Prognosis and immune function of Synaptotagmin-4 in gastric cancer and brain low-grade glioma

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Abstract

Background

Synaptotagmins (SYTs) are a family of proteins whose primary feature is the calcium sensor in vesicle transport and exocytosis. SYT4 is one of them, but the relationship between SYT4 and cancer remains unclear. We aim to explore the prognosis and immune function of SYT4 in gastric cancer and low-grade glioma.

Methods

These databases were used to study the immunological and prognostic role of SYT4 in cancers, including the Oncomine database, Kaplan-Meier plotter, GEPIA2, TIMER, and CGGA.

Results

The study suggested that the expression levels of SYT4 were lower in both gastric cancer and glioma, compared to the normal tissues. And SYT4 played a protective and harmful role in low-grade gliomas and gastric cancer, respectively. Moreover, we found that a difference between SYT4 expression and the levels of immune infiltration in stomach adenocarcinoma (STAD) and brain lower-grade glioma (LGG). Besides, after exploring the association between the expression levels of SYT4 and markers of immune cells in these two cancers, we found that markers of monocytes, M1/ M2, tumor-associated macrophages (TAMs), Treg cells and SYT4 expressions had an opposite correlation in STAD and LGG.

Conclusions

SYT4 had an effect on the prognosis of gastric cancer and glioma patients and was related to immune infiltration by regulating TAMs and Treg cells. SYT4 can be used as a prognostic biomarker for STAD and LGG.

Background

Calcium-mediated exocytosis is one of the methods of intercellular communication. For example, neuroendocrine cells can secrete hormones through calcium-mediated exocytosis[1]. Mast cells can also release granules through exocytosis under the mediation of calcium[2]. Similarly, for T lymphocytes, calcium-mediated exocytosis is essential for maintaining its normal function[3]. Synaptotagmins (SYTs) are a family of proteins whose primary feature is the calcium sensor in vesicle transport and exocytosis[4]. The SYTs have distinct structural characteristics, which determining that they can bind to calcium and affect exocytosis[5]. According to research by Zhiping P Pang et al. [6], at least 16 SYT subtypes have been identified, which have significantly different distribution patterns and functional characteristics. SYT4 is one of them, which is a post-synaptic protein and does not show calcium-dependent effects[7]. Previous studies have found that SYT4 is localized on the particles of islet B cells and can affect insulin secretion[8]. SYT4 is an essential vesicle protein that determines the secretion of brain-derived neurotrophic factors[9]. There is still no research to explore the relationship between SYT4 and cancer, but some studies have found the relationship between other members of SYTs and cancer. Qin Li et al. reported that SYT13 was helpful for the occurrence and development of colorectal cancer[10]. Wu Z et al. reported that SYT7 was beneficial for the development of osteosarcoma[11]. But the relationship between SYT4 and cancer remains unclear.

Tumor microenvironment includes tumor, stromal, immune, vascular endothelial cells, and extracellular matrix[12]. CD8 + or cytotoxic T lymphocytes play a tumor-killing function[13], while regulatory T cells attenuate effector T cell activity and promote the immunosuppression of TME[14]. Generally, M1 macrophages secrete Th1 cytokines, which play a pro-inflammatory and antitumor role[15]. However, M2-type macrophages, which can help tumor cells complete immune escape, promote tumor angiogenesis and promote lymph node and distant metastasis[16, 17]. In recent years, immunotherapy, including programmed cell death-1 and programmed cell death-ligand 1 inhibitor, has been used in clinical treatment as an alternative to traditional anticancer therapy[18]. But more potential therapeutic targets are still waiting to be discovered. Although SYT4 is a member of SYTs and has the potential to mediate exocytosis, it is still unknown whether it can affect the prognosis of cancer through immune infiltration.

In this study, the Oncomine database, Kaplan-Meier plotter, GEPIA2 dataset, and the CGGA dataset were used to explore the expression level of SYT4 in various cancers and the association with the prognosis. Also, TIMER was used to study the correlation between the expression levels of SYT4 and the levels of immune infiltration. Our results suggested that there was a different association between SYT4 expression and immune infiltration in STAD and LGG, which suggested that SYT4 might affect the prognosis of patients via immune infiltration.

Material And Methods

Oncomine database

Oncomine was a platform to explore data that contains multiple gene expression data sets[19]. The mRNA expression level of SYT4 in human cancers was analyzed in it. The gene name was "SYT4", the rank was top 10%, the fold change was 2, and the *p*-value was 0.001.

Kaplan-Meier Plotter Database

Kaplan-Meier plotter[20] assessed the impact of different genes on the prognosis of multiple cancers, including breast, ovarian, lung, and gastric cancer. The data were taken from Gene Expression Omnibus(GEO), European Genome-phenome Archive(EGA) and The Cancer Genome Atlas(TCGA) primarily. We used it to research the potential prognostic significance of SYT4 in different cancers. In this database, we used survival curves to show the final results.

TIMER Database

TIMER(Tumor IMmune Estimation Resource)[21], a web to explore tumor-infiltrating immune cells, collected data from 10,897 samples in 32 cancer types to study the B cells, CD4 + T cells, CD8 + T cells, neutrophils, macrophages, and dendritic cells in invasive cancers by a deconvolution method[22]. The DiffExp module in the web was used to explore the different levels of SYT4 in TCGA between tumor and normal tissues. We used box plots to show the results. The Wilcoxon test was used to evaluate whether the results were statistically significant. The Gene module was aimed to assess the association between the expression levels of SYT4 and the levels of immune infiltration in various cancers. Besides, the Correlation module was used to assess the association between SYT4 and immune cell markers. All immune cell markers were derived from the R & D Systems website. The results were expressed as scatter plots. The correlation was estimated using Spearman's rho value.

GEPIA2 Database

GEPIA2 was an updated version of Gene Expression Profiling and Interactive Analyses (GEPIA), including RNA sequencing data from 9,736 tumors and 8,587 normal samples in TCGA and Genotype-Tissue Expression (GTEx)[23]. We used it to explore the prognostic values of SYT4 and collected the hazard ratios (HRs) with 95% confidence intervals (CI) and the *p*-value. Besides, we performed a correlation analysis in SYT4 expression and immune cells' markers. The correlation was estimated using Spearman's rho value.

CGGA Database

Chinese Glioma Genome Atlas (CGGA) was a dataset with more than 2000 brain tumor samples from China, including whole-exome sequencing, DNA methylation, mRNA sequencing, mRNA microarray, microRNA microarrays and the clinical data. Two mRNA datasets, including mRNAseq_325 and mRNAseq_693, were used to analyze the prognostic significance of SYT4 in primary glioma patients.

Results

mRNA level of SYT4 in cancers

Firstly, we used the Oncomine database to explore mRNA expression levels of SYT4 in different tumors (Fig. 1A). The result suggested that mRNA expression of SYT4 was higher in the lung tumor tissues than the normal lung tissues. In contrast, the mRNA expressions of SYT4 were lower in the brain and nervous system cancer, colorectal, esophageal, gastric, and prostate cancer, as well as in sarcoma. Supplemental Table 1 explicitly presented the mRNA expression levels of SYT4 in tumors according to different researches.

Further, we used the TIMER database to explore the expression levels of SYT4 in different cancers (Fig. 1B). We found that a significant difference of the expression levels of SYT4 between the cancer tissues and the normal tissues in the following cancers: bladder urothelial carcinoma (BLCA), breast invasive carcinoma (BRCA), colon adenocarcinoma (COAD), esophageal carcinoma (ESCA), head and neck squamous cell carcinoma (HNSC), kidney chromophobe (KICH), kidney renal clear cell carcinoma (KIRC), kidney renal papillary cell carcinoma (KIRP), liver hepatocellular carcinoma (LIHC), lung adenocarcinoma (LUAD), lung squamous cell carcinoma (LUSC), prostate adenocarcinoma (PRAD), rectum adenocarcinoma (READ), stomach adenocarcinoma (STAD), thyroid carcinoma (THCA), as well as uterine corpus endometrial carcinoma (UCEC).

Prognostic Significance Of SYT4 In Different Cancers

We used the gene chip data which were derived from the Kaplan-Meier plotter database to explore the association between SYT4 expressions and the survival of breast, lung, gastric and ovarian cancer patients. The results were shown in Fig. 2. For patients with gastric cancer, SYT4 was associated with an unfavorable prognosis [Overall survival (OS): HR = 1.39(1.12–1.73), log-rank $P = 0.0025$; Progression-free survival (PFS): HR = 1.31(1.03–1.67), log-rank $P = 0.025$]. For breast cancer patients, SYT4 was beneficial for patients' recurrence free survival (RFS) [HR = 0.76(0.65–0.88), log-rank $P = 0.00042$] but had no significant impact on the OS [HR = 0.74(0.54–1.02), log-rank $P = 0.065$]. While SYT4 did not affect the OS and PFS of patients with lung cancer [OS: HR = 1.02(0.86–1.20), log-rank $P = 0.84$; PFS: HR = 1.12(0.86–1.47), log-rank $P = 0.40$] and ovarian cancer [OS: HR = 1.01(0.82–1.24), log-rank $P = 0.93$; PFS: HR = 1.07(0.89–1.29), log-rank $P = 0.46$].

After the exploration of SYT4 in the Kaplan-Meier plotter database, we analyzed the impact of SYT4 on the prognosis of different cancers by analyzing the RNA sequencing data which were from TCGA database through GEPIA2. (Supplemental Fig. 1). The results showed the impacts of SYT4 on the survival of LGG and STAD were consistency no matter the exploration of OS or disease-free survival (DFS). The expression levels of SYT4 were related to a poor prognosis in patients with STAD (OS: HR = 1.60, log-rank *P* = 0.006; DFS: HR = 1.80, log-rank *P* = 0.0041). In contrast, the expression levels of SYT4 were correlated with a good prognosis in LGG patients (OS: HR = 0.53, log-rank *P* = 0.00047; DFS: HR = 0.58, log-rank *P* = 6e-04).

To further explore the correlation between the expression levels of SYT4 and the survival of LGG patients, we verified it in the CGGA database (Supplemental Fig. 2). In the mRNAseq_325 dataset, the result suggested that a significant correlation between the expression level of SYT4 and all primary gliomas patients' survival, as well as the patients with WHO grade II and III, but there was no correlation with the fourth-grade patients' prognosis. Similarly, In the mRNAseq_693 dataset, the result indicated a correlation between the expression level of SYT4 and all primary gliomas patients' survival, as well as the patients with WHO grade III, but there was no correlation with the grade II and IV patients. These results suggested the prognostic value of SYT4 in LGG and STAD. Among them, SYT4 was beneficial for the prognosis of LGG patients. In contrast, SYT4 was detrimental to the survival of STAD patients.

The expression of SYT4 has an impact on the survival of gastric patients with lymphatic metastasis

In order to explore the mechanism in which the expression level of SYT4 affected the survival of gastric cancer patients, we used the Kaplan-Meier plotter database to explore the association between the expression levels of SYT4 and clinical factors of gastric cancer patients (Table 1). We conducted a stratified analysis of clinical factors for OS and PFS, such as gender, AJCC stage, T stage, N stage, as well as M stage. In terms of gender, the expression level of SYT4 was related to an unfavorable prognosis of OS in both male [HR = 1.49(1.11–2.01), log-rank *P* = 0.0082] and female[HR = 1.89(1.22–2.93), log-rank *P* = 0.0039], and an unfavorable prognosis of PFS in female patients [HR = 1.56(1.02–2.38), log-rank *P* = 0.038]. Besides, a significant correlation was also shown between the expression level of SYT4 and the lymphatic metastasis of patients. We did not find a significant association between the expression level of SYT4 and the survival of gastric cancer patients without lymph node metastasis [OS: HR = 1.10(0.47–2.57), log-rank *P* = 0.83; PFS: HR = 1.16(0.50–2.70), log-rank *P* = 0.73]. However, there was a significantly correlation between the expression level of SYT4 and the survival of gastric cancer patients with N1 stage [OS: HR = 1.85(1.21–2.81), log-rank *P* = 0.0036; PFS: HR = 1.90(1.27–2.83), log-rank *P* = 0.0014]. Moreover, compared with patients without lymph node metastasis, as long as there was lymph node metastasis in gastric cancer patients, their OS [HR = 1.31(1.01–1.70), log-rank *P* = 0.045]and PFS [HR = 1.32(1.02–1.70), log-rank *P* = 0.031] were correlated with the expression level of SYT4. It was suggested that the expression level of SYT4 might further affect the prognosis of gastric cancer patients via affecting lymph node metastasis.

Table 1
Correlation of SYT4 mRNA expression and clinical factors in gastric cancer.

	Overall survival (n = 881)			Progression-free survival (n = 645)		
	N	HR	*P*-value	N	HR	*P*-value
Sex						
Female	187	1.89(1.22–2.93)	0.0039	179	1.56(1.02–2.38)	0.038
Male	349	1.49(1.11–2.01)	0.0082	341	1.30(0.97–1.74)	0.073
Stage						
1	62	0.66(0.22–2.01)	0.46	60	0.69(0.23–2.11)	0.52
2	135	1.77(0.93–3.38)	0.077	131	1.79(0.97–3.31)	0.061
3	197	1.26(0.87–1.84)	0.22	186	1.21(0.84–1.78)	0.29
4	140	1.20(0.81–1.78)	0.46	141	1.03(0.70–1.51)	0.89
Stage T						
2	241	1.45(0.94–2.21)	0.088	239	1.26(0.83–1.90)	0.27
3	204	1.01(0.71–1.42)	0.97	204	1.11(0.80–1.55)	0.54
4	38	1.59(0.70–3.65)	0.27	39	0.92(0.43–1.98)	0.84
Stage N						
0	74	1.10(0.47–2.57)	0.83	72	1.16(0.50–2.70)	0.73
1	225	1.85(1.21–2.81)	0.0036	222	1.90(1.27–2.83)	0.0014
2	121	0.86(0.55–1.35)	0.52	125	0.82(0.53–1.25)	0.35
3	76	1.26(0.74–2.13)	0.4	76	1.04(0.62–1.76)	0.88
1 + 2 + 3	422	1.31(1.01–1.70)	0.045	423	1.32(1.02–1.70)	0.031
Stage M						
0	444	1.32(1.00-1.74)	0.052	443	1.33(1.02–1.73)	0.038
1	56	1.44(0.81–2.56)	0.21	56	1.10(0.61–1.96)	0.76

SYT4 expression is related to the level of immune infiltration in gastric cancer and low-grade brain glioma

Among various factors affecting survival and lymph node metastasis of cancer patients, lymphocyte infiltration is a significant independent predictor[24]. Therefore, we continued to analyze the asscociation between the expression levels of SYT4 and the levels of immune infiltration in 39 types of cancer in the TIMER database (Supplemental Fig. 3). According to the results, we found that tumor purity and the expression levels of SYT4 had significant correlations in 12 cancer types. Similarly, the levels infiltration of B lymphocytes, CD4 + T lymphocytes, CD8 + T lymphocytes, macrophages, neutrophils, and dendritic cells had significant correlations with the expression levels of SYT4 in 11, 14, 10, 19, 11 and 13 types of cancer, respectively.

Based on the findings in GEPIA2, Kaplan Meier plotter and CGGA, we focused on cancer types in which the expression level of SYT4 was negatively correlated with tumor purity in TIMER and had significant correlation with prognosis of patients in GEPIA2 and Kaplan Meier plotter, including STAD and LGG (Fig. 3). The BRAC was selected as a control. It was worth noting that the expression level of SYT4 was negatively related to the prognosis of STAD, but had a positive association with the infiltration of immune cells. We found that the expression level of SYT4 in STAD patients was negatively related to tumor purity (cor = − 0.172, P = 7.75e-04), but was positively associated with the following immune cell infiltration: B cells (cor = 0.192, P = 2.09e-04), CD4 + T cells (cor = 0.385, P = 2.38e-14), CD8 + T cells (cor = 0.122, P = 1.82e-02), macrophages (cor = 0.385, P = 1.72e-14), and dendritic cells (cor = 0.207, P = 6.01e-05). However, the expression level of SYT4 was positively related to the prognosis of LGG patients, but negatively associated with the following immune cell infiltration: B cells (cor = − 0.385, P = 6.54e-12), CD4 + T cells (cor = − 0.577, P = 1.34e-43), neutrophils (cor = − 0.375, P = 2.49e-17), macrophages (cor = − 0.505, P = 6.45e-32), and dendritic cells (cor = − 0.478, P = 1.37e-28). While we did not find similar correlations in BRCA. These above results suggested that the reasons why the expression level of SYT4 impacted the prognosis of STAD and LGG patients in the different way probably were that the different relationships between the expression level of SYT4 and the level of immune infiltration in STAD and LGG.

Correlations between the expression levels of SYT4 and markers of immune cells

We analyzed the correlations between the expression levels of SYT4 and immune markers of multiple immune cells in STAD and LGG based on the TIMER and GEPIA databases, whose aim was to further explore potential mechanisms of interaction between SYT4 and various immune infiltrating cells, such as CD8 + T cells, T cells (general), B cells, monocytes, TAMs, M1 / M2 macrophages, neutrophils, natural killer cells, and dendritic cells. Besides, we also performed correlation analysis on the immune marker of the following functional T cells: T helper cells, follicular helper T cell, regulatory T cells, and exhausted T cells. At the same time, its correlation coefficient was adjusted based on tumor purity[25] (Table 2). According to the correlation analysis between the expression levels of 56 immune cell markers and the expression levels of SYT4, we found that the purity-adjusted coefficients of 35 markers were statistically significant in STAD patients, and their purity-adjusted coefficients were all positive. While the purity-adjusted correlation analysis of 46 markers was statistically significant in LGG patients, and most of them were negatively correlated. And only ten markers had statistically significant purity-adjusted correlations with the expression levels of SYT4 in BRCA patients. Besides, we also found significant correlations between the expression levels of markers in monocytes, TAM and M2 macrophages and the expression levels of SYT4 in patients with STAD and LGG, but not in BRCA (Table 2, Fig. 4). In detail, these markers, such as CD115, CCL2, IL10, VSIG4, and MS4A4A, had significantly positive correlations with SYT4 expression levels in STAD ($P < 0.0001$, Fig. 4A-D). For LGG, the markers, such as CD163 of M2, NOS2, IRF5 and COX2 of M1, and CD86 of monocytes also showed a significant correlation with SYT4 expression levels except for these above markers ($P < 0.0001$, Fig. 4I-L). However, the expression of SYT4 in BRAC did not show significant correlations with the above markers (Fig. 4E-H). Then, to verify the results, we analyzed the correlation between monocytes, TAMs, M1, M2 macrophages immune markers and the expression levels of SYT4 in STAD, LGG, and BRAC based on the GEPIA2 database. And the results were similar to above those in TIMER (Table 3). What's more, it is worth noting that the correlations between the expression level of SYT4 and the levels of immune markers were positive and negative in STAD and LGG, respectively. Hence, we probably concluded that the expression levels of SYT4 interacted with various immune cells in STAD and LGG in the opposite way, which affected the prognosis of patients and makes the difference about the survival of STAD and LGG.

Table 2

Correlation analysis between the expression level of SYT4 and markers of immune cells in TIMER.

Dscription	Gene markers	STAD				LGG				BC			
		None		Purity		None		Purity		None		Purity	
		Cor	*p*-Value	Cor	*p*-Value	Cor	*p*-Value	Cor	*p*-Value	Cor	*p*-Value	Cor	*p*-Value
CD8 + T cell	CD8A	0.24	***	0.25	***	0.23	***	0.27	***	0.03	3.58E-01	0.02	4.37E-01
	CD8B	0.21	***	0.23	***	0.02	6.44E-01	0.03	4.57E-01	0.01	7.51E-01	0.01	7.50E-01
T cell (general)	CD3D	0.19	***	0.18	**	-0.22	***	-0.21	***	-0.01	7.72E-01	-0.02	5.78E-01
	CD3E	0.22	***	0.21	***	-0.25	***	-0.25	***	0.00	9.64E-01	-0.01	8.00E-01
	CD2	0.21	***	0.20	***	-0.25	***	-0.24	***	-0.01	8.24E-01	-0.01	7.07E-01
B cell	CD19	0.28	***	0.27	***	-0.33	***	-0.30	***	-0.02	4.88E-01	-0.04	2.68E-01
	CD79A	0.30	***	0.28	***	-0.40	***	-0.40	***	-0.02	4.84E-01	-0.04	1.87E-01
Monocyte	CD86	0.16	**	0.14	*	-0.51	***	-0.52	***	0.01	6.83E-01	-0.01	8.73E-01
	CD115(CSFIR)	0.26	***	0.24	***	-0.48	***	-0.50	***	0.03	3.99E-01	0.01	7.55E-01
TAM	CCL2	0.27	***	0.27	***	-0.34	***	-0.32	***	0.07	2.27E-02	0.06	4.41E-02
	IL10	0.23	***	0.23	***	-0.41	***	-0.38	***	0.07	1.92E-02	0.06	5.34E-02
M1 Macrophage	INOS(NOS2)	-0.09	6.59E-02	-0.10	6.29E-02	0.29	***	0.29	***	0.08	*	0.07	2.63E-02
	IRF5	0.12	1.24E-02	0.10	4.23E-02	-0.50	***	-0.52	***	0.08	1.26E-02	0.06	5.88E-02
	COX2(PTGS2)	0.13	*	0.13	*	0.14	*	0.18	***	0.13	***	0.14	***
M2 Macrophage	CD163	0.20	***	0.18	**	-0.39	***	-0.36	***	0.07	1.67E-02	0.06	6.48E-02
	VSIG4	0.20	***	0.20	***	-0.54	***	-0.53	***	0.04	1.63E-01	0.03	3.01E-01
	MS4A4A	0.24	***	0.24	***	-0.54	***	-0.53	***	0.08	*	0.07	2.67E-02
Neutrophils	CD66b(CEACAM8)	0.07	1.73E-01	0.07	1.91E-01	-0.10	2.37E-02	-0.09	6.09E-02	0.10	**	0.10	*
	CD11b(ITGAM)	0.18	**	0.16	*	-0.46	***	-0.47	***	-0.07	3.03E-02	-0.08	1.27E-02
	CCR7	0.33	***	0.33	***	-0.11	1.25E-02	-0.10	2.65E-02	0.02	5.72E-01	0.01	7.41E-01
Natural killer cell	KIR2DL1	0.06	2.39E-01	0.06	2.33E-01	-0.02	6.77E-01	-0.04	4.35E-01	0.05	1.05E-01	0.04	1.88E-01
	KIR2DL3	0.07	1.40E-01	0.05	3.58E-01	-0.12	*	-0.13	*	0.03	3.56E-01	0.03	4.22E-01
	KIR2DL4	-0.04	3.61E-01	-0.05	2.95E-01	-0.33	***	-0.33	***	0.00	9.55E-01	-0.02	5.96E-01

Dscription	Gene markers	STAD				LGG				BC			
		None		Purity		None		Purity		None		Purity	
		Cor	*p*-Value	Cor	*p*-Value	Cor	*p*-Value	Cor	*p*-Value	Cor	*p*-Value	Cor	*p*-Value
	KIR3DL1	0.13	*	0.10	6.29E-02	0.02	5.72E-01	0.03	5.12E-01	0.03	4.06E-01	0.00	9.65E-01
	KIR3DL2	0.10	3.82E-02	0.08	1.16E-01	-0.14	*	-0.15	**	0.02	4.97E-01	0.02	4.99E-01
	KIR3DL3	-0.03	5.53E-01	-0.01	8.78E-01	-0.03	4.44E-01	-0.03	5.45E-01	0.02	5.72E-01	0.01	7.47E-01
	KIR2DS4	-0.02	6.94E-01	-0.04	4.12E-01	-0.10	2.07E-02	-0.10	2.75E-02	0.02	4.91E-01	0.01	6.99E-01
Dendritic cell	HLA-DPB1	0.06	2.09E-01	0.07	1.55E-01	-0.34	***	-0.35	***	0.12	***	0.01	8.51E-01
	HLA-DQB1	0.00	9.76E-01	0.02	7.23E-01	-0.26	***	-0.28	***	0.11	**	0.03	3.65E-01
	HLA-DRA	-0.01	8.03E-01	0.00	9.89E-01	-0.32	***	-0.34	***	0.10	*	-0.01	6.59E-01
	HLA-DPA1	0.01	8.99E-01	0.01	7.75E-01	-0.31	***	-0.32	***	0.08	1.00E-02	-0.04	2.66E-01
	BDCA-1(CD1C)	0.20	***	0.21	***	-0.19	***	-0.19	***	0.13	***	0.03	2.86E-01
	BDCA-4(NRP1)	0.38	***	0.40	***	-0.13	*	-0.10	2.62E-02	0.19	***	0.13	***
	CD11c(ITGAX)	0.17	**	0.18	**	-0.23	***	-0.27	***	0.08	*	-0.02	6.12E-01
Th1	T-bet(TBX21)	0.09	7.70E-02	0.10	5.91E-02	-0.22	***	-0.20	***	0.11	**	0.00	9.71E-01
	STAT4	0.17	**	0.18	**	0.47	***	0.45	***	0.17	***	0.07	3.55E-02
	STAT1	-0.04	3.74E-01	-0.02	6.56E-01	-0.08	7.37E-02	-0.08	8.91E-02	0.03	3.50E-01	-0.02	5.51E-01
	IFNG(INF-γ)	-0.14	*	-0.12	2.02E-02	-0.13	*	-0.15	*	0.05	6.90E-02	-0.03	3.79E-01
	TNF(TNF-α)	0.08	1.20E-01	0.10	4.92E-02	-0.10	2.19E-02	-0.11	2.09E-02	0.02	4.81E-01	-0.01	7.89E-01
Th2	GATA3	0.19	***	0.21	***	-0.21	***	-0.22	***	-0.17	***	-0.13	***
	STAT6	0.09	6.78E-02	0.11	3.74E-02	0.15	**	0.10	2.35E-02	0.07	2.97E-02	0.05	1.32E-01
	STAT5A	0.16	**	0.20	**	-0.28	***	-0.33	***	0.07	1.30E-02	0.00	9.57E-01
	IL13	0.12	1.30E-02	0.13	1.11E-02	0.15	**	0.14	*	0.08	*	0.04	2.03E-01
Tfh	BCL6	0.32	***	0.34	***	-0.09	4.38E-02	-0.04	3.53E-01	0.07	2.16E-02	0.05	1.54E-01
	IL21	-0.03	5.42E-01	-0.01	7.90E-01	-0.13	*	-0.15	**	0.00	9.35E-01	-0.05	9.30E-02
Th17	STAT3	0.23	***	0.25	***	-0.24	***	-0.22	***	0.02	4.62E-01	0.00	9.84E-01
	IL17A	-0.05	3.23E-01	-0.05	3.06E-01	-0.05	2.38E-01	-0.04	4.41E-01	0.01	6.91E-01	-0.02	4.56E-01

Dscription	Gene markers	STAD				LGG				BC			
		None		Purity		None		Purity		None		Purity	
		Cor	*p*-Value	Cor	*p*-Value	Cor	*p*-Value	Cor	*p*-Value	Cor	*p*-Value	Cor	*p*-Value
Treg	FOXP3	0.10	4.81E-02	0.12	2.09E-02	0.15	**	0.14	*	0.13	***	0.04	1.66E-01
	CCR8	0.14	*	0.17	*	-0.19	***	-0.20	***	0.04	1.56E-01	-0.02	4.40E-01
	STAT5B	0.33	***	0.34	***	0.00	9.21E-01	0.07	1.06E-01	0.00	9.58E-01	-0.03	3.03E-01
	TGFB1(TGFβ)	0.38	***	0.38	***	-0.22	***	-0.26	***	0.19	***	0.10	*
T cell exhaustion	PDCD1(PD-L1)	0.06	2.34E-01	0.09	7.94E-02	-0.27	***	-0.27	***	0.12	***	0.02	4.86E-01
	CTLA4	0.01	8.88E-01	0.04	4.23E-01	-0.11	*	-0.11	1.79E-02	0.07	1.71E-02	-0.02	5.58E-01
	LAG3	-0.01	9.13E-01	0.03	6.23E-01	-0.14	*	-0.13	*	0.04	1.66E-01	-0.01	8.03E-01
	TIM-3(HAVCR2)	0.13	1.07E-02	0.14	*	-0.29	***	-0.35	***	0.06	4.89E-02	-0.03	4.30E-01
	GZMB	-0.07	1.64E-01	-0.05	3.09E-01	-0.02	5.89E-01	-0.03	4.85E-01	0.08	*	-0.01	7.38E-01

STAD: Stomach adenocarcinoma; LGG: Brain lower grade glioma; BC: Breast cancer; TAM: tumour-associated macrophage; Th: T helper cell; Tfh: Follicular helper T cell; Treg: regulatory T cell; Cor: R value of Spearman's correlation; None: correlation without adjustment; Purity: correlation adjusted by purity. *$p < .01$; **$p < .001$; ***$p < .0001$.

Table 3

Correlation analysis between the expression level of SYT4 and markers of monocytes, TAM, M1 and M2 in GEPIA.

Dscription	Gene markers	STAD		BRCA		LGG	
		Tumor		Tumor		Tumor	
		Cor	*p*-Value	Cor	*p*-Value	Cor	*p*-Value
Monocyte	CD86	0.16	*	0.01	0.74	-0.48	***
	CD115(CSFIR)	0.25	***	0.027	0.37	-0.45	***
TAM	CCL2	0.25	***	0.055	0.07	-0.32	***
	IL10	0.20	***	0.099	*	-0.36	***
M1	NOS2	-0.07	0.16	0.073	0.016	0.31	***
	IRF5	0.11	0.027	0.10	**	-0.48	***
	COX2(PTGS2)	0.16	*	0.12	***	0.14	*
M2	CD163	0.15	*	0.056	0.064	-0.39	***
	VSIG4	0.18	**	0.045	0.14	-0.51	***
	MS4A4A	0.22	***	0.09	*	-0.52	***

STAD: Stomach adenocarcinoma; BRCA: Breast invasive carcinoma; LGG: Brain lower grade glioma; TAM: tumour-associated macrophage; Cor: R value of Spearman's correlation; *$p < 0.01$; **$p < 0.001$; ***$p < 0.0001$.

Discussion

Calcium-mediated exocytosis was an integral part of the human immune system. Exocytosis of mast cells, natural killer cells, and cytotoxic lymphocytes protected human beings from pathogen invasion, infected cells and malignant cells[26]. SYTs, a large class of membrane transporters, are the primary calcium sensors during exocytosis. And it had significant regulatory effects in nerves, endocrine, and immunity[27].

Baram D et al. confirmed that SYT1 expression was observed in mouse bone marrow-derived mast cells (BMMCs) and rat abdominal mast cells (RPM-Cs) by immunoblotting, and played a decisive regulatory role in mast cell exocytosis. While SYT2 played a negative supervisory role in lysosomal exocytosis of mast cells[28]. Lindmark IM et al. confirmed that SYT2 was expressed in human neutrophils (PMN), while SYT1, SYT3, and SYT4 were not, and SYT2 was involved in PMN phagocytosis and exocytosis[29]. These studies reflected the effects of the SYTs family on immune cells and the diversity of their functions. In the terms of the relationship between SYTs and tumors, a research found that SYT13 was helpful for the occurrence and development of colorectal cancer[10]. SYT7 was beneficial for the development of osteosarcoma[11]. But the relationship between SYT4 and cancer, as well as its relationship with tumor immunity remained unclear.

This study used the following databases to explore the associations between SYT4 and cancers: Oncomine, Kaplan-Meier plotter, GEPIA2, TIMER, and CGGA database. Data from Oncomine showed that SYT4 was lowly expressed in brain and nervous system cancer, colorectal, esophageal, gastric, prostate cancer, as well as sarcoma compared to normal tissues. Only one data set showed higher expression levels of SYT4 in lung cancer (Fig. 1A). The results from TIMER showed that compared to normal adjacent tissues, the expression levels of SYT4 were statistically different in the following cancers: BLCA, BRCA, COAD, ESCA, HNSC, KICH, KIRC, KIRP, LIHC, LUAD, LUSC, PRAD, READ, STAD, THCA, and UCEC (Fig. 1B). Throughout these databases, we determined the consistency of SYT4 expression in LGG and STAD. In the analysis of patient prognosis, we selected the Kaplan-Meier plotter and GEPIA2 database. The result suggested that the expression level of SYT4 was related to a favorable prognosis of LGG patients. In contrast, the high expression level of SYT4 was correlated with an unfavorable prognosis in STAD. At the same time, the CGGA database was verified the above result, and similar results were obtained. These findings suggested that the relationships between the expression level of SYT4 and the prognosis of cancers were different, indicating that SYT4 might be seen as a prognostic biomarker, at least in STAD and LGG.

After finding the expression of SYT4 had an impact on the survival of gastric patients with lymphatic metastasis (Table 1), we explored the associations between the expression level of SYT4 and the levels of immune infiltration in STAD and LGG. What's more, the association was also analyzed in BRCA as a control, because the prognosis of BRCA patients was not related to the expression of SYT4. Our findings indicated that the expression of SYT4 was related to the infiltration levels of B cells, CD4 + T cells, macrophages, and DCs in STAD and LGG, but this correlation was not presented in BRCA (Fig. 3). Interestingly, there was a positive correlation coefficient between the levels of immune cells infiltration and SYT4 expression levels in STAD, while a negative correlation coefficient existed in LGG. Although causality cannot be established through current research, we did find different correlations between the expression levels of SYT4 and the levels of immune cell infiltration, and the different correlations between the expression of SYT4 and the prognosis of STAD and LGG.

Besides, there was no correlation between SYT4 expression and tumor purity in LGG, but negative correlation with tumor purity in STAD. We speculated that the difference might be due to the different enrichment patterns of SYT4 in TME. Genes, which had a high expression in cells of the TME instead of tumor cells, were negatively related to the level of tumor purity, while genes with a high expression in tumor cells had a positive correlation with tumor purity[25]. In this study, the expression of SYT4 had different associations with tumor purity under different circumstances, indicating that SYT4 had different functions in various tumors. Also, we explored the correlations between the expression level of SYT4 and immune cells' molecular markers in STAD, LGG and BRAC. The focus was on monocytes, M1, M2, and TAM (Fig. 4). The expression level of SYT4 was positively related to the molecular markers of TAM (CCL2, IL10) and M2 (CD163, VSIG4, MS4A4A) in STAD, but the opposite result existed in LGG. TAM helped tumor cell migration, whose mechanism involved macrophages secreted epidermal growth factor family ligands and tumor cells secreted colony stimulating factor-1[30]. What's more, M2 macrophages were detrimental to prognosis of cancer patients because of stimulating lymphangiogenesis and angiogenesis[31]. The above studies helped us to explain the results of this study. The TAM and M2 macrophages were positively associated with the expression level of SYT4, and the expression level of SYT4 was also associated with poor prognosis in STAD patients. What's more, M1, as a macrophage with significant antitumor effect[15], had a strong correlation with the expression of SYT4 in LGG. However, the correlation was weak in STAD. Therefore, we had reasons to speculate that SYT4 might affect the prognosis of cancer patients through TAM.

In addition, we found that Treg cell markers TGFB1 had the highest correlation coefficient (cor = 0.38) with the SYT4 expression levels, and other Treg cells' markers, such as: CCR8, FOXP3 and STAT5B, also had significantly positive correlations with SYT4 expression in STAD. However, Treg cells' markers: CCR8 and TGFB1, showed a negative association with the expression level of SYT4 in LGG. Treg cells had been shown to be associated with poor prognosis[32]. Activated TGF-β existed widely in the TME, and inhibited the activity of NK cells and CTLs, the proliferation of Teff cells and the production of cytokines, as well as inhibited the differentiation of Teff cells into Th1 and Th17 cells[33]. These findings were also consistent with our results, suggesting that SYT4 interacted with Treg cells, and the interaction probably had effect on the prognosis of patients.

But the study had some limitations. Firstly, Biases might exist in the exploration of multiple databases. Secondly, due to the different sources of data from various databases, there were some contradictory aspects. Thirdly, our study conducted a bioinformatics analysis of SYT4, and the results did not be verified in vivo or in vitro. Fourthly, causal inferences cannot be made from our current research results, and follow-up prospective studies were needed.

Conclusions

In general, SYT4 can affect the prognosis of gastric cancer and glioma patients and is related to immune infiltration. The mechanism may be that SYT4 has different TAM patterns in TME. SYT4 can be used as a prognostic biomarker for STAD and LGG.

Declarations

Ethics approval and consent to participate

The human data in this study were all from the online database, which does not require ethical approval.

Consent for publication

Not applicable

Availability of data and materials

Our study analyzed all publicly available datasets. All these data could be found in the following

websites: https://www.oncomine.org, https://kmplot.com/analysis/, http://gepia2.cancer-pku.cn/#index, https://cistrome.shinyapps.io/timer/, http://cgga.org.cn/.

List Of Abbreviations

Synaptotagmins (SYTs), Synaptotagmin-4 (SYT4), Gene Expression Omnibus(GEO), European Genome-phenome Archive(EGA), The Cancer Genome Atlas(TCGA), TIMER(Tumor IMmune Estimation Resource); Gene Expression Profiling and Interactive Analyses (GEPIA), Genotype-Tissue Expression (GTEx), Chinese Glioma Genome Atlas (CGGA), hazard ratios (HRs), confidence intervals (CI), bladder urothelial carcinoma (BLCA), breast invasive carcinoma (BRCA), colon adenocarcinoma (COAD), esophageal carcinoma (ESCA), head and neck squamous cell carcinoma (HNSC), kidney chromophobe (KICH), kidney renal clear cell carcinoma (KIRC), kidney renal papillary cell carcinoma (KIRP), liver hepatocellular carcinoma (LIHC), lung adenocarcinoma (LUAD), lung squamous cell carcinoma (LUSC), prostate adenocarcinoma (PRAD), rectum adenocarcinoma (READ), stomach adenocarcinoma (STAD), thyroid carcinoma (THCA), brain lower-grade glioma (LGG), uterine corpus endometrial carcinoma (UCEC), Overall survival (OS), Progression-free survival (PFS), recurrence free survival (RFS), disease-free survival (DFS), tumor-associated macrophages (TAMs), Breast cancer(BC), tumor-associated macrophage(TAM), T helper cell(Th), Follicular helper T cell (Tfh), regulatory T cell(Treg).

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Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

SY-J: Collection and/or assembly of data, data analysis and interpretation, manuscript writing, methodology and software. LZ-Z: Data analysis and interpretation, manuscript editing. SB-Y: Data analysis, interpretation. C-J: Manuscript writing and project administration. B-W: Conception/design. Y-R: Conception/design, supervision and editing.

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Additional Files

Supplemental Figure 1. Survival analysis of SYT4 in different cancers in GEPIA2 database. A, B: The impact of SYT4 on the prognosis of patients with STAD. C, D: The impact of SYT4 on the prognosis of patients with LGG. E: Survival analysis of SYT4 in 33 types of cancer in GEPIA2 database. ACC: Adrenocortical carcinoma; BLCA: Bladder Urothelial Carcinoma; BRCA: Breast invasive carcinoma; CESC: Cervical squamous cell carcinoma and endocervical adenocarcinoma; CHOL: Cholangiocarcinoma; COAD: Colon adenocarcinoma; DLBC: Lymphoid Neoplasm Diffuse Large B-cell Lymphoma; ESCA: Esophageal carcinoma; GBM: Glioblastoma multiforme; HNSC: Head and Neck squamous cell carcinoma; KICH: Kidney Chromophobe; KIRC: Kidney renal clear cell carcinoma; KIRP: Kidney renal papillary cell carcinoma; LAML: Acute Myeloid Leukemia; LGG: Brain Lower Grade Glioma; LIHC: Liver hepatocellular carcinoma; LUAD: Lung adenocarcinoma; LUSC: Lung squamous cell carcinoma; MESO: Mesothelioma; OV: Ovarian serous cystadenocarcinoma; PAAD: Pancreatic adenocarcinoma; PCPG: Pheochromocytoma and Paraganglioma; PRAD Prostate adenocarcinoma; READ: Rectum adenocarcinoma; SARC: Sarcoma; SKCM: Skin Cutaneous Melanoma; STAD: Stomach adenocarcinoma; TGCT: Testicular Germ Cell Tumors; THCA: Thyroid carcinoma ; THYM: Thymoma; UCEC: Uterine Corpus Endometrial Carcinoma; UCS: Uterine Carcinosarcoma; UVM: Uveal Melanoma.

Supplemental Figure 2. Survival analysis of SYT4 in primary glioma in CGGA database. A-D: Survival analysis of SYT4 in primary glioma in the mRNAseq_325 dataset. E-H: Survival analysis of SYT4 in primary glioma in the mRNAseq_693 dataset.

Supplemental Figure 3. The correlation between the expression level of SYT4 and the level of immune infiltration in 39 types of cancer based on TIMER database. ACC, adrenocortical carcinoma; BLCA, bladder urothelial carcinoma; BRCA, breast invasive carcinoma; BRCA-Basal, breast invasive carcinoma-basal; BRCA-Luminal, breast invasive carcinoma-luminal; BRCA-Her2, breast invasive carcinoma-her2; CESC, cervical and endocervical cancer; CHOL, cholangiocarcinoma; COAD, colon adenocarcinoma; DLBC, diffuse large B-cell lymphoma; ESCA, esophageal carcinoma; GBM, glioblastoma multiforme; HNSC, head and neck cancer; HNSC-HPVpos, head and neck cancer-HPV positive; HNSC-HPVneg, head and neck cancer-HPV negative; KICH, kidney chromophobe; KIRC, kidney renal clear cell carcinoma; KIRP, kidney renal papillary cell carcinoma; LGG, lower grade glioma; LIHC, liver hepatocellular carcinoma; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; MESO, mesothelioma; OV, ovarian serous cystadenocarcinoma; PAAD, pancreatic adenocarcinoma; PCPG, pheochromocytoma and paraganglioma; PRAD, prostate adenocarcinoma; READ, rectum adenocarcinoma; SARC, sarcoma; SKCM, skin cutaneous melanoma; SKCM-Primary, skin cutaneous melanoma-primary; SKCM-Metastasis, skin cutaneous melanoma-metastasis; STAD, stomach adenocarcinoma; TGCT, testicular germ cell tumors; THCA, thyroid carcinoma; THYM, thymoma; UCEC, uterine corpus endometrial carcinoma; UCS, uterine carcinosarcoma; UVM, uveal melanoma.

Supplemental Table 1. SYT4 expression in cancers verus normal tissue in Oncomine.

Figures

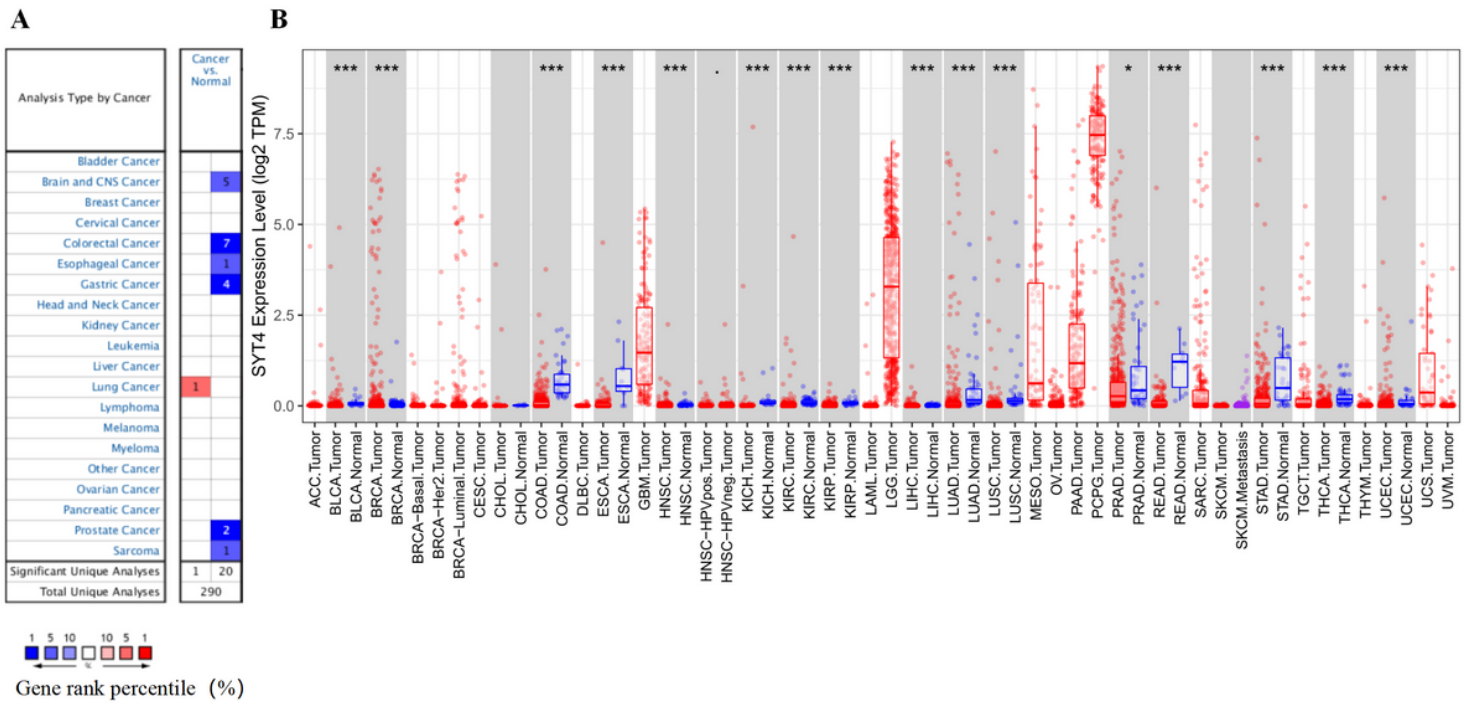

Figure 1

The expression levels of SYT4 in cancers. A. The data from Oncomine show the differences between the expression levels of STY4 in tumors and normal tissues. B. The data from TMIER show the expression level of SYT4 in different cancers.

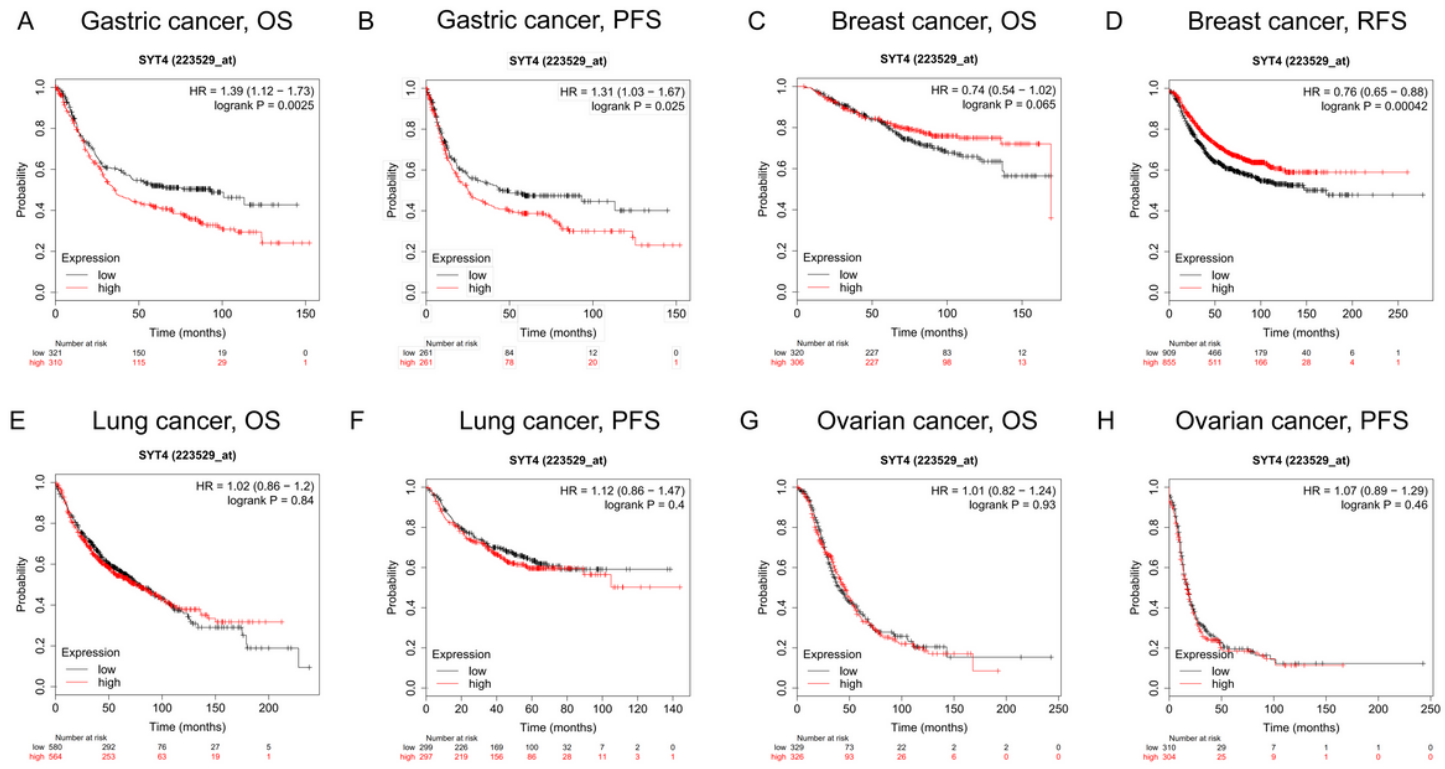

Figure 2

Survival analysis of SYT4 in different cancers in Kaplan-Meier plotter database. A, B: The impact of SYT4 on the prognosis of patients with gastric cancer. C, D: The impact of SYT4 on the prognosis of patients with breast cancer. E, F: The impact of SYT4 on the prognosis of patients with lung cancer. G, H: The impact of SYT4 on the prognosis of patients with ovarian cancer. (OS: Overall survival; PFS: Progression free survival; RFS: Recurrence free survival)

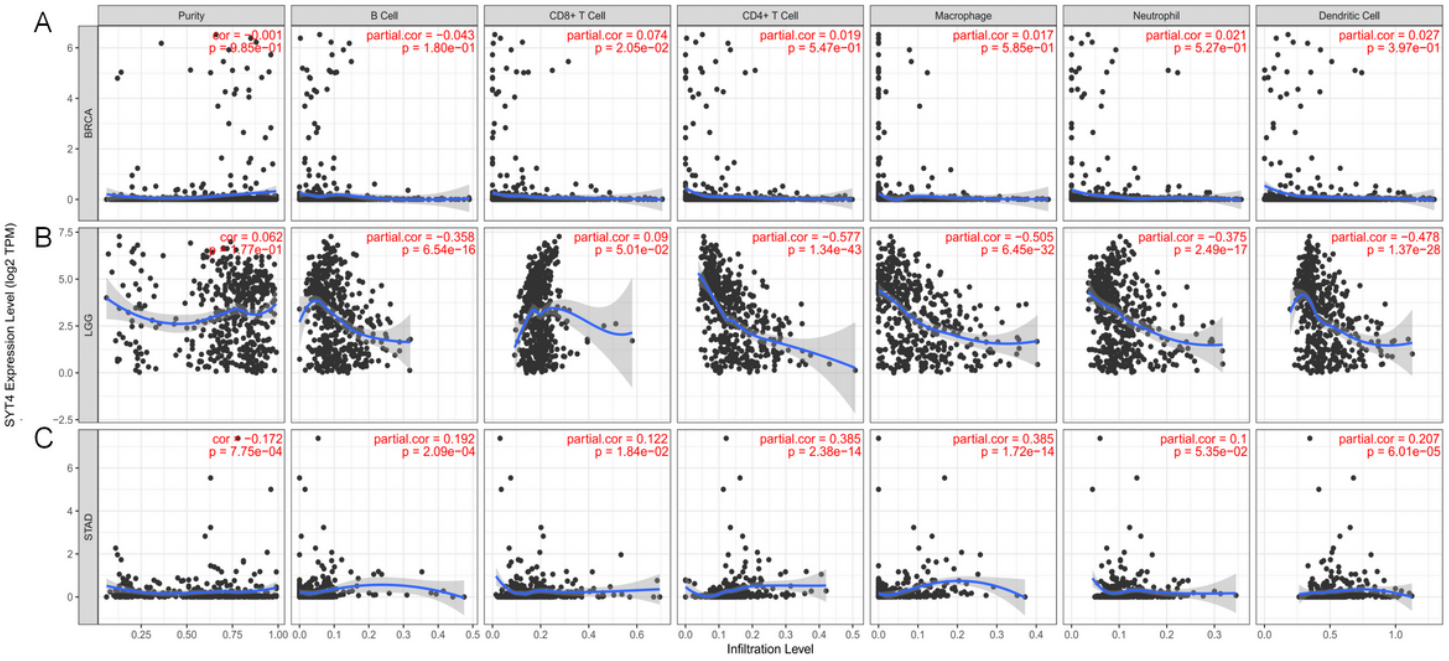

Figure 3

Correlation analysis of the expression level of SYT4 and the level of immune infiltration in STAD, LGG and BRAC. A. No correlation exists between the expression level of SYT4 and tumor purity, and the infiltration of 5 immune cells in BRCA, but there was a positive correlation between SYT4 and CD8 + T cells. B. The expression level of SYT4 has no association with tumor purity in LGG, but has a negative correlation with the level of infiltration of 5 immune cells, except for CD8 + T cells. C. The expression level of SYT4 has a negative association with tumor purity in STAD, but has a positive correlation with the level of infiltration of 5 immune cells, except for neutrophils.

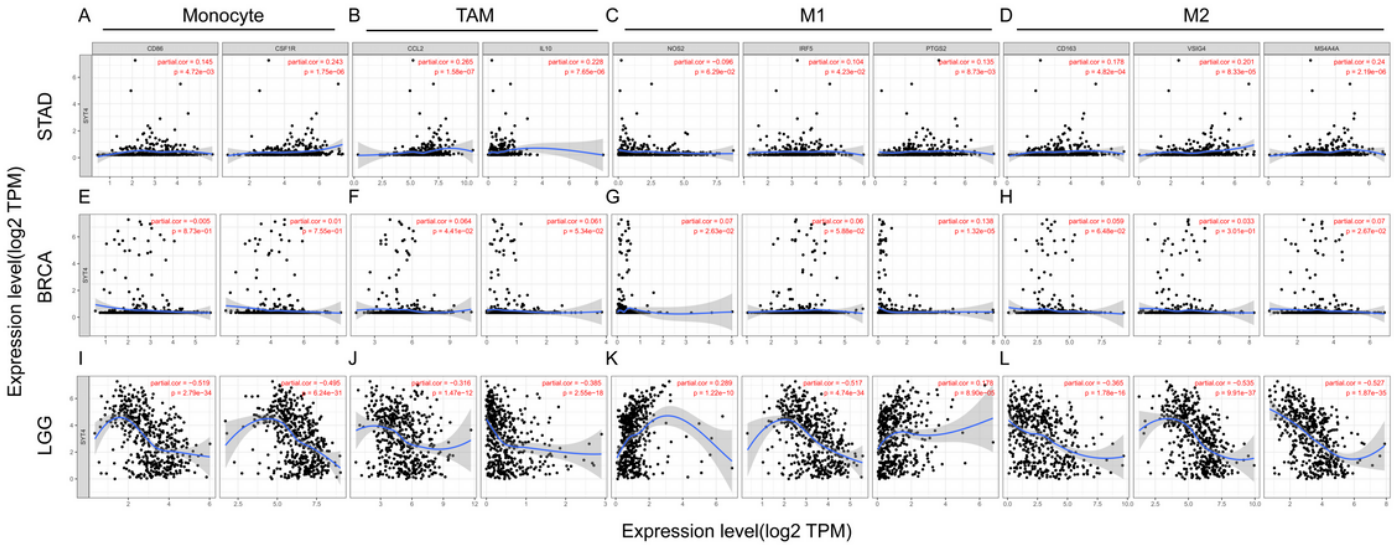

Figure 4

The correlation between the expression level of SYT4 and markers of immune cells, such as Monocytes (CD86, CSFIR), tumor-associated macrophages (CCL2,IL10), M1 macrophages (NOS2, IRF5, PTGS2) and M2 macrophages(CD163, VSIG4, MS4A4A), in STAD(A-D), BRCA(E-H), and LGG(I-L).

Supplementary Files

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

- SupplementalFigure2.png
- SupplementalFigure1.png
- SupplementalTable1.docx
- SupplementalFigure3.png