78550_Auto_Edited-check.docx

Basic Study

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

Zhao ZX *et al*. SYPL2 LNM CRC

Abstract

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 ($P = 0.0321$, $c^2 = 4.644$) 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

Zhao ZX, Liu QL, Yuan Y, Wang FS. Synaptophysin-like 2 expression correlates with lymph node metastasis and poor prognosis in colorectal cancer patients. *World J Gastrointest Oncol* 2022; In press

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.

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) 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') and 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 ºC for 15 s, followed by 40 cycles of denaturation at 95 ºC for 15 s, annealing at 55 ℃ for 30 s, and extension at 72 ºC 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). 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/), and GraphPad Prism 8 (GraphPad Software, San Diego, CA, United States), with P < 0.05 defined as statistically significant.

RESULTS

Expression of SYPL2 and its association with prognosis in CRC

GEPIA showed that the mean expression of SYPL2 was significantly lower in 275 colon cancer tissue samples than in 349 normal colon tissue samples and was lower in 92 rectal cancer tissue samples compared to 318 normal rectal tissue samples (P < 0.05, Figure 1A). Similar findings were observed when SYPL2 expression was compared between 203 CRC and 160 normal colorectal tissue samples (GSE87211) (P < 0.001,

Figure 1B) and between 98 CRC samples, 98 adjacent normal colorectal tissue samples, and 50 healthy normal tissue samples ($P < 0.0001$, Figure 1C). These findings were confirmed by comparing SYPL2 expression by qRT-PCR in 20 freshly obtained CRC and adjacent normal tissue samples ($P < 0.05$, Figure 1D). A high expression of SYPL2 was significantly associated with poorer OS and DFS ($P < 0.05$, Figures 1E and 1F), with these results confirmed by Kaplan-Meier analysis ($P < 0.05$, Figures 1G and 1H). Furthermore, high expression of SYPL2 was significantly associated with a worse OS in both stage II and III CRC (Figures 1I and 1J).

Correlations of SYPL2 expression with clinicopathological characteristics of patients with CRC

Associations between SYPL2 expression and the clinicopathological characteristics of patients with CRC were assessed by dividing CRC patients into two groups based on the median SYPL2 expression. High expression of SYPL2 was significantly associated with greater tumor depth ($P = 0.0140$, $c^2 = 6.078$), LNM ($P = 0.0321$, $c^2 = 4.644$), and greater American Joint Committee on Cancer stage ($P = 0.0228$, $c^2 = 5.182$) (Table 1). An analysis of the 20 paired CRC and normal colon tissue samples showed that SYPL2 expression was significantly correlated with LNM ($P < 0.001$, Figure 2B). Univariate Cox regression analysis showed that high expression of SYPL2 was associated with poorer OS [hazard ratio (HR) = 2.078; 95% confidence interval (CI): 1.162-3.716; $P = 0.014$], older age ($P < 0.05$), and higher TNM stage ($P < 0.05$) (Table 2), with multivariate Cox analysis revealing that high SYPL2 expression remained an independent risk factor for OS (HR = 1.754; 95%CI: 1.103-2.790; $P = 0.018$).

Correlation between SYPL2 expression and the expression of key target genes
Correlation analyses were performed to determine whether SYPL2 could act as a biomarker to predict the outcomes of targeted therapy in patients with CRC. SYPL2 expression correlated significantly with the mutation of $BRAF^{V600E}$ ($P < 0.05$) (Figure 3A) and the expression levels of *KDR* ($P < 0.0001$, $r = 0.47$), *EGFR* ($P < 0.0001$, $r = 0.33$),

CTLA4 (*P* < 0.01, *r* = 0.15), and *PDCD1* (*P* < 0.01, *r* = 0.15) (Figure 3B). We collected a total of 16 tumor samples entered into GSE60331 prior to undergoing treatment with bevacizumab; the responder group included seven samples and the non-responder group contained nine samples. Some detailed information can be retrieved from GSE60331[17]. Moreover, high SYPL2 expression was found to correlate with the response to bevacizumab treatment (*P* < 0.05, Figure 4). Taken together, these findings indicate that SYPL2 may be a potential biomarker of response of CRC to targeted therapy.

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 (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 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 *BRAF*[V600E] 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[24]. The mutation of *BRAF*[V600E] 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 BRAF[V600E] mutation group, and associated with poor prognosis. The mutation of *BRAF*[V600E] is also extremely associated with targeted therapy of metastatic CRC[25]. 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[26]. 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 cancer-related pathways, including pathways in basal cell carcinoma, renal cancer, and small-cell lung cancer; and several vital tumor-related signaling pathways, including the hedgehog, MAPK, NOTCH, and TGF-β signaling pathways.

In this study, SYPL2 expression in tumor tissues was significantly lower than that in normal tissue. However, higher SYPL2 expression was associated with worse survival in CRC. The paradox of the opposite effect of SYPL2 expression might be due to the following reasons. First, compared to normal tissue, tumor tissue can abnormally activate a series of signaling pathways and have special tumor microenvironment[27,28]. Some genes (including SYPL2) may play roles in promoting or suppressing cancer in specific signal pathways in the tumor microenvironment. Second, SYPL2 might work as a biomarker in CRC. Higher expression of SYPL2 could be associated with some specific and powerful activated oncogenic genes and enhanced malignant behavior of tumors. Finally, tumor-infiltrating immune cells are closely related to tumorigenesis, angiogenesis, and tumor cell growth and metastasis, which may in turn regulate the quantity and differentiation of immune cells[29]. CD8+ T-cells are typically thought to be a homogenous group of cytotoxic cells that produce interferon-γ[30]. In addition, CD8+ T lymphocytes are the major anti-tumor effector cells[31]. 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 (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.

78550_Auto_Edited-check.docx

8   Zuolei Jing, Xinyuan He, Zhirong Jia, Yunli Sa, Bolin Yang, Ping Liu. "NCAPD2 inhibits autophagy by regulating Ca2+/CAMKK2/AMPK/mTORC1 pathway and PARP-1/SIRT1 axis to promote colorectal cancer", Cancer Letters, 2021
16 words — < 1%
Crossref

9   f6publishing.blob.core.windows.net
15 words — < 1%
Internet

10 www.anatoljcardiol.com
15 words — < 1%
Internet

11 acsjournals.onlinelibrary.wiley.com
14 words — < 1%
Internet

12 www.frontiersin.org
14 words — < 1%
Internet

13 www.researchgate.net
14 words — < 1%
Internet

14 Kang-Ming Zhu, Hui Yuan, Jing Li. "Decreased expression of KPNA2 in Hepatocellular carcinoma infers favorable prognosis and high immune infiltrating level", Research Square Platform LLC, 2021
13 words — < 1%
Crossref Posted Content

15 Yifei Dai, Weijie Qiang, Kequan Lin, Yu Gui, Xun Lan, Dong Wang. "An Immune-Related Gene Signature for Predicting Survival and Immunotherapy Efficacy in Hepatocellular Carcinoma", Research Square Platform LLC, 2020
13 words — < 1%
Crossref Posted Content

16 Zhenjiang Wang, Mingyi Guo, Xinbo Ai, Jianbin Cheng, Zaiwei Huang, Xiaobin Li, Yuping Chen.
13 words — < 1%

"Identification of Potential Diagnostic and Prognostic Biomarkers for Colorectal Cancer Based on GEO and TCGA Databases", Frontiers in Genetics, 2021
Crossref

17   tessera.spandidos-publications.com
      Internet   13 words — < 1%

18   Sisi Chen, Ying Li, Muyao Wu, Lian Xue et al.   12 words — < 1%
      "Nucleolar and Coiled-Body Phosphoprotein 1 Is
      Associated With Stemness and Represents a Potential
      Therapeutic Target in Triple-Negative Breast Cancer", Frontiers
      in Oncology, 2022
      Crossref

19   academic-accelerator.com
      Internet   12 words — < 1%

20   citeseerx.ist.psu.edu
      Internet   12 words — < 1%

21   www.cambridge.org
      Internet   12 words — < 1%

22   www.pubfacts.com
      Internet   12 words — < 1%