Original Article

Expression and clinical significance of *ENC1* in gastrointestinal tumors: Bioinformatics analysis based on a public gene database

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Background: To investigate the expression characteristics of ectodermal-neural cortex 1 (*ENC1*) in gastrointestinal tumors and its potential value in judging the prognosis of patient survival.

Methods: RNA sequence (RNA-seq) data and patient survival data related to stomach adenocarcinoma (STAD) and colon adenocarcinoma (COAD) in gastric cancer and colon cancer from The Cancer Genome Atlas (TCGA) were downloaded for expression difference analysis and Cox survival regression analysis. A Kaplan–Meier (KM) survival curve was plotted to analyze the tumor invasion level of patients with different *ENC1* expression levels, and the main influencing pathways of *ENC1* were analyzed by Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis and protein network analysis.

Results: The data of 405 STAD samples and 494 COAD clinical samples of TCGA were analyzed, and it was found that the expression of *ENC1* in tumor tissues of patients with both types of cancer was significantly higher than that in normal tissues, with Log_2 fold change values of 1.97 and 2.06, respectively, P<0.001. Cox analysis revealed that the high expression of *ENC1* was not significantly correlated with the prognosis and survival time of patients with gastric cancer and patients with colon cancer: overall survival (OS) hazard ratio (HR): 1.039, 95% confidence interval (CI): 0.890–1.213, P=0.627 for gastric cancer, OS HR: 0.886, 95% CI: 0.702–1.111, P=0.306 for colon cancer. KEGG pathway enrichment analysis of gene *ENC1* revealed that *ENC1* was mainly involved in neuroactive ligand-receptor interaction. The high expression of *ENC1* was associated with various immune cells and different *T* cells such as basophils, $CD4^+$ memory T cells, $CD4^+$ TEM, and MV endothelial cells in gastric and colon cancers. The results of *ENC1* protein interaction network analysis suggested that *ENC1* may be involved in regulating neurite formation and neural crest cell differentiation.

Conclusions: *ENC1* expression is elevated in both gastric and colon cancers, and ENC1 is associated with various immune cells and different *T* cells such as basophils, $CD4^+$ memory T cells, $CD4^+$ TEM, and MV endothelial cells in both gastric and colon cancers, but *ENC1* does not affect the survival and prognosis of patients.

Keywords: Ectodermal-neural cortex 1 (ENC1); gastric cancer; colon cancer

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Introduction

Gastrointestinal tumors include tumors arising in the stomach, small intestine, colon, and rectum, among which gastric cancer and colon cancer are the most common (1). Gastric carcinoma (GC) is a malignant tumor originating from the gastric mucosal epithelium, which can occur anywhere in the stomach and then spread to other parts of the body, and more than 60% of gastric cancers are caused by *Helicobacter pylori* infection (2). At present, the mortality rate of gastric cancer is extremely high, ranking third in cancer in the world and difficult to cure, but the 5-year survival probability of patients with early gastric cancer is as high as more than 90%, so early detection and treatment are the key. Colon adenocarcinoma (COAD) is a primary malignant tumor of the colonic epithelium with no obvious early symptoms. With the progression of the tumor, patients gradually exhibit abdominal pain, hematochezia, changes in stool characteristics, weight loss, and other symptoms. At present, comprehensive treatment regimens based on surgery for colon cancer have the best efficacy, but their therapeutic effect is still poor for patients with advanced or advanced disease (3,4). The symptoms of patients with early gastrointestinal tumors are insidious, and most patients have already reached the later stages by the time of diagnosis. Therefore, it is important to reveal the key molecular mechanism of gastrointestinal tumorigenesis for early diagnosis, prognosis, and precise treatment. Ectodermal-neural cortex 1 (*ENC1*) is a nervous system-specific expressed gene belonging to the KELCH family of genes, which was first discovered in mammals in 1997 by Hernandez *et al.* (5). This gene is involved in and encodes a binding protein for actin and plays an important role throughout nervous system development (6). In recent years, with the deepening of genetics in cancer research, it has been found that *ENC1* may play a promoting role in the development of tumors. It has been shown that the expression of *ENC1* is significantly increased in breast cancer compared with normal breast tissue, and is significantly associated with the proliferation, migration, and invasion of breast cancer cells (7). A study on cervical cancer patients showed that the higher the *ENC1* expression level in cancer tissues, the worse the prognosis (8). Based on the few studies on the effect of *ENC1* on gastrointestinal tumors, this study aimed to investigate the expression, clinical significance, and prognosis of *ENC1* in gastrointestinal tumors by analyzing the data in The Cancer Genome Atlas (TCGA), providing a new target for the precise treatment of gastrointestinal tumors. We present the following article in accordance with the REMARK reporting checklist (available at https://jgo.amegroups.com/article/view/10.21037/jgo-23-217/rc).

Highlight box

Key findings

- *ENC1* expression is elevated in both gastric and colon cancers.
- *ENC1* is not a key gene that affects the survival and prognosis of patients.

What is known and what is new?

- *ENC1* expression was found to be significantly higher in several cancer tissues.
- *ENC1* is not a key gene for gastrointestinal tumors.

What is the implication, and what should change now?

- Although *ENC1* may be a gene that is involved with the development of gastrointestinal tumors, it does not affect the prognosis of patients.
- Other key genes should be further studied for targeted therapy.

Methods

Data acquisition

Data acquisition was logged on Xena website (https://xenabrowser.net/datapages/) and RNA sequence (RNA-seq) clinical expression profile data were obtained from stomach adenocarcinoma (STAD) and COAD samples source from the TCGA database (https://portal.gdc.cancer.gov/). The count data, fragments per kilobase million (FPKM) data, and survival data were downloaded. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

Software platform and analysis tools

The software platform and analysis tools were programmed in R language version 4.2.2 (The R Foundation for Statistical Computing Platform, Vienna, Austria) for data processing and figure presentation, the compilation tool used was Rtools42, and the integrated development environment was RStudio-2022.12.0-353.

Gene differential expression analysis

R language was used to filter out the messenger RNA (mRNA) genes in the dataset, and the matrix was divided into 2 groups: "Tumor" and "Normal" according to the last 3 digits of "01A" and "11A" listed in the organization of the matrix. The "DESeq2" module of BiocManager software package was used to perform differential analysis on the 2 groups of gene matrices. The screening threshold was $|\log_2 \text{fold change (FC)}| > 1$, false discovery rate (FDR) <0.001. Eligible patients were those understood to possess a significantly differentially expressed gene (DEG), and the line named "*ENC1*" in the search results was used to obtain the up-regulation information of this gene. *ENC1* expression in the "Tumor" and "Normal" groups was extracted, and group comparison plots were used to describe the information of the 2 groups.

Prognostic survival analysis

We obtained the survival data of STAD and COAD Phenotype from the same Xena website, collated the data using R language, and performed Cox regression analysis using the "survival" package, and the difference was considered significant at P<0.05. The hazard ratio (HR) information of *ENC1* for overall survival (OS) could be found in the results. These samples were divided into a high expression group and a low expression group according to the median *ENC1* expression value. The survival rate of patients in the high and low expression groups was compared. A Kaplan-Meier (KM) survival curve was drawn using the R survival package and log rank test was performed for the curve. P<0.05 suggested that the *ENC1* expression level had a statistically significant difference in the prognosis and survival between the 2 groups.

Gene function enrichment analysis

We used the "DESeq2" model again to perform the difference analysis between the high expression group and low expression group divided by gene *ENC1* and obtained the DEGs. Gene function enrichment analysis was performed by Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis of the dataset through the "clusterprofiler" package based on these DEGs, and the results with P<0.05 were screened as significant enrichment results.

Correlation between gene expression and tumor immune infiltration

The FPKM datasets of STAD and COAD were divided into high and low expression groups according to the median expression of *ENC1*, and immune cell calculation and analysis were performed using the "xCell" package and plotted using "ggpubr".

Construction of protein-protein interaction (PPI) network interacting with ENC1

A PPI network was constructed by predicting proteins interacting with *ENC1* using the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) database. We entered "*ENC1*" in the database search page "Protein by name" option, selected "*Homo sapiens*" in the "organism" option, and selected the "Medium confidence (0.400)" option in the option of "minimum required interaction score" in the result set to obtain the analysis results and a PPI network was constructed.

Statistical analysis

The difference of *ENC1* gene expression between gastric cancer, colon cancer, and normal tissues was analyzed by *t*-test. The relationship between *ENC1* expression and the prognosis of gastric cancer and colon cancer was analyzed by Log rank test with test parameter P=0.05, and all tests were statistically analyzed by SPSS 22.0 (IBM Corp., Armonk, NY, USA).

Results

Data acquisition results

A total of 405 samples were collected from the STAD data chip set in the TCGA database, including 373 tumor tissue samples and 32 normal tissue samples; a total of 494 samples were collected from the COAD data chip set, including 453 tumor tissue samples and 41 normal tissue samples.

Differential gene expression analysis and display between tumor tissues and normal tissues

Through data mining and analysis of the dataset, it was found that the expression of *ENC1* in tumor tissues of both cancer patients was significantly higher than that

Table 1 Gene expression of *ENC1* in microarray datasets from two cancers

Cancer type	Log_2FC	P adj.	Change
STAD	1.97	9.08e-31	Up
COAD	2.06	1.10e-109	Up

STAD, stomach adenocarcinoma; COAD, colon adenocarcinoma; FC, fold change.

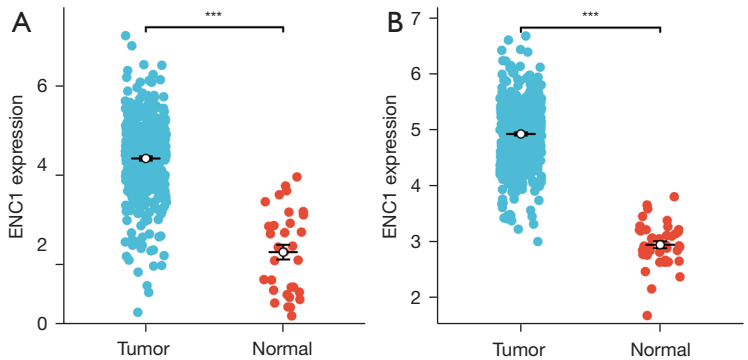

Figure 1 *ENC1* expression levels in different tumor types: (A) gastric cancer; (B) colon cancer. The number of dots indicates the significant differences between the two comparing groups. ***, P<0.001.

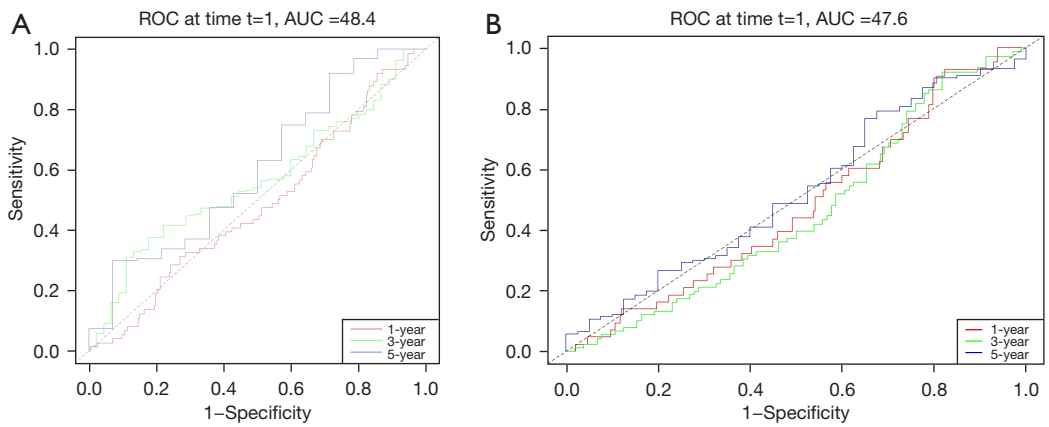

Figure 2 ROC curve for survival of patients with high *ENC1* expression: (A) gastric cancer; (B) colon cancer. t=1 indicates the 'Year-1' line, AUC num with % as the actual area. ROC, receiver operating characteristic; AUC, area under the curve.

in normal tissues, and its Log_2FC values were 1.97 and 2.06, respectively (as shown in *Table 1*), as represented by punctate plots in *Figure 1*.

Prognostic survival analysis

Cox regression analysis of the survival data of STAD and COAD using the R language's survival package revealed that high expression of *ENC1* was not significantly associated with the prognosis and survival time of gastric cancer patients and colon cancer patients: OS HR: 1.039, 95% confidence interval (CI): 0.890–1.213, P=0.627 for gastric cancer; OS HR: 0.886, 95% CI: 0.702–1.111, P=0.306 for colon cancer, and its 1-, 3-, and 5-year survival receiver operating characteristic (ROC) curves are shown in *Figure 2* and timeROC curves are shown in *Figure 3*.

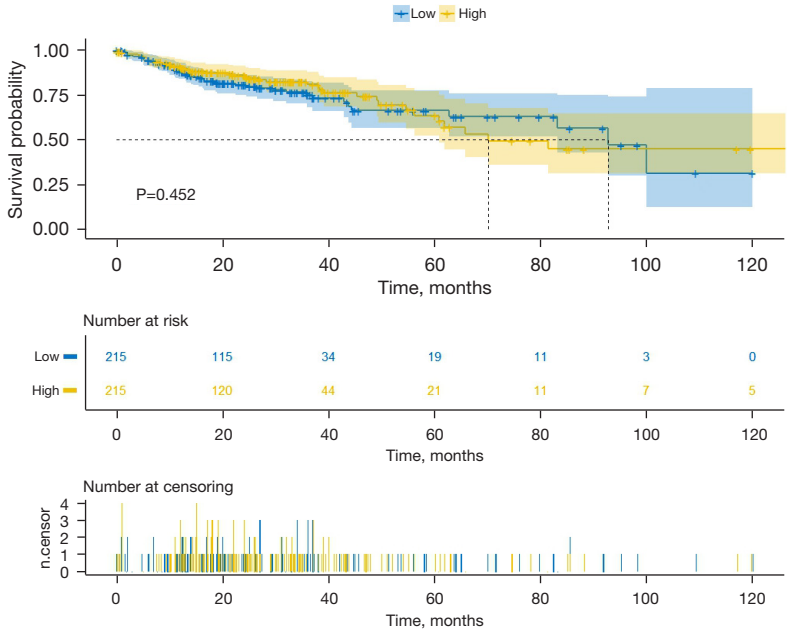

Figure 3 Comparison of timeROC curves of high *ENC1* expression with low *ENC1* expression in colon cancer patients. timeROC, time dependent receiver operating characteristic.

Functional enrichment analysis

For gene *ENC1*, KEGG pathway enrichment analysis showed that *ENC1* was mainly involved in neuroactive ligand-receptor interaction, as shown in *Figure 4*.

Correlation with tumor immunity

Through excavation analysis of tumor infiltration in the dataset, it was found that high expression of *ENC1* was significantly associated with each immune cell and different T cells such as basophils, $CD4^+$ memory T cells, $CD4^+$ TEM, and MV endothelial cells in gastric and colon cancers, as shown in *Figure 5*.

PPI network interacting with ENC1

A search of the STRING database to investigate the *ENC1* PPI network identified a total of 10 genes interacting with *ENC1*, comprising *KLHL20*, *KLHL25*, *SPOPL*, *LZTR1*, *SPOP*, *KLHL42*, *KEAP1*, *CUL3*, *RBX1*, and *TNFAIP1*, as shown in *Figure 6*, suggesting that *ENC1* may be involved in regulating neurite formation and neural crest cell differentiation.

Discussion

Gastric cancer and colon cancer are common tumors worldwide, but because their early symptoms are not obvious, most patients have reached an advanced stage by the time of diagnosis, resulting in poor prognosis and a serious threat to the health of patients (9,10). In recent years, the mechanism of various genes in the process of cancer development has been continuously revealed, which provides a solid theoretical basis for the treatment and diagnosis of gastric cancer and colon cancer, but no specific gene has been found to be used as an early diagnostic criterion or prognostic marker for these gastrointestinal malignant tumors.

Named for its expression pattern in the ectoderm and neurocortex, *ENC1* is expressed in the expected neuroectodermal region of the ectoderm during early embryogenesis and continues to be expressed throughout the development of the nervous system (11). Analysis of *ENC1* showed that it is a homolog of *KELCH*, an eosinophil gene essential for oogenesis, and *ENC1*, a BTB-Kelch protein belonging to the same family as Keap1, is mainly involved in the differentiation process of human central nervous cells and malignant transformation of cells and is

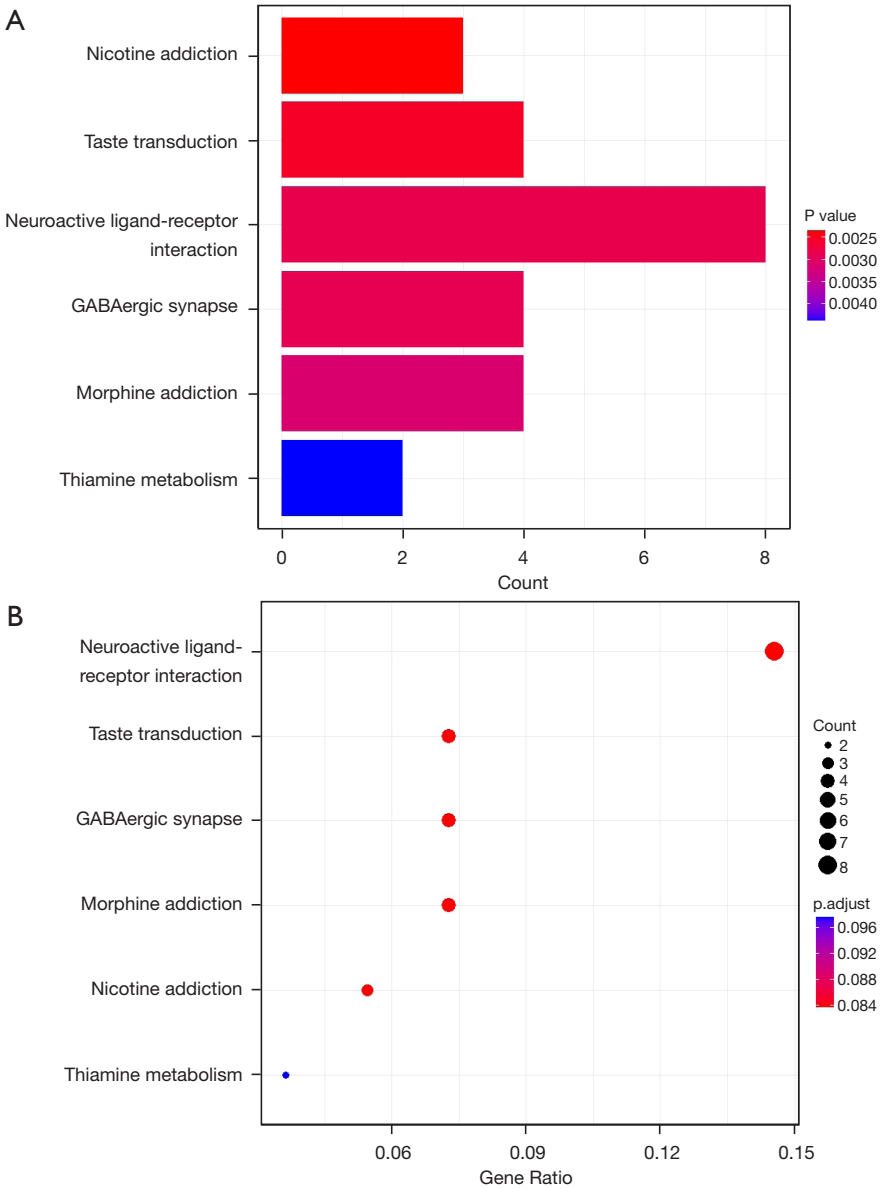

Figure 4 KEGG pathway functional enrichment results for DEGs between the different *ENC1* expressed groups (colon cancer). (A) The bar graph. (B) The bubble graph. KEGG, Kyoto Encyclopedia of Genes and Genomes; DEGs, differently expressed genes.

abnormally expressed in a variety of brain tumor tissues including gliomas and astrocytomas (12). *ENC1* is the only member of this family that is expressed in the nervous system and encodes a cytoplasmic protein that interacts with the actin cytoskeleton (13,14). *ENC1* is overexpressed in a variety of malignancies and is thought to play an important regulatory role in the malignant transformation of a variety of tumors. It has also been found that abnormal expression of *ENC1*, including gene mutation, overexpression, and localization errors, can promote cell proliferation and inhibit apoptosis, thus promoting the occurrence of brain tumors. Fan *et al.* found that down-regulation of *ENC1* expression significantly inhibited the proliferation, migration, and invasion of ovarian cancer cells, whereas low expression of *ENC1* was associated with a good prognosis in ovarian cancer patients (15). Therefore, it is speculated that *ENC1* is a crucial oncogene in tumor development.

In this study, we analyzed the expression of *ENC1* in

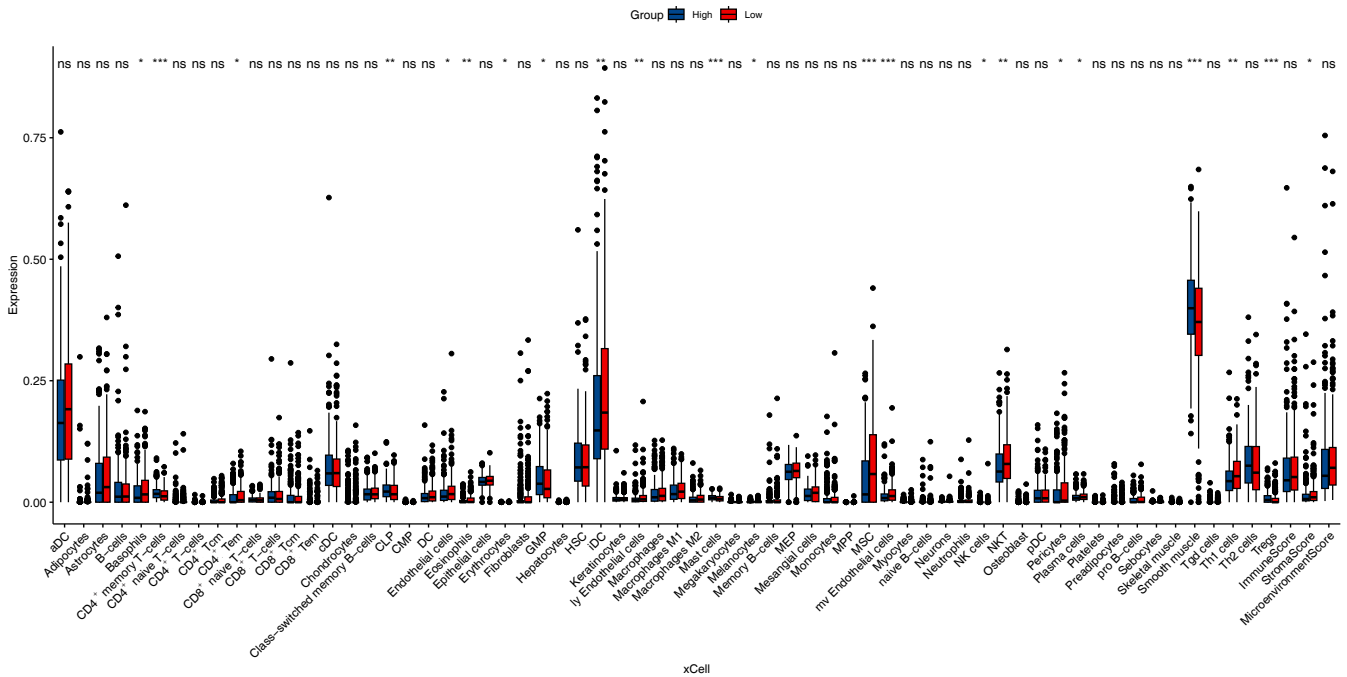

Figure 5 Correlation between *ENC1* expression and level of immune infiltration (colon cancer). The symbol *, P<0.05; **, P<0.01; ***, P<0.001; ns, P>0.05.

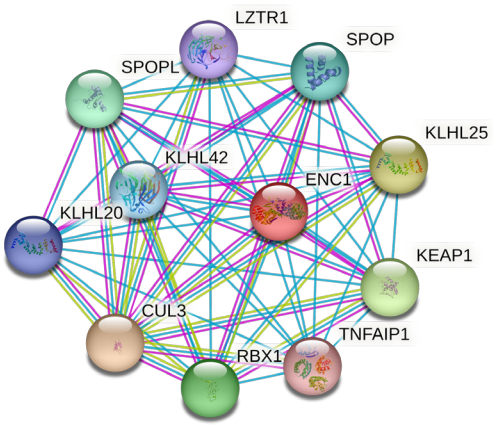

Figure 6 PPI network predicted by STRING database of *ENC1*. PPI, protein-protein interaction.

gastric cancer tissues and colon cancer tissues by data mining of a large sample composed of multiple datasets in TCGA, and the results showed that the expression of *ENC1* was higher than that in normal tissues in both STAD and COAD (Log_2FC =1.97 and 2.06, respectively). However, survival analysis showed that high and low expression of *ENC1* did not show a significant effect on the survival and prognosis of patients, and KM curves also showed that there was no significant difference between the 2 cancers, indicating that for gastrointestinal tumors, although *ENC1* expression in tumor tissues was higher than that in normal tissues, *ENC1* did not have a significant effect on prognosis. It is speculated that *ENC1* is mainly involved in the differentiation and malignant transformation of nerve cells in human heat stroke, and plays an obvious role in the proliferation and regulation of craniocerebral tumors, but *ENC1* does not play a major role in gastrointestinal tumors. As can also be seen from the KEGG functional enrichment of *ENC1*, *ENC1* has a huge impact on the neuroactive ligand-receptor interaction, but remains too small for other pathways. The PPI network of *ENC1* also showed that this gene was mainly involved in regulating neurite formation and neural crest cell differentiation, which echoed the aforementioned conclusions. However, although there was no significant correlation between *ENC1* and the prognosis of gastrointestinal cancer, in immune infiltration analysis, we still found that high and low *ENC1* expression showed significant differences at the level of some immune cells and *T* cells, suggesting that *ENC1* can predict some immune infiltration levels. In summary, *ENC1* does not become a key prognostic oncogene in the development of

gastrointestinal tumors, nor a gene that significantly affects cancer cell proliferation, migration, and invasion.

In this study, only STAD and COAD datasets were mined, and no other types of gastrointestinal tumors were involved. In the study by Cui *et al.*, *ENC1* promoted the occurrence and metastasis of colorectal cancer through epithelial-mesenchymal transition mediated by the JAK2/STAT5/AKT axis, indicating that *ENC1* may become a reasonable diagnostic marker and targeted therapy point for colorectal cancer (16). In another study on a mouse lung cancer model, reduced *ENC1* levels inhibited lung tumor growth, suggesting that *ENC1* is involved in proliferation, migration, and invasion of lung cancer cells and may therefore be an effective diagnostic target for lung cancer (17). A study by Zhang *et al.* also showed that *ENC1* played an important role in the mitogen-activated protein kinase (*MEK*)/extracellular signal-regulated kinase (*ERK*) pathway to promote cervical cancer (18). Evidence has shown that *ENC1* also was one of the key genes involved in the pathogenesis of Alzheimer's disease (19). He *et al.* found that upregulated *ENC1* predicts unfavorable prognosis and correlates with immune infiltration in endometrial cancer (8). Further investigation of *ENC1* is warranted.

In this study, we mined and analyzed the STAD and COAD datasets in TCGA and found that *ENC1* expression was increased in gastrointestinal tumors, but *ENC1* did not affect the survival and prognosis of patients, and speculated that *ENC1* was not a key gene affecting the occurrence and progression of gastrointestinal tumors. There are still some shortcomings in this study: (I) we only selected 2 datasets, STAD and COAD, and did not analyze the data of other types of gastrointestinal tumors (such as rectal cancer and small intestinal cancer), which may have biased the conclusions; (II) TCGA data have some shortcomings because they are provided by various countries or laboratories, the data of some tumors have not been updated recently, and due to sequencing technology or sequencing quality, RNA-seq data of TCGA do not fully represent the expression of tumor genes; (III) there is no significant demarcation line between the high and low expression of *ENC1*, and we used the median as the demarcation line, which may have caused data bias. Therefore, the conclusions reached in this study still need further experimental validation.

Conclusions

ENC1 expression is elevated in both gastric and colon cancers, and *ENC1* is associated with various immune cells and different T cells such as basophils, $CD4^+$ memory T cells, $CD4^+$ TEM, and MV endothelial cells in both gastric and colon cancers; however, *ENC1* does not affect the survival and prognosis of patients.

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Footnote

Reporting Checklist: The authors have completed the REMARK reporting checklist. Available at https://jgo.amegroups.com/article/view/10.21037/jgo-23-217/rc

Peer Review File: Available at https://jgo.amegroups.com/article/view/10.21037/jgo-23-217/prf

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://jgo.amegroups.com/article/view/10.21037/jgo-23-217/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).

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