Synaptophysin-like 2 expression correlates with lymph node metastasis and poor prognosis in colorectal cancer patients

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BACKGROUND
Colorectal cancer (CRC) is one of the most common and fatal cancers worldwide. Synaptophysin-like 2 (SYPL2) is a neuroendocrine-related protein highly expressed in skeletal muscle and the tongue. The involvement of SYPL2 in CRC, including its level of expression and function, has not been evaluated.

AIM
To evaluate the correlations of SYPL2 expression with lymph node metastasis (LNM) and prognosis in patients with CRC.

METHODS
The levels of expression of SYPL2 in CRC and normal colorectal tissues were analyzed in multiple public and online databases. The associations between clinical variables and SYPL2 expression were evaluated statistically, and the associations between SYPL2 expression and prognosis in patients with CRC were analyzed using the Kaplan-Meier method and univariate/multivariate Cox regression analyses. SYPL2 expression was assessed in 20 paired CRC tissue and adjacent normal colorectal tissue samples obtained from Fuyang People’s Hospital, and the associations between SYPL2 expression and the clinical characteristics of these patients were investigated. Correlations between the levels of expression of SYPL2 and key targeted genes were determined by Pearson’s correlation analysis. The distribution of immune cells in these samples was calculated using the CIBERSORT algorithm. Gene set enrichment analysis (GSEA) was performed to evaluate the biofunction and pathways of SYPL2 in CRC.

RESULTS
SYPL2 expression was significantly lower in CRC tissue samples than in normal
colorectal tissue samples ($P < 0.05$). High SYPL2 levels in CRC tissues correlated significantly with LNM ($P < 0.05$) and a poorer patient prognosis, including significantly shorter overall survival (OS) [hazard ratio (HR) = 1.9, $P < 0.05$] and disease-free survival (HR = 1.6, $P < 0.05$). High SYPL2 expression was an independent risk factor for OS in both univariate (HR = 2.078, $P = 0.014$) and multivariate (HR = 1.754, $P = 0.018$) Cox regression analyses. In addition, SYPL2 expression correlated significantly with the expression of KDR ($P < 0.0001$, $r = 0.47$) and the $BRAF^V600E$ mutation ($P < 0.05$). Higher SYPL2 expression was associated with the enrichment of CD8 T-cells and M0 macrophages in the tumor microenvironment. GSEA revealed that SYPL2 was associated with the regulation of epithelial cell migration, vasculature development, pathways in cancer, and several vital tumor-related pathways.

**CONCLUSION**
SYPL2 expression was lower in CRC tissue than in normal colorectal tissue. Higher SYPL2 expression in CRC was significantly associated with LNM and poorer survival.

**Key Words:** Synaptophysin-like 2; Colorectal cancer; Lymph node metastasis; Prognosis; Immune microenvironment; Bevacizumab

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**Core Tip:** In this research, we reported the expression and biofunctions of synaptophysin-like 2 (SYPL2) in colorectal cancer (CRC) for the first time. SYPL2 correlated with lymph node metastasis and a poor prognosis (both overall and disease-free survival) in CRC. SYPL2 mainly influence CD8 T-cell and M0 macrophage enrichment in the tumor microenvironment. Gene set enrichment analysis indicated that SYPL2 might also influence the tumor vasculature development. In addition, we found that SYPL2 was correlated with the effect of bevacizumab therapy.

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**INTRODUCTION**
Colorectal cancer (CRC), which is responsible for an estimated 8% of new cancer diagnoses and 8% of cancer deaths annually, is the third-most common cause of cancer deaths worldwide[1]. The stage at diagnosis is the most important predictor of survival, with 5-year relative survival rates of 90% for patients diagnosed with localized disease compared to 14% for patients diagnosed with distant-stage disease[2]. Complete mesocolic excision is the cornerstone of CRC treatment, showing good pathological outcomes as well as improvements in overall survival (OS), disease-free survival (DFS) and local recurrence[3]. Lymph node metastasis (LNM) is important in CRC staging and patient prognosis [4], with regional LNM being one of the most important indications for adjuvant chemotherapy[5,6]. Risk factors for LNM include lymphovascular invasion, histological grade, submucosal invasion depth, and tumor budding[7-9]. Although LNM of CRC is usually evaluated by radiologic methods, including computed tomography, magnetic resonance imaging, and positron emission tomography/computed tomography, these imaging methods cannot accurately evaluate LNM using criteria like short-axis diameter, signal heterogeneity, shape, and boundaries[10,11]. Several key biomarkers, however, have been reported to be predictive of LNM and prognosis in patients with CRC[12,13].

Immunotherapy and targeted therapy play important roles in the management of CRC. The molecular targets of CRC identified to date include epithelial growth factor receptor (EGFR), vascular endothelial growth factor (VEGF), human EGFR2, V-raf murine sarcoma viral oncogene homolog B1 (BRAF), Kirsten rat sarcoma (KRAS), P53 mutation, programmed cell death protein 1, and cytotoxic T-lymphocyte-associated protein 4[14,15]. Targeting these proteins in clinical practice has provided survival benefits for patients.

Synaptophysin-like 2 (SYPL2) is a neuroendocrine-related cytosolic protein enriched primarily in skeletal muscles and the tongue. The role of SYPL2 in cancer, including CRC, has not been determined. Thus, the present study comprehensively and systematically compared SYPL2 expression in CRC and normal colorectal tissues. Survival (Cox regression) analyses were also performed to assess the prognostic value of SYPL2 expression, along with other clinicopathological features. The correlation
between SYPL2 expression and the expression of key targeted genes in CRC was analyzed. Moreover, gene set enrichment analysis (GSEA) was performed to evaluate the SYPL2-associated biological pathways involved in CRC pathogenesis, providing clues about the function of SYPL2.

**MATERIALS AND METHODS**

**Data collection**

The gene-expression profiles and associated clinicopathological data of patients with CRC were downloaded from the Cancer Genome Atlas (TCGA) Genomic Data Commons Data Portal ([https://portal.gdc.cancer.gov/repository](https://portal.gdc.cancer.gov/repository)) on March 25, 2022. RNA-sequencing gene-expression HTSeq-FPKM data for 571 CRC tissue samples and 44 normal adjacent tissue samples were collected for further analysis. The GSE87211, GSE44076, GSE60331, and GSE103479 datasets were obtained from Gene Expression Omnibus microarrays. In addition, CRC and normal adjacent tissue samples were collected from 20 patients who underwent surgery for CRC at the Fuyang People’s Hospital. Demographic and clinical characteristics of these patients, including their age, sex, cancer stage, and lymph node status, were also recorded and analyzed. All participating patients provided written informed consent, and the study protocol was approved by the ethics review committees of Fuyang People’s Hospital.

**Gene Expression Profiling Interactive Analysis**

Gene Expression Profiling Interactive Analysis (GEPIA) is a newly developed, interactive web server that includes the RNA-sequencing expression data of 9736 tumors and 8587 normal samples from the TCGA and Genotype-Tissue Expression datasets, utilizing a standard processing pipeline. GEPIA offers customizable functions, such as tumor/normal differential expression analysis, profiling according to cancer type or pathological stage, patient survival analysis, detection of similar genes, correlation analysis, and dimensional reduction analysis. In the present study, GEPIA was used to perform differential expression, survival, and correlation analyses, the latter of which was performed with key targeted genes using Pearson’s test.

**Quantitative real-time polymerase chain reaction**

Total RNA extracted from cells using RNAprep Pure Tissue kits (Tiangen, Beijing, China) was reverse-transcribed to complementary DNA using the FastKing gDNA Dispelling RT SuperMix for quantitative polymerase chain reaction (qPCR) (Tiangen). The samples were subjected to quantitative real-time PCR (qRT-PCR) using 2 × SYBR Green qPCR Master Mix (Tiangen) and primers specific for SYPL2 (forward, 5’-CGCTGGTGGACTTCTGTG-3’; reverse, 5’-GCTGGATGGTCGTGTGG-3’), GAPDH (forward, 5’-AAGGTCGGAGTCAACGGA-3’; reverse, 5’-TTAAAAGCAGCCCTGGTGA-3’), with all gene primers obtained from Aoke Dingsheng Biotechnology (Beijing, China). Thermal cycling conditions consisted of an initial denaturation at 95 °C for 15 s, followed by 40 cycles of denaturation at 95 °C for 15 s, annealing at 55 °C for 30 s, and extension at 72 °C for 30 s. Relative messenger RNA expression levels were calculated using the \(2^{-\Delta\Delta CT}\) method, with the average level of SYPL2 expression in the 20 normal colorectal tissues defined as the reference for normalization and comparison with the 20 CRC tissues.

**Cox regression analyses and immune cell enrichment analyses**

Univariate and multivariate Cox analyses were used to investigate the association between SYPL2 expression and other clinical characteristics, such as age, sex, cancer stage, distant metastasis status, and lymph node status. OS was assessed by univariate Cox regression analyses, with factors significantly associated with OS subsequently entered into a multivariate Cox model. In addition, survival was directly analyzed using the Kaplan-Meier method (KM plotter: [http://kmplot.com](http://kmplot.com)). The presence of 22 types of immune cells in the CRC microenvironment was assessed using the CIBERSORT algorithm [16].

**GSEA**

Datasets and phenotype label files from TCGA were generated and uploaded into the GSEA software program. The phenotype labels were SYPL2 high expression and SYPL2 low expression (grouped relative to the median SYPL2 expression). Gene set permutations were conducted 1000 times for each analysis. Gene sets with ES > 0.6 and FWER \(P < 0.05\) were considered enriched.

**Statistical analysis**

Survival curves were plotted using the Kaplan-Meier method and compared using the log-rank test. Cox regression analyses were performed using the R “survival” package. Correlation analyses were performed by determining Pearson’s correlation coefficients. Categorical variables were compared using the chi-squared and Fisher’s exact tests, parametric continuous variables were compared using Student’s \(t\) tests, and non-parametric continuous variables were compared using Mann-Whitney \(U\) tests. Statistical analyses were performed using R version 4.1.0 (R Foundation for Statistical Computing, Vienna, Austria), Bioconductor ([https://www.bioconductor.org/](https://www.bioconductor.org/)), and GraphPad Prism 8 (GraphPad...
SYPL2 predicts the infiltration of immune cells into the CRC microenvironment

To assess the roles of SYPL2 in the tumor immune microenvironment, it was necessary to investigate the types of infiltrating immune cells in CRC patients. CIBERSORT evaluation of the relative proportions of 22 types of immune cells in all CRC specimens from TCGA showed high infiltration of regulatory T-cells and M0 macrophages in tumors with high SYPL2 expression (Figure 5A) and high infiltration of CD8 T-cells, activated CD4 memory T-cells, activated natural killer cells, and activated dendritic cells in tumors with low SYPL2 expression (Figure 5A). The level of SYPL2 expression had no effect on OS in tumors enriched with CD8 T-cells (P > 0.05, Figure 5B), whereas high expression of SYPL2 was closely associated with poorer OS in tumors unenriched with CD8 T-cells (P < 0.05, Figure 5C). High expression of SYPL2 was also associated with poorer OS in tumors both enriched (P < 0.05, Figure 5D) and unenriched (P < 0.05, Figure 5E) with M0 macrophages. Collectively, these findings show that SYPL2 expression correlates with the level of infiltration of most immune cells, possibly indicating the state of the tumor immune microenvironment and suggesting that SYPL2 might play different roles in different immune microenvironments.

GSEA identified functions and signaling pathways

GSEA was performed to determine the biological characteristics shared by tissue samples displaying different levels of SYPL2 expression and to identify the functions and pathways in which SYPL2 may be involved. Gene Ontology (GO) enrichment analyses indicated that SYPL2 was associated with the enrichment of genes involved in the positive regulation of vasculature development, epithelial cell migration, development growth, JUN kinase activity, MAP kinase activity, phospholipase activity, and single-organism cell adhesion (Figure 6A). In addition, Kyoto Encyclopedia of Genes and Genomes

| Characteristics | SYPL2 level | P value |
|----------------|-------------|---------|
|                | Low (n = 285) | High (n = 286) |       |
| Age (yr)       | 68.00 ± 12.14 | 65.31 ± 13.37 | 0.1148 |
| Gender         |             |         |       |
| Female         | 125         | 128     | 0.8273 |
| Male           | 145         | 143     |       |
| Unknow         | 20          | 15      |       |
| T              |             |         |       |
| T1 + T2        | 61          | 37      | 0.0140 |
| T3 + T4        | 179         | 192     |       |
| Unknow         | 45          | 57      |       |
| N              |             |         |       |
| N0             | 153         | 123     | 0.0312 |
| N1-2           | 87          | 105     |       |
| Unknow         | 45          | 56      |       |
| M              |             |         |       |
| M0             | 185         | 164     | 0.0955 |
| MI             | 29          | 39      |       |
| Unknow         | 71          | 83      |       |
| AJCC stage     |             |         |       |
| I-II           | 146         | 114     | 0.0228 |
| III-IV         | 88          | 106     |       |
| Unknow         | 51          | 66      |       |

*P < 0.05.
AJCC: American Joint Commission on Cancer; SYPL2: Synaptophysin-like 2.

(KEGG) analysis found that genes involved in basal cell carcinoma; cell-adhesion molecules; extracellular matrix receptor interactions; the epidermal growth factor receptor, hedgehog, mitogen-activated protein kinases (MAPK), NOTCH, transforming growth factor (TGF)-β, and WNT signaling pathways; GAP junctions; and vascular smooth muscle contraction were significantly enriched in CRC samples expressing high levels of SYPL2 (Figure 6B).

**DISCUSSION**

Members of the synaptophysin-like family, including SYPL1 and SYPL2, are synaptic vesicle membrane proteins. SYPL1 was originally considered a neuroendocrine-related protein but was found to be expressed in both neuronal and non-neuronal tissues[18]. A recent immunohistochemistry-based study showed that SYPL1 was prognostic of poor outcomes in patients with hepatocellular carcinoma and was associated with the epithelial-mesenchymal transition[19]. SYPL1 is also upregulated in pancreatic ductal adenocarcinoma, with higher SYPL1 expression being associated with tumor cell proliferation and poorer prognosis[20]. Serum SYPL1 may be a diagnostic marker for CRC, especially in patients with low serum carcinoembryonic antigen concentrations[21]. SYPL2, also called MG29, is primarily expressed in skeletal muscles and the tongue and is functionally thought to participate in cellular calcium ion homeostasis[22]. In addition, the SYPL2 gene has been associated with morbid obesity and may be involved in the development of excess body fat[23]. However, the roles and functions of SYPL2 in cancer and its related molecular mechanisms remain unknown.

The present study, using multiple public databases and donor-matched CRC and adjacent normal tissues, showed that the level of SYPL2 expression was lower in cancerous tissues than in normal tissues. An analysis of the associations between SYPL2 expression and clinical pathologic features
Table 2 Cox regression analysis of synaptophysin-like 2 expression and clinical pathological characteristics

| Characteristics | Univariate Cox | Multivariate Cox |
|-----------------|----------------|-----------------|
|                 | HR  | 95%CI         | P value | HR  | 95%CI         | P value |
| Age             | 1.037 | 1.015-1.059   | 0.0001* | 1.051 | 1.027-1.075   | 0.0001* |
| Gender (male)   | 1.160 | 0.746-1.803   | 0.509   | 0.987 | 0.621-1.571   | 0.957   |
| T               |     |               |         | 1     |               |         |
| T1              | 0.819 | 0.169-3.953   | 0.804   | 0.354 | 0.069-1.805   | 0.212   |
| T2              | 1.324 | 0.322-5.456   | 0.697   | 0.424 | 0.096-1.863   | 0.256   |
| T3              | 4.661 | 1.082-20.075  | 0.039*  | 0.906 | 0.189-4.348   | 0.901   |
| N               |     |               |         | 1     |               |         |
| N0              | 2.547 | 1.484-4.368   | 0.0001* | 1.988 | 1.048-3.770   | 0.035*  |
| N1              | 4.195 | 2.479-7.099   | 0.0001* | 2.253 | 1.179-4.304   | 0.014*  |
| N2              | 4.482 | 2.829-7.100   | 0.0001* | 2.585 | 1.468-4.660   | 0.001*  |
| M (M1)          |     |               |         | 1     |               |         |
| ACJJ            |     |               |         | 1     |               |         |
| I               | 1.474 | 0.548-3.961   | 0.442   | 2.858 | 1.086-7.627   | 0.033*  |
| III              | 2.253 | 1.179-4.304   | 0.014*  | 2.585 | 1.468-4.660   | 0.001*  |
| IV              | 8.096 | 3.135-20.903  | 0.0001* | 6.123 | 3.246-10.793  | 0.0001* |
| SYPL2 (high)    | 2.078 | 1.162-3.716   | 0.014*  | 1.754 | 1.103-2.790   | 0.018*  |

*P < 0.05.

AJCC: American Joint Commission on Cancer; SYPL2: Synaptophysin-like 2; HR: Hazards ratio; CI: Confidence interval.

revealed that higher SYPL2 levels in CRC patients were associated with lymph node metastases (N stage) and more advanced tumors (T stage), although qRT-PCR analysis found that higher SYPL2 expression was associated only with LNM. Univariate and multivariate Cox analyses and survival analyses indicated that SYPL2 expression level is a potential independent marker of poor prognosis in patients with CRC. Correlation analyses showed that the SYPL2 gene-expression level was significantly associated with the expression of KDR (also called VEGFR) (R > 0.4) and EGFR (R > 0.3) and with BRAFV600E mutation (P < 0.05). Carvalho et al.[24] reported that VEGFR expression is associated with the effect of bevacizumab therapy, and Szablewski et al.[25] found that EGFR overexpression and mutations in KRAS and BRAF contribute to colorectal carcinogenesis. Moreover, EGFR-directed molecular treatments could be investigated in a subset of patients affected by intestinal-type adenocarcinoma. These results of above studies were consistent with our research.

Agents targeting VEGFR or EGFR and multiple tyrosine kinase inhibitors play an important role in CRC management[26]. The mutation of BRAFV600E residue occurs in approximately 10% of CRCS, constituting a group with a particularly poor prognosis. And our result also found that SYPL2 was higher expression in the BRAFV600E mutation group, and associated with poor prognosis. The mutation of BRAFV600E is also extremely associated with targeted therapy of metastatic CRC[27]. Our result suggests that SYPL2 may be a biomarker for predicting targeted treatment of CRC patients. We collected a total of 16 tumor samples prior to bevacizumab treatment from GSE60331 for validation because there is greater clinical practice value in predicting the treatment response by considering pre-treatment genes. Our result revealed that the SYPL2 higher-expression group seemed more likely to respond to bevacizumab. Existing evidence indicates that tumor progression may result from the escape of cancer cells from host immunosurveillance[28]. Therefore, clarifying the infiltrating immune cells in the tumor microenvironment may help to elucidate the underlying mechanism involving SYPL2 in CRC. Moreover, SYPL2 may be involved in the immune microenvironment by enriching CD8 T-cells and M0 macrophages. These data indicate that SYPL2 might influence the infiltration of immunocytes and lead to a worse prognosis in CRC.

GO enrichment analysis of the biological functions of SYPL2 in CRC indicated that SYPL2 might regulate epithelial cell migration, vasculature development, MAPK kinase activity, and cell adhesion. Furthermore, KEGG enrichment analysis found that SYPL2 might participate in cell adhesion; several
**Figure 1** Synaptophysin-like 2 expression levels and survival analyses in colorectal cancer. A: Synaptophysin-like 2 (SYPL2) expression in 275 colon cancer tissue and 349 normal colon tissue samples and in 92 rectal cancer tissue and 318 normal rectal tissue samples from Gene Expression Profiling Interactive Analysis (GEPIA) (\(P < 0.05\)); B: SYPL2 expression in 203 colorectal cancer (CRC) tissue and 160 normal colorectal tissue samples from GSE87211 (\(P < 0.01\)); C: SYPL2 expression in tumour tissues and adjacent normal colon tissue samples from 20 CRC patients who underwent tumour resection at Fuyang People’s Hospital (\(P < 0.05\)). The average expression of SYPL2 in the 20 normal tissue samples was regarded as a reference; E: Associations of SYPL2 expression with the overall survival (OS) of CRC patients from GEPIA (\(P < 0.05\)); F: Associations of SYPL2 expression with the disease-free survival (DFS) of CRC patients from GEPIA (\(P < 0.05\)); G: Associations of SYPL2 expression with the OS of CRC patients from the Kaplan-Meier plotter (\(P < 0.05\)); H: Associations of SYPL2 expression with the DFS of CRC patients from the Kaplan-Meier plotter (\(P < 0.05\)); I: Associations of SYPL2 expression with the OS of stage II CRC patients from TCGA (\(P < 0.05\)); J: Associations of SYPL2 expression with the OS of stage III CRC patients from TCGA (\(P < 0.05\)).

**Figure 2** Correlations of tumor expression of synaptophysin-like 2 among the 20 colorectal cancer patients who underwent tumor resection at Fuyang People’s Hospital. A: T stage; B: Lymph node metastasis. SYPL2: Synaptophysin-like 2; LNM: Lymph node metastasis. *\(P < 0.01\).
Figure 3 Correlations of levels of synaptophysin-like 2 expression with Kirsten rat sarcoma $BRAF^{V600E}$, and $P53$ mutations from GSE103479 and levels of expression of the key target genes $KDR$, epidermal growth factor receptor, vascular endothelial growth factor A, $CD274$, $PDCD1$, and $CTLA4$ from Gene Expression Profiling Interactive Analysis. A: Association of synaptophysin-like 2 (SYPL2) expression with $KRAS$, $BRAF^{V600E}$, $P53$ mutation; B: Association of SYPL2 expression with KDR, epidermal growth factor receptor, vascular endothelial growth factor A, CD274, PDCD1, cytotoxic T-lymphocyte-associated protein 4. SYPL2: Synaptophysin-like 2; MT: Mutation type; WT: Wild type; EGFR: Epidermal growth factor receptor; VEGFA: Vascular endothelial growth factor A; CTLA4: Cytotoxic T-lymphocyte-associated protein 4.

Figure 4 Levels of synaptophysin-like 2 expression in colorectal cancer patients from GSE60331 who did and did not respond to bevacizumab treatment. $aP < 0.05$. 

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Figure 5 Effects of synaptophysin-like 2 expression level on the immune microenvironment in colorectal cancer from the Cancer Genome Atlas. A: Statistical chart after using the CIBERSORT method, showing the different proportions of immune cells in groups of patients with high (red) and low (blue) synaptophysin-like 2 expression; B-E: Survival analyses of patient subgroups. SYPL2: Synaptophysin-like 2; HR: Hazards ratio.
Figure 6 Enrichment plots from the Gene set enrichment analysis. A: Gene Ontology; B: Kyoto Encyclopedia of Genes and Genomes.
which may in turn regulate the quantity and differentiation of immune cells[31]. CD8+ T-cells are typically thought to be a homogenous group of cytotoxic cells that produce interferon-γ[32]. In addition, CD8+ T lymphocytes are the major anti-tumor effector cells[33]. In this study, CD8+ T-cell counts in the SYPL2 high-expression group were significantly lower than those in the SYPL2 low-expression group. Therefore, SYPL2 might contribute to the poor prognosis of CRC by affecting immune cell infiltration. However, the relevant molecular and pathway mechanisms still necessitate further experiments for verification.

The present study was designed to evaluate SYPL2 gene expression in CRC and reveal the associations of SYPL2 with pathologic features and survival outcomes. This study used only GSEA to analyze biological functions and the molecular mechanism of SYPL2 in CRC. Further studies of SYPL2 protein expression and its associations with biological functions and molecular mechanisms in CRC are warranted.

CONCLUSION

SYPL2 expression was lower in CRC than in adjacent normal tissue, suggesting that SYPL2 may be a potential diagnostic and prognostic CRC-specific molecular marker. High SYPL2 expression was significantly associated with lymph node metastases and poorer survival.

ARTICLE HIGHLIGHTS

Research background
Colorectal cancer (CRC) is the third-most common cause of cancer deaths worldwide and lymph node metastasis (LNM) is important in CRC staging and patient prognosis. Risk factors for LNM include lymphovascular invasion, histological grade, submucosal invasion depth, and tumor budding. In addition, LNM of CRC is usually evaluated by radiologic methods, including computed tomography, magnetic resonance imaging etc. However, these imaging methods cannot accurately evaluate LNM. It was necessary to investigate key biomarkers to predict LNM and prognosis in patients with CRC. Moreover, synaptophysin-like 2 (SYPL2) is a neuroendocrine-related cytosolic protein enriched primarily in skeletal muscles and the tongue. The role of SYPL2 in cancer, including CRC, has not been determined.

Research motivation
The role of SYPL2 in CRC has not been studied. The present study comprehensively and systematically compared SYPL2 expression and potential functions. The relationship between SYPL2 expression and clinicopathological characteristics was completed. And we found that high expression of SYPL2 was significantly associated with LNM and worse prognosis. And we verified the results by experiment. In addition, we analyzed the correlation between SYPL2 expression and the expression and mutation of target genes.

Research objectives
This study aimed to investigate the SYPL2 expression, potential biological functions and pathways, correlation clinicopathological characteristics and prognosis in CRC.

Research methods
The gene expression profiles and associated clinicopathological data of patients with CRC were downloaded from multiple public and online databases {The Cancer Genome Atlas, GEO, Gene Expression Profiling Interactive Analysis [gene set enrichment analysis (GSEA)]}. The associations between clinical variables, prognosis and SYPL2 expression were analyzed statistically using the Kaplan-Meier method, univariate/multivariate Cox regression analyses, chi-squared and Fisher’s exact tests. In addition, we collected 20 paired CRC tissue and adjacent normal colorectal tissue samples for validation by quantitative real-time polymerase chain reaction (qRT-PCR). GSEA was performed to evaluate the biofunction and pathways of SYPL2 in CRC.

Research results
SYPL2 expression was significantly lower in CRC tissue samples than in normal colorectal tissue samples. High SYPL2 levels in CRC tissues correlated significantly with LNM and worse prognosis. High SYPL2 expression was an independent risk factor for overall survival in both univariate and multivariate Cox regression analyses. SYPL2 expression correlated significantly with the expression of KDR and high SYPL2 expression was correlate with the response to bevacizumab treatment. Higher SYPL2 expression was associated with the enrichment of CD8 T-cells and M0 macrophages. GSEA
revealed that SYPL2 was associated with the regulation of epithelial cell migration, vasculature development, pathways in cancer, and several vital tumor-related pathways.

Research conclusions
SYPL2 expression was lower in CRC than in adjacent normal tissue. However, high SYPL2 expression was significantly associated with lymph node metastases and poorer survival.

Research perspectives
The SYPL2 gene expression and the correlations between clinical variables, prognosis were analyzed by multiple public and online databases. Furthermore, we collected 20 paired CRC tissue and adjacent normal colorectal tissue samples for validation by qRT-PCR.

FOOTNOTES

Author contributions: Zhao ZX and Liu QL collected data and completed the manuscript; Yuan Y collected relevant reference and completed a part of experiment; Wang FS designed the study and assisted in writing the manuscript; and all authors read and approved the final manuscript.

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