High Expression of ISYNA1 Predicts Unfavourable Prognosis in Patients with Colorectal Cancer

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Abstract

Background: Mining the prognostic biomarkers of colorectal cancer (CRC) has important clinical and scientific significance. Inositol-3-phosphate synthase 1 (ISYNA1) has been reported in very few previous literature and its role in cancer remains controversial. However, the prognostic role of ISYNA1 in CRC still remains to be characterized.

Methods: The potential prognostic factor ISYNA1 was screened out through TCGA database analysis. Then its expression and associations between clinicopathological variables were assessed in TCGA CRC cohort. The prognostic value of ISYNA1 was assessed by multiple methods, such as ROC curve, Kaplan-Meier method and univariate/multivariate Cox regression analysis. Then, functional enrichment and annotation among the high and low ISYNA1 groups were achieved by GO and KEGG analysis and GSEA. CRC tissue microarray and fresh CRC tissue samples obtained clinically were used for further validation.

Results: ISYNA1 was highly expressed in CRC tissues compared to normal tissues. Meantime, overexpression of ISYNA1 correlated with higher N stage, M stage, AJCC stage, more lymphatic invasion and more R2 resection. Besides, high expression of ISYNA1 was closely correlated to less favorable OS, DSS and PFI. Moreover, ISYNA1 was identified an independent prognostic factor of CRC by performing univariate/multivariate Cox regression analysis. The analysis of CRC tissues microarray further confirmed the conclusion drawn from TCGA data analysis.

Conclusion: ISYNA1 is notably upregulated in CRC tissues and may serves as a potential biomarker of CRC.

Introduction

Colorectal cancer (CRC) is the third most common type of malignant tumor and the second leading cause of cancer-related deaths worldwide[1]. The mortality rate among patients with CRC has gradually decreased owing to advances in medical technology. Nevertheless, the prognosis for patients with advanced CRC remains extremely poor, with the 5-year survival rate being 14%[2]. CRC is caused by numerous factors that involve multiple genetic and epigenetic changes. Over the past several decades, many researchers have attempted to unveil the underlying mechanism. Despite this, our understanding of the associated molecular mechanisms remains limited, and additional studies are urgently required. Numerous molecular biomarkers have been unearthed, and they have contributed to the early detection and selection of appropriate therapeutic options in the diagnosis and treatment of CRC. Therefore, it is of great scientific interest and clinical importance to identify additional biomarkers of CRC.

Inositol or cyclohexanol, a type of water-soluble vitamin, is widely distributed in animals and plants. It serves as a growth factor and performs multiple functions, such as lipid metabolism[3], carbohydrate metabolism[4], hair growth[5], and cancer cell proliferation[6]. Inositol-3-phosphate synthase 1 (ISYNA1) catalyzes the rate-limiting conversion of glucose 6-phosphate to myoinositol 1-phosphate, thus playing an important role in the biosynthesis pathway of inositol[7]. As reported in the literature, ISYNA1

overexpression promotes the proliferation and inhibits the apoptosis of bladder cancer cells[8]. However, ISYNA1 has been identified as a tumor suppressor in pancreatic cancer[9]. Accordingly, ISYNA1 may exert distinct functions in distinct types of tumors. Nevertheless, thus far, the causative role of ISYNA1 has not been reported in the CRC literature.

In the present study, we perform bioinformatic analysis of the ribonucleic acid (RNA)-sequencing dataset obtained from The Cancer Genome Atlas (TCGA) database to determine the impact potential and prognostic value of ISYNA1 for CRC patients. Moreover, we use clinically obtained specimens and tissue microarrays for further verification of the conclusion drawn from the analysis of TCGA CRC cohort. Gene set enrichment analysis (GSEA), and gene ontology (GO) and Kyoto Encyclopedia Genes and Genomes (KEGG) analyses, are performed to evaluate the biological pathways involved in the ISYNA1 regulatory networks in CRC progression.

Methods

Experimental design and data acquisition

The specific experimental process is shown in the flowchart (Fig.1). Level 3 RNA-seq V2 data sets and matched clinicopathological information were downloaded from TCGA CRC cohort (<https://portal.gdc.cancer.gov/>; December 15, 2018), and then the transcriptome data were normalized by using DESeq package as previous literature described. The limma R package and survival R package were used for screening for differentially expressed genes and prognosis-correlated genes respectively.

Patients specimens and tissue microarray

Fresh cancerous tissues and matched adjacent non-tumorous tissues were obtained from CRC patients who underwent surgery and confirmed by histopathological examination in Fujian Cancer Hospital and then were cryopreserved in liquid nitrogen for further analysis. The human CRC tissue microarrays (TMA) containing 40 pairs of CRC tissues and matched adjacent normal tissues were also collected from surgery and histopathological validation.

Gene set enrichment analysis

GSEA of microarray data was carried out by using the GSEA v.3.0 desktop software (Broad Institute) to explore the potential biological mechanisms of ISYNA1. If they reached the preset threshold of $P < 0.05$, the biological processes were considered to be apparently enriched.

GO and KEGG enrichment analyses

R package was used for GO and KEGG enrichment analyses and visualization as previous described[10]. ClusterProfiler R package was used for enrichment analysis, Org. Hs.eg.db R package [3.10.0 version] was used for ID conversion and GOplot R package [1.0.2 version] was used for calculating Zscore.

Western blot

Fresh surgical specimens were grounded and then lysed in RIPA buffer (Beyotime, China) with added protease inhibitor cocktail for 20 minutes in an ice bath. Then the lysis mixture was centrifuged for 10 minutes at 15,000 rpm and supernatants were collected for protein quantification with BCA Protein Assay Kit (abcam, USA). 20 micrograms of protein sample were electrophoresed by 10% SDS-polyacrylamide gels, and , blotted on PVDF membranes (Beyotime, China). The blotted PVDF membranes were blocked with 5% milk/TBST at room temperature for 1h and then incubated overnight at 4°C with primary antibody against ISYNA1 (14142-1-AP, Proteintech, China). Then, PVDF membranes was incubated at room temperature for 1h with secondary antibody and then detected by chemi-luminescence HRP substrate kit (Millipore, GER).

Immunohistochemistry

The TMA was used to performed immunohistochemistry (IHC) according to the methods described in previous literature[11]. The expression of ISYNA1 was detected with a special antibody dilution (proteintech, China, 1:500) . Then the stained TMA were examined by two expert pathologists independently, who were both blinded to the detail clinicopathological data. Protein expression of ISYNA1 was determined by evaluating the staining intensity of positive staining, scored as negative, weak, medium, and strong.

Statistical analysis

Analysis was performed by using SPSS 20.0. The data in figures and tables were presented as the means $\pm$ SD. The Kaplan-Meier method and Log-rank test were used to determine the overall survivals (OS), disease-free survivals (DFS) and progress free interval (PFI) between different groups. Spearman's correlation coefficient was utilized to analyze the correlation between ISYNA1 expression and the promoter methylation level. Fisher's exact tests were used to analyze the correlation between ISYNA1 expression in CRC tissues and that in paired non-tumorous tissues. Student's two-tailed unpaired t-test was performed to analyze the ISYNA1 expression in different groups. Differences at $P\leq 0.05$ were considered to be significantly different.

Results

ISYNA1 is screened as a differentially expressed prognosis-related gene.

Data were obtained from TCGA database and a microarray analysis was then performed by using Limma R package to compare the gene expression profiles of CRC and normal tissue groups. A total of 2792 upregulated genes were detected based on the screening parameters set with a $p\leq 0.05$ and Fold change ≥ 2 . The survival R package used for screening for OS-related genes and PFI-related genes based on the parameters set with $p\leq 0.05$. Then 309 genes and 496 genes that can affect OS and PFI were detected respectively. In order to further study differentially expressed genes associated with different

prognosis, these three high-throughput analysis data were overlapped and only 29 candidate genes left. Then genes, of which functions have been elaborated previously or the association with CRC has been confirmed in previous study, were eventually abandoned. ISYNA1 was finally found (Fig. 1).

Expression of ISYNA1 was remarkably overexpressed in CRC and multiple types of cancers

The analysis of TCGA CRC data revealed that ISYNA1 was notably upregulated in CRC tissues compared to normal tissues ($P < 0.001$) (Fig. 2B). Meantime, ISYNA1 was highly expressed in CRC tissues compared to matched adjacent normal tissues in TCGA cohort ($P < 0.001$) (Fig. 2C). Moreover, the expression of ISYNA1 was significantly negatively correlated with its promoter methylation level (Spearman correlation = -0.217, $P = 0.001$) (Fig. 2D). We also showed that ISYNA1 was upregulated in multiple types of cancers, such as bladder Urothelial Carcinoma (BLCA), breast invasive carcinoma (BRCA), cholangiocarcinoma (CHOL), esophageal carcinoma (ESCA), glioblastoma multiforme (GBM), head and Neck squamous cell carcinoma (HNSC), lung adenocarcinoma (LUAD), lung squamous cell carcinoma (LUSC), pheochromocytoma and paraganglioma (PCPG), skin cutaneous melanoma (SKCM), thyroid carcinoma (THCA), thymoma (THYM), stomach adenocarcinoma (STAD) etc., but downregulated in urinary system tumors, such as kidney chromophobe (KICH), kidney renal clear cell carcinoma (KIRC), kidney renal papillary cell carcinoma (KIRP), prostate adenocarcinoma (PRAD) (Fig. 2E).

Association of ISYNA1 expression and clinicopathological characteristics

Clinicopathological data obtained from TCGA CRC cohort, including gender, serum carcinoembryonic antigen (CEA) level, perineural invasion, lymphatic invasion, residual tumor, TNN classification, were analyzed. The results revealed that the expression level of ISYNA1 in the tissues of patients with N2 stage was significantly higher than that in patients with N1 ($P = 0.05$) and N0 stages ($P = 0.001$) (Fig. 3B). Moreover, ISYNA1 expression was significantly associated with AJCC stage ($P = 0.01$) (Fig. 3D) and metastasis (M stage) ($P = 0.05$) (Fig. 3C). Meantime, ISYNA1 expression in the CRC tissues with R2 resection was significantly higher than that with R0 resection ($P = 0.01$) (Fig. 3E). ISYNA1 expression was strongly associated with lymphatic invasion ($P = 0.001$) (Fig. 3G) but not with perineural invasion ($P = 0.16$) (Fig. 3F). Interestingly, ISYNA1 expression levels were significantly higher in female CRC tissues than that in male CRC tissues ($P = 0.05$) (Fig. 3H). Then Logistic regression analysis revealed that ISYNA1 expression level was significantly correlated with N stage (OR = 1.426 (1.042-1.956), $p = 0.027$), M stage (OR = 1.652 (1.044-2.647), $p = 0.034$), AJCC stage (OR = 1.529 (1.113-2.104), $p = 0.009$), gender (OR = 0.696 (0.509-0.949), $p = 0.022$), residual tumor (OR = 2.723 (1.376-5.788), $p = 0.006$), Lymphatic invasion (OR = 2.356 (1.680-3.320), $p = 0.034$) (Table 1.).

Overexpression of ISYNA1 predicted poor prognosis in TCGA CRC cohort

Then we analysis the association between ISYNA1 expression and the prognosis of CRC patients. As shown in Figure.4, low expression of ISYNA1 exhibited more favorable OS, PFI, DSS outcomes ($P = 0.05$) (Fig. 4A-C). In order to evaluate the diagnostic value of ISYNA1, a receiver operating characteristic (ROC) curve was plotted according to data obtained from TCGA cohort. As shown in Figure. 4D, the area under

ROC curve was 0.706 [95% confidence interval (CI): 0.671-0.745]. Then univariate/multivariate Cox regression analysis were further performed to clarify the prognostic value of ISYNA1. The results showed that ISYNA1 was an independent unfavorable prognostic factor of CRC patients in TCGA cohort (Fig. 4E-F).

ISYNA1 was highly expressed in CRC tissues and correlated with Infiltrating and metastatic ability in CRC microarray tissues.

Firstly, the expression level of ISYNA1 in CRC tissues and adjacent normal tissues was compared by constructing and staining tissue microarray analysis. Representative pictures are shown in Fig. 5A. In addition, we analyzed the correlation between the expression level of ISYNA1 and the clinicopathological parameters of patients with CRC. ISYNA1 is closely related to the tumor size ($P=0.033$) and lymphatic metastasis ($P=0.024$) of CRC (Fig. 5B). Moreover, TMA revealed that ISYNA1 was strongly expressed in 8 of 40, moderately expressed in 23 of 40 and weakly expressed in 9 of 40 CRC tissues respectively. By contrast, ISYNA1 was strongly expressed in 1 of 40, moderately expressed 10 of 40, weakly expressed in 28 of 40, and negatively expressed in 1 of 40 adjacent normal tissues respectively ($P<0.001$) (Fig. 5C). Then, Western blot was performed to detect ISYNA1 protein expression levels in 4 paired fresh surgical specimens and the results revealed that ISYNA1 was highly expressed in CRC tissues compared to matched normal tissues (Fig. 5D).

Functional Annotation among the High and Low ISYNA1 Expression Groups

At first, 752 differently expressed genes (DEGs) were identified by performing genome-wide coexpression analysis with setting the cutoff values ($P_{\text{adj}}\leq 0.05$ and $|\text{Log}_2\text{-fold change}|\geq 1$) between ISYNA1 high expression group and low expression group. 416 up-regulated genes and 336 down-regulated genes were identified and presented in the volcano plot (Fig. 6A). The top 20 positively correlated genes and the top 20 negatively correlated genes, ordered by Pearson correlation coefficient, are presented in a gene co-expression heatmap (Fig. 6B). The results of GO and KEGG pathway analyses were present in the bubble diagram that coexpression of ISYNA1 was mainly enriched in autophagy, cell growth, Ras protein signal transduction, post-translational protein modification, collagen-containing extracellular matrix (ECM), cell-cell junction, cell-substrate junction, cell-substrate adherens junction, ubiquitin-like protein transferase activity, ubiquitin-protein protein ligase activity, ubiquitin-protein protein ligase activity, mitogen-activated protein kinases (MAPK) signaling pathway and mammalian target of rapamycin (mTOR) signaling pathway (Fig. 6B-C). Meantime, the GSEA was performed to further identify the potential functions of ISYNA1. The GSEA revealed that ISYNA1 was enriched in substantial gene sets, including phosphoinositide 3-kinase/AKT (PI3/AKT signaling pathway (NES= 1.589, FDR ≤ 0.05) (Fig. 7B), KEGG pathways in cancer (NES= 1.472, FDR ≤ 0.05) (Fig. 7C), MAPK signaling pathway (NES= 1.485, FDR ≤ 0.05) (Fig. 7D), epithelial-to-mesenchymal transition (EMT) in colorectal cancer (NES= 1.866, FDR ≤ 0.05) (Fig. 7E), extracellular matrix organization (NES= 2.211, FDR ≤ 0.05) (Fig. 7F) and immunoregulatory interactions (NES= 1.994, FDR ≤ 0.05) (Fig. 7G).

Correlation Between ISYNA1 and tumor microenvironment in CRC

The tumor microenvironment (TME), including cellular and non-cellular components, is increasingly recognized to play a critical role in tumorigenesis[12]. In order to characterize the immunological role of ISYNA1 in TME, we evaluated the relationship between ISYNA1 and immune cell infiltration in CRC by analyzing the data set in TCGA cohort and performing single-sample Gene set enrichment analysis (ssGSEA) according to previous literature[13]. The relationship between ISYNA1 expression and immune cell infiltration was intuitively represented in a bubble diagram (Fig. 8A). The analysis revealed that ISYNA1 expression was positively correlated with the abundance of multiple immune cells, such as NK cells, macrophages, dendritic cell, Mast cells, neutrophils, B cells, etc., whereas it was negatively correlated with Th17 cells, T helper cells, Th2 cells, Central Memory T cell (Tcm) (Fig. 8B). Meantime, the difference in the abundance of immune/stromal cells between high ISYNA1 group and low ISYNA1 group was shown in Figure 8B.

Correlation between ISYNA1 and immune-related modulators in CRC

To better characterize the potential role of ISYNA1 in modulating TME, the relationship between ISYNA1 immune modulators was further explored by analyzing the association between ISYNA1 expression and makers genes of related immune cells. Cancer-associated fibroblasts (CAFs), one of the important cellular components of the TME, have been proved to play critical roles in cancer cell proliferation, tumour immunity, extracellular matrix remodelling as well as inflammatory responses by secreting cell growth factors, inflammatory factors, and extracellular matrix[14]. As shown in gene co-expression heatmap, a significantly positive correlation between ISYNA1 and CAF-associated marker genes was observed (Fig. 8C).

Macrophages can be divided into classically activated macrophages (M1 macrophages) and alternatively activated macrophages (M2 macrophages) according to their activation state. Tumor-associated macrophages (TAMs) often display characteristics similar to M2 macrophages, which can exert multiple cancer-promoting functions in TME[15]. As shown in Figure 8D, significant positive correlations between ISYNA1 expression and the majority of genes related to M2 macrophages were clearly observed.

It is well documented that exhausted T cell signature was very important for immunotherapy response, especially immune checkpoint inhibitors and chimeric antigen receptor T-cell immunotherapy[16]. The association between ISYNA1 expression and T cell exhaustion genes was in further explored. We found that ISYNA1 expression was positively correlated with the gene set associated with T cell exhaustion (Fig. 8E). Meantime, it was also confirmed that there was a significant positive correlation between most immunosuppression-associated genes and ISYNA1 expression (Fig. 8F).

Immune checkpoint receptors inhibit the activation of T cell via delivering co-inhibitory signaling, thereby modulating the duration and intensity of the immune response[17]. In our study, the relation between ISYNA1 expression and a variety of immune checkpoint molecules in CRC was explored. The results revealed that there existed strongly correlation between ISYNA expression and the majority of immune checkpoint molecules in CRC (Fig. 8G).

Discussion

CRC is the most common type of gastrointestinal cancer worldwide, and of late, its incidence has been increasing year-on-year. Owing to advances in diagnosis and treatment technologies, the mortality rate of CRC patients has decreased. Nevertheless, their five-year survival rate remains less than 60%[2]. Tumor recurrence and metastasis are the key factors affecting their poor prognosis. Therefore, from the clinical standpoint, it is important to explore the relationship between tumor recurrence and metastasis-related molecular markers.

ISYNA1 catalyzes the rate-limiting conversion of glucose 6-phosphate to inositol 1-phosphate, thus playing a vital role in the biosynthetic pathway of inositol. Although the unexpected role of inositol in multiple diseases, such as gynecological diseases[18], respiratory distress syndrome[19], Alzheimer's disease[20], metabolism-related diseases[21], and cancer[22], has been reported, reports on its catalytic synthetase ISYNA1 are scarce in the context of tumors. Guo et al. reported that ISYNA1 is strongly expressed in bladder cancer, and it promotes the proliferation and inhibits the apoptosis of bladder cancer cells. However, ISYNA1 has been identified as a tumor suppressor in pancreatic cancer, and weak ISYNA1 expression has been found to be correlated to poor prognosis for pancreatic cancer patients. Therefore, ISYNA1 may play distinct roles in distinct types of tumors.

Reports on the role of ISYNA1 are scarce in the CRC literature. In this study, through data mining of the TCGA database, we discovered that ISYNA1 was overexpressed in CRC tissues. Moreover, we found that strong ISYNA expression was significantly correlated to poor OS, DSS, and PFI in CRC. Subsequently, we further detected the expression level of ISYNA1 in clinical fresh CRC tissue samples by using the western blot method and analyzed the relationship between ISYNA1 expression and clinicopathological characteristics by using the tissue microarray technique. We demonstrated that the strong expression of ISYNA1 was strongly associated with the tumor size and lymphatic metastasis which confirmed the conclusions drawn from our analysis of the TCGA database.

GO and KEGG analysis is a computational method for identifying potential biological processes through the collection and analysis of gene expression datasets extracted from numerous sources, including sequencing data and published experimental datasets[23]. GO and KEGG analysis is usually used to nominate the potential related pathway in gene expression datasets. In this study, enrichment of GO and KEGG terms revealed that coexpression of ISYNA1 was mainly enriched in autophagy, cell growth, Ras protein signal transduction, post-translational protein modification, ECM, MAPK signaling pathway, mTOR signaling pathway, etc. Numerous studies have confirmed the relationship between these biological mechanisms and cancer prognosis. According to recent literature, autophagy, which is an intracellular evolutionarily conserved, self-degradation process, can play complex and diverse roles in tumorigenesis, necrosis and metastasis, depending on the type and stage of the tumor. Meantime, recent studies has indicated that cancer cell behavior and differentiation can be influenced by changes in the composition or organization of the ECM[24]. Meanwhile, a variety of studies have confirmed that MAK, PI3K-AKT-mTOR and Ras pathways all play important roles in in the field of oncology, such as tumor proliferation,

invasion, metastasis, drug resistance, and cancer immunotherapy[25–29]. Besides, post-translational protein modifications have been widely studied and proved to exert a wide variety of functions in cancer, by altering the biological function of tumor-associated molecules (such as stability, localization, etc.)[30].

In addition, to further elucidate the underlying biological mechanism of ISYNA1 in CRC, GSEA was conducted and revealed that coexpression of ISYNA1 was mainly enriched in PI3/AKT signaling pathway, MAPK signaling pathway, EMT, ECM organization and immunoregulation. EMT is a well-known transdifferentiation program which play a critical role in cancer metastasis[31] and chemoresistance[32]. Therefore, we speculate that ISYNA1 may serve as an oncogene that influences the prognosis of patients with CRC through these biological pathways. However, further research is needed to verify its tumor-related function and clarify the potential mechanism.

In recent years, the tumor microenvironment has gained increasing attention, largely because of its close association with the effectiveness of immunotherapy. Currently, immunotherapy has revolutionized the cancer therapy and rapidly become the mainstay of treatment for a variety of solid cancers. However the immunotherapy of CRC presents enormous challenges to personalized treatment regimen mainly due to the heterogeneity and dynamics of TME. Tumor-infiltrating immune cells (TIICs) in TME have been shown to play a vital role in tumor development and progression[33]. Accordingly, it is of great importance to comprehensive analysis of tumor-infiltrating immune cells immune cell components in TME. GSEA identified that coexpression of ISYNA1 was enriched in immunomodulatory response. Therefore, we analyzed and compared the components of TIICs between the high and low ISYNA1 expression groups. Our research demonstrated that ISYNA1 expression was significantly positively correlated with the majority of the genes of CAF and M2-like macrophage, which are both immunosuppressive cells[34–35]. Moreover, a significantly positive correlation was observed between most immunosuppression-associated genes and ISYNA1 expression. Clinically, T cell exhaustion, characterized by the gradual functional deficits of exhausted T cells, has been considered to be a cause of the failure of immunotherapy (such as Chimeric Antigen Receptor T-Cell Immunotherapy (CAR-T) and immune checkpoint inhibitor)[36]. In our research, ISYNA1 expression correlated positively with T cell exhaustion genes. Thus, we speculated that high ISYNA1 may lead to the formation of an immunosuppressive TME which can promote immune escape and fosters tumor growth. Targeted inhibition of ISYNA1 may reverse the immunosuppression of TME to improve outcomes for CRC patients.

Conclusions

In conclusion, we confirmed that ISYNA1 overexpression was closely related to the poor prognosis of patients with CRC. Moreover, we identified ISYNA1 as a biomarker for evaluating the prognosis of patients with CRC. In the light of these findings, further studies on the specific function and potential mechanism of ISYNA1 are urgently warranted, and the development of an efficient strategy to suppress ISYNA1 expression may improve the prognosis of patients with CRC in the future.

Abbreviations

ISYNA1
Inositol-3-phosphate synthase 1
CRC
Colorectal cancer
TCGA
The Cancer Genome Atlas
GSEA
Gene set enrichment analysis
GO
gene ontology
KEGG
Kyoto Encyclopedia Genes and Genomes
TMA
tissue microarrays
IHC
immunohistochemistry
OS
overall survivals, DFS:disease-free survivals
PFI
progress free interval
CEA
carcinoembryonic antigen
OR
odds ratio
CI
confidence interval
DEGs
differently expressed genes
MAPK
mitogen-activated protein kinases
mTOR
mammalian target of rapamycin
PI3/AKT
phosphoinositide 3-kinase/AKT
EMT
epithelial-to-mesenchymal transition
FDR
false discovery rate
TME
tumor microenvironment

ssGSEA
single-sample Gene set enrichment analysis
CAFs
Cancer-associated fibroblasts
TAMs
Tumor-associated macrophages
ROC
receiver operating characteristic
ECM
extracellular matrix
TIICs
Tumor-infiltrating immune cells.

Declarations

Ethics approval and consent to participate

This study was approved by the Ethics Committee of Fujian Cancer Hospital. The processing of clinical tissue samples is in strict compliance with the ethical standards of the Declaration of Helsinki. All patients signed written informed consent.

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analysed during this study are included in this published article and its supplementary information files.

Competing Interests

The authors declare that they have no competing interests.

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Author's contributions

Xiaopeng Wang, Zaisheng Ye, Zeng Yi and Yi Wang completed data analysis wrote the main manuscript text. Shenhong Wei and Zhitao Lin prepared Figure 1-3. Shu Chen and Yi Zeng prepared Figure 4-5. Hang

Chen and Zhiwei Wang prepared Figure 6-8 and Table 1. Ruirong Lin and Luchuan Chen guides other authors in topic selection and data analysis . All authors reviewed the manuscript.

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References

1. Jemal A, Bray F, Center M M. Global cancer statistics, 2012.[J]. *Ca A Cancer Journal for Clinicians*, 2013, 65(2):87–108.
2. Siegel R, Desantis C, Jemal A. Colorectal cancer statistics, 2014.[J]. *Ca A Cancer Journal for Clinicians*, 2014, 64(2):104–117.
3. Liu Z X, You S, Tang B P, et al. Inositol as a new enhancer for improving lipid production and accumulation in *Schizochytrium* sp. SR21[J]. *Environmental Science and Pollution Research*, 2019, 26(1).
4. Khodakovskaya M, Sword C, Qian W, et al. Increasing inositol (1,4,5)-trisphosphate metabolism affects drought tolerance, carbohydrate metabolism and phosphate-sensitive biomass increases in tomato.[J]. *Plant Biotechnology Journal*, 2010, 8(2):170–183.
5. Q Zhang, VW Ringo, Z Xavier, et al. Knock-Down of *Arabidopsis* PLC5 Reduces Primary Root Growth and Secondary Root Formation While Overexpression Improves Drought Tolerance and Causes Stunted Root Hair Growth[J]. *Plant & Cell Physiology*, 2018.
6. Mound A, Rodat-Despoix L, Bougarn S, et al. Molecular interaction and functional coupling between type 3 inositol 1,4,5-trisphosphate receptor and BKCa channel stimulate breast cancer cell proliferation[J]. *European Journal of Cancer*, 2013, 49(17):3738–3751.
7. Seelan R S, Parthasarathy L K, Parthasarathy R N. E2F1 regulation of the human myo-inositol 1-phosphate synthase (ISYNA1) gene promoter[J]. *Archives of Biochemistry & Biophysics*, 2004, 431(1):95–106.
8. Guo X, Li H H, Hu J, et al. ISYNA1 is overexpressed in bladder carcinoma and regulates cell proliferation and apoptosis[J]. *Biochemical and Biophysical Research Communications*, 2019, 519(2).
9. Musashi2 promotes the progression of pancreatic cancer through a novel ISYNA1-p21/ZEB-1 pathway[J]. *Journal of Cellular and Molecular Medicine*, 2020.
10. Yu G, Wang L G, Han Y, et al. clusterProfiler: an R package for comparing biological themes among gene clusters[J]. *Omics: a journal of integrative biology*, 2012, 16(5): 284–287.

11. Loss of ID3 drives papillary thyroid cancer metastasis by targeting E47-mediated epithelial to mesenchymal transition[J]. *Cell Death Discovery*.
12. Quail D F, Joyce J A. Microenvironmental regulation of tumor progression and metastasis[J]. *Nature Medicine*, 2013, 19(11):1423–1437.
13. Bindea, Gabriela, et al. Spatiotemporal dynamics of intratumoral immune cells reveal the immune landscape in human cancer. *Immunity* 39.4 (2013): 782–795.
14. Costa A, Kieffer Y, Scholer-Dahirel A, et al. Fibroblast Heterogeneity and Immunosuppressive Environment in Human Breast Cancer[J]. *Cancer Cell*, 2018, 33(3):463-479.e10.
15. Cao Q, Yan X, Kai C, et al. Macrophages as a potential tumor-microenvironment target for noninvasive imaging of early response to anticancer therapy[J]. *Biomaterials*, 2018, 152:63.
16. Lynn R C, Weber E W, Sotillo E, et al. c-Jun overexpression in CAR T cells induces exhaustion resistance[J]. *Nature*, 2019, 576(7786).
17. Yu J, Qin B, Moyer A M, et al. Regulation of sister chromatid cohesion by nuclear PD-L1[J]. *Cell Research*, 2020:1–12.
18. Wojciechowska A, Osowski A, M Jówik, et al. Inositols' Importance in the Improvement of the Endocrine–Metabolic Profile in PCOS[J]. *International Journal of Molecular Sciences*, 2019, 20(22).
19. Howlett A, Ohlsson A, Plakkal N. Inositol for respiratory distress syndrome in preterm infants[J]. *Cochrane Database of Systematic Reviews*, 2000, 3(3):CD000366.
20. Bizzarri M, Fuso A, Dinicola S, et al. Pharmacodynamics and pharmacokinetics of inositol(s) in health and disease[J]. *Expert Opinion on Drug Metabolism & Toxicology*, 2016:17425255.2016.1206887.
21. Croze M L, Soulage C O. Potential role and therapeutic interests of myo-inositol in metabolic diseases[J]. *Biochimie*, 2013, 95(10):1811–1827.
22. Ivana, VUCENIK. Anticancer Properties of Inositol Hexaphosphate and Inositol: An Overview[J]. *Journal of Nutritional Science and Vitaminology*, 2019, 65(Supplement):S18-S22.
23. Thomas P D, Hill D P, Mi H, et al. Gene Ontology Causal Activity Modeling (GO-CAM) moves beyond GO annotations to structured descriptions of biological functions and systems[J]. *Nature Genetics*, 51(10):1429–1433.
24. Mohan V, Das A, Sagi I. Emerging roles of ECM remodeling processes in cancer[J]. *Seminars in Cancer Biology*, 2020, 62:192–200.
25. Qiang Z, Hong B, Lei Z, et al. Pyruvate kinase M2 inhibits the progression of bladder cancer by targeting MAKP pathway[J]. *Journal of Cancer Research and Therapeutics*, 2018, 14(Supplement).
26. Yan L, Gu H, Jia L, et al. RKIP and 14-3-3 ϵ exert an opposite effect on human gastric cancer cells SGC7901 by regulating the ERK/MAPK pathway differently[J]. *Digestive Diseases & Sciences*, 2013, 58(2):389–396.
27. Saeed, Noorolyai, Neda, et al. The relation between PI3K/AKT signalling pathway and cancer.[J]. *Gene*, 2019.

28. Liu R, Chen Y, Liu G, et al. PI3K/AKT pathway as a key link modulates the multidrug resistance of cancers[J]. Cell Death & Disease.

29. O'Donnell J S, Massi D, Teng M, et al. PI3K-AKT-mTOR inhibition in cancer immunotherapy, redux[J]. Seminars in Cancer Biology, 2017, 48.

30. Han Z J, Feng Y H, Gu B H, et al. The post-Translational modification, SUMOylation, and cancer (Review)[J]. International Journal of Oncology, 2018, 52(4).

31. Neelakantan D, Zhou H, Oliphant M, et al. EMT cells increase breast cancer metastasis via paracrine GLI activation in neighbouring tumour cells[J]. Nature Communications, 2017, 8:15773.

32. Rivas J, Brozovic A, Izraely S, et al. Cancer drug resistance induced by EMT: novel therapeutic strategies[J]. Archives of Toxicology, 2021(4).

33. Leick K M, Rodriguez A B, Melssen M M, et al. The Barrier Molecules Junction Plakoglobin, Filaggrin, and Dystonin Play Roles in Melanoma Growth and Angiogenesis[J]. Annals of Surgery, 2019, 270(4):1.

34. Biffi G, Oni T E, Spielman B, et al. IL-1-induced JAK/STAT signaling is antagonized by TGF-beta to shape CAF heterogeneity in pancreatic ductal adenocarcinoma[J]. Cancer Discovery, 2018, 9.

35. Mingen, Liu, Roddy, et al. Metabolic rewiring of macrophages by CpG potentiates clearance of cancer cells and overcomes tumor-expressed CD47-mediated 'don't-eat-me' signal.[J]. Nature Immunology, 2019.

36. Park S H. VEGF-A drives TOX-dependent T cell exhaustion in anti-PD-1-resistant microsatellite stable colorectal cancers. 2020.

Tables

Table 1. Clinicopathological factors and ISYNA1 expression in TGCA cohort

Characteristics	Total(N)	Odds Ratio(OR)	P value
T stage (T3&T4 vs. T1&T2)	641	1.184 (0.806-1.743)	0.389
N stage (N1&N2 vs. N0)	640	1.426 (1.042-1.956)	0.027
M stage (M1 vs. M0)	564	1.652 (1.044-2.647)	0.034
Pathologic stage (Stage III&Stage IV vs. Stage I&Stage II)	623	1.529 (1.113-2.104)	0.009
Gender (Male vs. Female)	644	0.696 (0.509-0.949)	0.022
CEA level (>5 vs. ≤5)	415	0.846 (0.567-1.261)	0.412
Residual tumor (R1&R2 vs. R0)	510	2.723 (1.376-5.788)	0.006
Perineural invasion (Yes vs. No)	235	1.187 (0.659-2.140)	0.566
Lymphatic invasion (Yes vs. No)	582	2.356 (1.680-3.320)	<0.001

Figures

Figure 1

The flow chart for selected candidate genes which were significantly up-regulated in CRC tissues and associated with prognosis of CRC patients.

Figure 2

ISYNA1 was highly expressed in CRC tissues and most tumors. (A) Venn diagram showing genes screened by different methods. (B) Expression differences of ISYNA1 between 41 cancerous tissues and 480 non-tumorous tissues in TCGA cohort. *** $P < 0.001$. (C) Expression differences of ISYNA1 between 41 cancerous tissues and 41 matched adjacent normal tissues in TCGA cohort. *** $P < 0.001$. (D) ISYNA1

expression was negatively correlated with promoter methylation ($r=-0.217$, $P<0.001$). (E) ISYNA1 was highly expressed in most tumors in TCGA cohort.

Figure 3

The relationship between ISYNA1 expression and clinicopathological characteristics in TCGA CRC cohort. (A-I) The ISYNA1 mRNA expression level was closely associated with N stage (B), M stage (C) and AJCC stage (D), but showed no significant correlations with T stage (A). Significant correlations were detected

between ISYNA1 expression and residual tumor (E), lymphatic invasion (G), and gender (H), however not detected between ISYNA1 expression and perineural invasion (F) and serum CEA level (I). * $P < 0.05$

Figure 4

Overexpression of ISYNA1 in CRC tissues was associated with poor prognosis in TCGA cohort. (A) Kaplan-Meier analyses for OS according to ISYNA1 expression in CRC patients in TCGA CRC cohort. (B) Higher expression of ISYNA1 was correlated with more favorable DSS in CRC patients in TCGA CRC cohort. (C) ISYNA1 expression level and PFI in CRC patients in TCGA cohort. (D) The predictive value of ISYNA1 was evaluated by ROC curve. (E-F) ISYNA1 was identified as an independent prognostic factor by performing univariate (E) and multivariate (F) Cox regression analysis

Figure 5

ISYNA1 protein was highly expression in CRC. (A) Representative image of immunohistochemical staining of ISYNA1 on a tissue microarray containing 40 pairs of CRC and adjacent normal tissues. .Scale bars = 500 μ m (top) or 100 μ m (bottom). (B) Comparing age, gender, differentiation, tumor size, lymph node metastasis, invasion depth, tumor metastasis among 40 CRC patients according to the ISYNA1 protein expression on a CRC tissue microarray. (C) Statistical analysis of IHC staining results for ISYNA1 from the CRC tissue microarray slide. *** $P < 0.001$. (D) Detection of ISYNA1 protein expression levels in 4 pairs of CRC and matched adjacent normal tissues by western blot.

Figure 6

Functional and pathway annotation among the high and low ISYNA1 groups by GO and KEGG analysis. (A) The volcano plot showed the DEGs identified between high and low ISYNA1 groups in TCGA cohort. The red and blue dot represents the up-regulated gene and down-regulated gene with significance respectively, and each grey dot represents genes without significant difference. (B) Gene expression heatmap and correlations for ISYNA1 co-expressed genes. * $P < 0.05$, ** $P < 0.01$ ** and *** $P < 0.001$. (C) The top

14 significant terms of GO and KEGG pathway analyses. (D) Network visualization of GO and KEGG pathway analyses.

Figure 7

Functional and pathway annotation among the high and low ISYNA1 groups by GSEA. (A-G) The GSEA revealed that PI3K/Akt signaling pathway (B), pathways in cancer (C), MAPK signaling pathway (D), EMT

(E), extracellular matrix organization (F), immunoregulatory interactions between lymphoid and non-lymphoid cells (G).

Figure 8

The relationship of ISYNA1 expression to immune cell infiltration and immune-related modulators in CRC. (A) Bubble diagrams revealed the correlation between differentially enriched composition of infiltrated immune cells and ISYNA1 expression in TCGA CRC cohort. (B) The relationship between ISYNA1 expression levels and the abundance of different types of immune cells. (C-F) ISYNA1 was significantly positively with majority of CAF-related genes (C), M2-like macrophage genes (D), T cell exhaustion genes (E) and immunosuppressive genes (F). (G) ISYNA1 expression level is notably correlated with the expression of immune checkpoint molecules in CRC. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, ns: no significant difference.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [COADREADclinicalraw.xlsx](#)
- [DEGsidentifiedbetweenhighandlowISYNA1groups.xlsx](#)
- [DSSrelatedgenesinTCGA.xlsx](#)
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- [PFIrelatedgenesinTCGA.xlsx](#)
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