCNB1 in COAD, READ and STAD ($P < .05$). Therefore, in this study, we detected the expressions of CCNA2 and CCNB1 in colorectal cancer and gastric cancer tissues, respectively, and found that they both had significantly higher expression compared with normal tissues. We analyzed the correlation between PNPLA7 expression level and CCNA2 and CCNB1 expression levels in tumor tissues, and found that PNPLA7 was significantly negatively correlated with CCNA2 and CCNB1 in digestive tract tumor tissues. Consistently, we also used survival curves from the KM Plotter database to demonstrate that the overall survival rate of patients with gastric and colon cancers with high PNPLA7 expression was significantly higher than that of patients with low expression.

In fact, our research still has some shortcomings. First, the manuscript lacks detailed mechanistic insights into how PNPLA7 functions in the context of colorectal and gastric cancers. While the study highlights the correlation between PNPLA7 expression and cancer, it does not delve into the underlying molecular pathways or mechanisms through which PNPLA7 contributes to tumorigenesis. Second, the study focuses on the diagnostic potential of PNPLA7 but does not explore any potential therapeutic implications. Investigating how manipulating PNPLA7

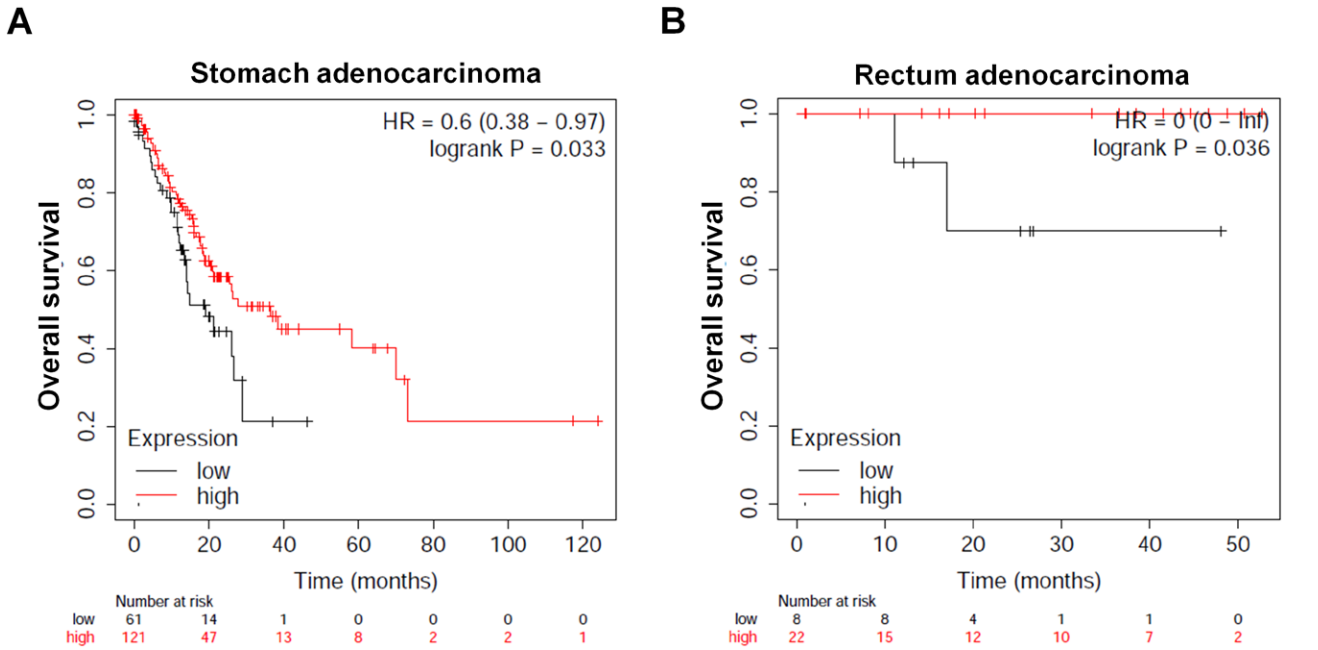


**Figure 6.** PNPLA7 is predicted as a positive indicator for patients with colorectal and gastric cancers. (A and B) The overall survival of patients with stomach adenocarcinoma (A) or rectum adenocarcinoma (B) was analyzed by Pan-cancer RNA-seq from Kaplan–Meier Plotter (http://kmplot.com). The detailed information about the database was stated in the material section. PNPLA7 = Patatin-like phospholipase domain containing protein 7.

expression affects cancer growth, metastasis, or response to treatment could provide valuable insights into its therapeutic relevance. Moreover, the manuscript relies heavily on publicly available databases for gene expression analysis and survival curves. While these databases are valuable resources, they might have limitations in terms of data quality, patient diversity, and accuracy. We will continue to focus on these issues in our future research.

## 5. Conclusion

We demonstrated that phospholipase PNPLA7 can be used as a positive diagnostic indicator for colorectal and gastric cancers. We report that PNPLA7 is statistically under-expressed in colorectal and gastric cancers with an AUC more than 0.5 for ROC analysis. In addition, the survival curves from the KM Plotter database demonstrates that the overall survival rate of patients with gastric and colon cancers with high PNPLA7 expression was significantly higher than that of patients with low expression.

## Author contributions

**Data curation:** Yang Bai, Kunlun Luo, Weixuan Xie.
**Funding acquisition:** Kunlun Luo.
**Investigation:** Kunlun Luo.
**Resources:** Kunlun Luo.
**Supervision:** Kunlun Luo.
**Validation:** Yang Bai, Kunlun Luo, Weixuan Xie.
**Writing – original draft:** Yang Bai, Kunlun Luo, Weixuan Xie.
**Writing – review & editing:** Yang Bai, Kunlun Luo, Weixuan Xie.

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