Comprehensive analysis on expression profile and prognostic values of SYTs family members and their methylation in gastric cancer

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Abstract

Background: Synaptotagmins (SYTs) family members, consisting of 17 isoforms, play crucial roles in the development and progression of human cancer. However, there are few studies on the expression and prognostic values of SYTs family in gastric cancer (GC).

Methods: We comprehensively evaluate the expression, methylation, prognosis and immune significance of SYTs family members through bioinformatics analysis from the online databases in GC.

Results: The expressions of SYT4, SYT9 and SYT14 were up-regulated, and negatively associated with their methylation levels in GC. Both SYT4, SYT9 and SYT14 overexpression and their hypomethylation levels contributed to unsatisfactory overall survival (OS) and progression-free survival (PFS) in GC. Moreover, the low expression levels of methylation cg sites (cg02795029, cg07581146, cg15149095, cg19922137, cg25371503, cg26158959, cg02269161, cg03226737, cg08185661, cg16437728, cg22723056 and cg24678137) were significantly correlated with unfavorable OS and PFS in GC. Furthermore, the expression of SYT4, SYT9 and SYT14 played a pivotal role in immune cells infiltration in GC.

Conclusion: SYT4, SYT9 and SYT14 might be potent prognostic indicators and promising immunotherapy targets for the patients with GC.

Introduction

Gastric cancer (GC) has, worldwide, remained a tricky health problem over a million new cases per year¹. According to the World Health Organization (WHO) data shows in 2018, GC ranks as the fifth most frequently diagnosed cancer, nevertheless, which is responsible for the third of cancer deaths¹. GC usually has symptoms at an advanced stage, which contributes to poor prognosis with the five years survival rate of less than 30%². Despite recent progresses and advancements made in the management of GC, the long-term efficacy is still unfavorable³. This is mainly because of GC pathogenesis are still unknown⁴. Therefore, identification of an effective biomarker for the diagnosis, individualized treatment and prognosis evaluation of GC is of urgent clinical significance.

Synaptotagmins (SYTs) family members are abundant, evolutionarily conserved integral membrane proteins and consist of 17 isoforms (SYT1–SYT17), which mainly serve as sensors of calcium signaling in cellular secretions^{5,6}. The SYTs family members are found primarily in tissues and have diversity functional significance⁷. Previous studies have showed that SYTs family members play an essential role in postsynaptic receptor endocytosis, vesicle trafficking, membrane repair, synaptic plasticity, and protection against neurodegeneration in the brain^{7,8}. Additionally, several studies have recently discovered that SYTs family members play an oncogenic role in the pathogenesis and progression of human cancer^{9,10}. However, the clinical and prognostic significance of SYTs family members in the occurrence and development of GC remain largely unknown.

In this study, we first explored the differential expression and prognostic value of SYTs family members in GC *via* The Cancer Genome Atlas (TCGA). Then, we evaluated the correlation between SYTs family expression and their methylation cg sites, and assessed the prognostic significance of their methylation. Univariate and multivariate regression analysis was performed to explore the SYTs family members expression and their methylation levels correlated with clinical characteristics. Finally, Tumor Immune Estimation Resource (TIMER) dataset was used to assess the potential correlation between SYTs family members expression and immune infiltration levels in GC.

Methods

Data source

TCGA database is conducted by the National Cancer Institute and National Human Genome Research Institute, and contains gene expression database and corresponding clinical information data. The high-throughput sequencing (HTSeq) Fragments Per Kilobase of transcript per Million mapped reads (FPKM) data, methylation450 profile and clinical data with GC were downloaded from TCGA database *via* UCSC Xena (<https://xena.ucsc.edu/>). Thus, a total of 375 cases of GC and 32 normal controls were included in this study.

Comparison of the mRNA expression and methylation level of SYTs family members in GC and normal controls

The mRNA expression levels of the SYTs family members were extracted from the HTSeq of GC using Perl 5.26 software. The different expression of the SYTs family members in GC compared with normal control was calculated using *ggpubr* package in R 4.0 software.

The methylation level of the SYTs family members were extracted from the methylation450 of GC using Perl 5.26 software. The SYTs family members methylation levels of cg site in the DNA promoter regions of GC were analyzed using *plyr* and *ggpubr* packages in R 4.0 software.

Correlation between mRNA expression and methylation of the SYTs family members in CG

The relationship between SYTs family members mRNA expression and their methylation cg sites were evaluated through *ggplot2* and *ggpubr* packages in R 4.0 software.

Survival analysis of mRNA expression and methylation cg site of SYTs family members

According to the median values of mRNA expression and methylation levels of SYTs family members, patients with GC were divided into high and low expression groups. Then, survival analysis was performed compared high expression with low expression group using *survival* and *survminer* package in R 4.0 software.

Timer Analysis

TIMER is an online tool for comprehensively assessing the immune infiltration status of different cancer types. Therefore, we utilized the TIMER software to explore the correlation between SYTs family members expression and six immune infiltration fluids (B cells, CD4⁺ T cells, CD8⁺ T cells, neutrophils, macrophages, and dendritic cells).

Statistical analysis

All statistical analysis analyses and plots were performed using R 4.0. Based on between-group differences, categorical variables were calculated using the Chi-squared test or Fisher's exact test; continuous variables were calculated using the student *t*-test or Mann-Whitney test. Pearson's correlation analysis was explored for the relationship between mRNA expression of SYTs family members and their methylation. Kaplan-Meier analyses were used to evaluate prognostic factors. In all cases, $P < 0.05$ was considered statistically significant.

Results

Expression status of SYTs family members in GC

We explored the mRNA expression levels of SYTs family members from 375 cases of GC and 32 normal controls, which originated from TCGA database. As shown in Fig. 1, SYT4, SYT9, SYT13 and SYT14 were significantly upregulated in GC samples compared to normal control ($P = 0.022$, $P = 0.023$, $P < 0.001$ and $P = 0.037$, respectively), while SYT8, SYT10, SYT12, SYT15 and SYT16 were significantly downregulated in GC samples ($P < 0.001$, $P < 0.001$, $P = 0.00083$, $P = 0.00062$ and $P = 0.00083$, respectively). However, there were not significantly different in SYT1, SYT2, SYT3, SYT5, SYT6, SYT7, SYT11 and SYT17 expressions compared GC samples with normal controls ($P = 0.53$, $P = 0.46$, $P = 0.92$, $P = 0.093$, $P = 0.84$, $P = 0.43$, $P = 0.99$ and $P = 0.28$, respectively).

Validation the prognostic value of SYTs family members in GC

Subsequently, we utilized Kaplan-Meier survival analysis to evaluate the prognostic values between SYTs family members and overall survival (OS) and progression-free survival (PFS) in patients with GC from TCGA database. We discovered that the high expression of SYT3, SYT4, SYT9 and SYT14 was associated with worse OS ($P = 0.026$, $P = 0.046$, $P = 0.046$ and $P = 0.002$, respectively), whereas the expression levels of SYT1, SYT2, SYT5, SYT6, SYT7, SYT8, SYT10, SYT11, SYT12, SYT13, SYT15, SYT16 and SYT17 were not correlated with OS for the patients with GC ($P = 0.248$, $P = 0.897$, $P = 0.585$, $P = 0.248$, $P = 0.748$, $P = 0.164$, $P = 0.778$, $P = 0.662$, $P = 0.115$, $P = 0.221$, $P = 0.064$, $P = 0.414$ and $P = 0.794$, respectively) (Fig. 2). Moreover, we found that up-regulated SYT4, SYT9 and SYT14 was significantly correlated with worse PFS ($P = 0.032$, $P = 0.007$ and $P < 0.001$, respectively), whereas the expression levels of SYT1, SYT2, SYT3, SYT5, SYT6, SYT7, SYT8, SYT10, SYT11, SYT12, SYT13, SYT15, SYT16 and SYT17 were not associated with PFS for the patients with GC ($P = 0.994$, $P = 0.059$, $P = 0.124$, $P = 0.094$, $P = 0.182$, $P = 0.611$, $P = 0.514$, $P = 0.289$, $P = 0.141$, $P = 0.08$, $P = 0.398$, $P = 0.069$, $P = 0.051$ and $P = 0.064$) (Fig. 3).

Prognostic value of SYTs family members for assessing overall survival in GC

Based on the above-mentioned results, we obtained the SYT4, SYT9 and SYT14 as candidate genes for further research in GC. Then, we utilized receiver operating characteristic curve (ROC) to evaluate the capabilities of SYT4, SYT9 and SYT14 predicting OS for patients with GC at 1-year, 3-year and 5-year. The area under curve (AUC) values of SYT4, SYT9 and SYT14 were 0.606, 0.603 and 0.564, respectively, at 1-year. Moreover, the AUC values of SYT4, SYT9 and SYT14 were 0.538, 0.626 and 0.570, respectively, at 3-year. Furthermore, the AUC values of SYT4, SYT9 and SYT14 were 0.500, 0.590 and 0.671, respectively, at 5-year (Fig. 4).

Correlation of SYTs family members expression and methylation in GC

Methylation of DNA promoter regions is one of crucial factors that regulates gene expression during the pathogenesis of human cancer. Then, we observed the methylation cg sites of SYT4, SYT9 and SYT14 in GC from TCGA database. There were 3 (cg27485084, cg12053284 and cg16222762), 10 (cg08913010, cg02269161, cg03226737, cg08185661, cg14243481, cg16437728, cg18560328, cg22723056, cg24678137 and cg26945996) and 8 (cg04932544, cg15389528, cg02795029, cg07581146, cg15149095, cg19922137, cg25371503 and cg26158959) methylation cg sites corresponded to SYT4, SYT9 and SYT14, respectively (Fig. 5A, 5B and 5C). The Pearson's correlation analysis demonstrated the inverse correlation between mRNA expression and methylation levels of SYT4, SYT9 and SYT14 ($R=-0.16$, $P = 0.0025$; $R=-0.37$, $P < 0.001$ and $R=-0.17$, $P = 0.0016$, respectively) (Fig. 5D, 5E and 5F).

Indeed, We found that except for 1 methylation cg site (cg27485084) ($P = 0.12$) (Fig. 6A), 2 methylation cg sites (cg12053284 and cg16222762) negatively correlated with the expression of SYT4 ($P = 0.016$ and $P = 0.023$) (Fig. 6B and 6C). Moreover, we discovered that except for 1 methylation cg site (cg08913010) ($P = 0.38$) (Fig. 6D), 9 methylation cg sites (cg02269161, cg03226737, cg08185661, cg14243481, cg16437728, cg18560328, cg22723056, cg24678137 and cg26945996) inversely correlated with the expression of SYT9 (all $P < 0.001$) (Fig. 6E, 6F, 6G, 6H, 6I, 6J, 6K, 6L and 6M). Furthermore, we demonstrated that except for 2 methylation cg sites (cg04932544 and cg15389528) ($P = 0.13$ and $P = 0.051$) (Fig. 6N and 6O), 6 methylation cg sites (cg02795029, cg07581146, cg15149095, cg19922137, cg25371503 and cg26158959) were negative association with the expression of SYT14 (all $P < 0.001$) (Fig. 6P, 6Q, 6R, 6S, 6T and 6U).

Validation the prognostic value of different methylation cg sites in GC

Then, Kaplan-Meier survival analysis was used to investigate the prognostic values of methylation cg sites of SYT4, SYT9 and SYT14 in patients with GC from TCGA database. We found that the methylation cg sites levels (cg27485084, cg12053284 and cg16222762) of SYT4 were not associated with OS ($P = 0.108$, $P = 0.283$ and $P = 0.145$, respectively) (Fig. 7A, 7B and 7C). Moreover, we discovered that the low methylation cg sites levels (cg02269161, cg03226737, cg08185661, cg18560328, cg22723056 and cg24678137) of SYT9 were correlated with unfavorable OS ($P = 0.004$, $P = 0.014$, $P = 0.037$, $P = 0.001$, $P = 0.005$ and $P = 0.007$, respectively) (Fig. 7D, 7E, 7F, 7G, 7H and 7I), whereas others (cg08913010,

cg14243481, cg16437728 and cg26945996) did not have prognostic value for patients with GC ($P = 0.926$, $P = 0.065$, $P = 0.059$ and $P = 0.056$, respectively) (Fig. 7J, 7K, 7L and 7M). Furthermore, we demonstrated that the low methylation cg sites levels (cg07581146, cg15389528 and cg25371503) of SYT14 were associated with unsatisfactory OS ($P = 0.026$, $P = 0.027$ and $P = 0.031$, respectively) (Fig. 7N, 7O and 7P), whereas others (cg02795029, cg04932544, cg15149095, cg19922137 and cg26158959) were not associated with prognostic value for patients with GC ($P = 0.160$, $P = 0.390$, $P = 0.382$, $P = 0.346$ and $P = 0.164$) (Fig. 7Q, 7R, 7S, 7T and 7U).

Similarly, we found that the methylation cg sites levels (cg27485084, cg12053284 and cg16222762) of SYT4 were not associated with PFS ($P = 0.359$, $P = 0.828$ and $P = 0.584$, respectively) (Fig. 8A, 8B and 8C). Moreover, we discovered that the low methylation cg sites levels (cg02269161, cg03226737, cg08185661, cg16437728, cg18560328, cg22723056 and cg24678137) of SYT9 were associated with unfavorable PFS ($P < 0.001$, $P = 0.044$, $P = 0.002$, $P = 0.015$, $P = 0.004$, $P = 0.028$ and $P = 0.005$, respectively) (Fig. 8D, 8E, 8F, 8G, 8H, 8I and 8J), whereas others (cg08913010, cg14243481 and cg26945996) were not associated with prognostic value for patients with GC ($P = 0.564$, $P = 0.075$ and $P = 0.122$, respectively) (Fig. 8K, 8L and 8M). Furthermore, we demonstrated that the low methylation cg sites levels (cg07581146 and cg19922137) of SYT14 were correlated with unfavorable PFS ($P = 0.041$ and $P = 0.010$) (Fig. 8N and 8O), whereas others (cg02795029, cg04932544, cg15149095, cg15389528, cg25371503 and cg26158959) were not correlated with prognostic value for patients with GC ($P = 0.112$, $P = 0.139$, $P = 0.635$, $P = 0.059$, $P = 0.205$ and $P = 0.061$, respectively) (Fig. 8P, 8Q, 8R, 8S, 8T and 8U).

Association with mRNA expression and methylation levels and clinicopathologic characteristics

We further explored the detailed association of SYT4, SYT9 and SYT14 expression as well as their methylation levels with clinicopathologic characteristics. As shown in Table 1 and Table 2, the expression of SYT4 was closely correlated with T stage ($P = 0.0029$) and pathological stage ($P = 0.0181$), while the methylation levels of SYT4 were closely correlated with T stage ($P = 0.0192$). As shown in Table 3 and Table 4, the expression of SYT9 was closely associated with T stage ($P = 0.0159$), meanwhile the methylation levels of SYT9 were closely associated with M stage ($P = 0.0032$). As shown in Table 5 and Table 6, the expression of SYT14 was closely correlated with T stage ($P = 0.0129$), meanwhile the methylation levels of SYT14 were closely correlated with age ($P = 0.0208$) and pathological stage ($P = 0.0273$).

Table 1
Correlation between SYT4 mRNA expression and clinicopathologic characteristics
in TCGA database

		Total	High	Low	P-value
Age ^a	<=65	155(45.86%)	84(49.7%)	71(42.01%)	0.156
Age	> 65	179(52.96%)	82(48.52%)	97(57.4%)	
Age	unknow	4(1.18%)	3(1.78%)	1(0.59%)	
Gender ^a	female	118(34.91%)	60(35.5%)	58(34.32%)	0.9092
Gender	male	220(65.09%)	109(64.5%)	111(65.68%)	
Grade ^a	G2	123(36.39%)	46(27.22%)	77(45.56%)	
Grade	G3	198(58.58%)	114(67.46%)	84(49.7%)	
Grade	unknow	9(2.66%)	5(2.96%)	4(2.37%)	
M ^a	M0	301(89.05%)	148(87.57%)	153(90.53%)	0.6162
M	M1	19(5.62%)	11(6.51%)	8(4.73%)	
M	unknow	18(5.33%)	10(5.92%)	8(4.73%)	
N ^a	N0	103(30.47%)	45(26.63%)	58(34.32%)	0.3329
N	N1	87(25.74%)	44(26.04%)	43(25.44%)	
N	N2	71(21.01%)	35(20.71%)	36(21.3%)	
N	N3	69(20.41%)	40(23.67%)	29(17.16%)	
N	unknow	8(2.37%)	5(2.96%)	3(1.78%)	
T ^a	T1	18(5.33%)	3(1.78%)	15(8.88%)	0.0029
T	T2	67(19.82%)	27(15.98%)	40(23.67%)	
T	T3	160(47.34%)	84(49.7%)	76(44.97%)	
T	T4	93(27.51%)	55(32.54%)	38(22.49%)	
Stage ^a	Stage I	46(13.61%)	13(7.69%)	33(19.53%)	0.0181
Stage	Stage II	108(31.95%)	58(34.32%)	50(29.59%)	
Stage	Stage III	145(42.9%)	73(43.2%)	72(42.6%)	
Stage	Stage IV	29(8.58%)	17(10.06%)	12(7.1%)	

Note: a, Chi-squared test

		Total	High	Low	<i>P</i> -value
Stage	unknow	10(2.96%)	8(4.73%)	2(1.18%)	
Note: a, Chi-squared test					

Table 2
Correlation between SYT4 methylation levels and clinicopathologic characteristics
in TCGA database

		Total	High	Low	P-value
Age ^a	<=65	155(45.86%)	70(41.42%)	85(50.3%)	0.1245
Age	> 65	179(52.96%)	97(57.4%)	82(48.52%)	
Age	unknow	4(1.18%)	2(1.18%)	2(1.18%)	
Gender ^a	female	118(34.91%)	51(30.18%)	67(39.64%)	0.087
Gender	male	220(65.09%)	118(69.82%)	102(60.36%)	
Grade ^a	G1	8(2.37%)	3(1.78%)	5(2.96%)	0.2182
Grade	G2	123(36.39%)	56(33.14%)	67(39.64%)	
Grade	G3	198(58.58%)	108(63.91%)	90(53.25%)	
Grade	unknow	9(2.66%)	2(1.18%)	7(4.14%)	
M ^a	M0	301(89.05%)	151(89.35%)	150(88.76%)	1
M	M1	19(5.62%)	10(5.92%)	9(5.33%)	
M	unknow	18(5.33%)	8(4.73%)	10(5.92%)	
N ^a	N0	103(30.47%)	52(30.77%)	51(30.18%)	0.5057
N	N1	87(25.74%)	43(25.44%)	44(26.04%)	
N	N2	71(21.01%)	40(23.67%)	31(18.34%)	
N	N3	69(20.41%)	30(17.75%)	39(23.08%)	
N	unknow	8(2.37%)	4(2.37%)	4(2.37%)	
T ^a	T1	18(5.33%)	11(6.51%)	7(4.14%)	0.0192
T	T2	67(19.82%)	28(16.57%)	39(23.08%)	
T	T3	160(47.34%)	77(45.56%)	83(49.11%)	
T	T4	93(27.51%)	53(31.36%)	40(23.67%)	
Stage ^a	Stage I	46(13.61%)	18(10.65%)	28(16.57%)	0.2368
Stage	Stage II	108(31.95%)	55(32.54%)	53(31.36%)	
Stage	Stage III	145(42.9%)	79(46.75%)	66(39.05%)	

Note: a, Chi-squared test

		Total	High	Low	<i>P</i> -value
Stage	Stage IV	29(8.58%)	12(7.1%)	17(10.06%)	
Stage	unknow	10(2.96%)	5(2.96%)	5(2.96%)	
Note: a, Chi-squared test					

Table 3
Correlation between SYT9 mRNA expression and clinicopathologic characteristics
in TCGA database

		Total	High	Low	P-value
Age ^a	<=65	155(45.86%)	88(52.07%)	67(39.64%)	0.065
Age	> 65	179(52.96%)	81(47.93%)	98(57.99%)	
Age	unknow	4(1.18%)	0(0%)	4(2.37%)	
Gender ^a	female	118(34.91%)	53(31.36%)	65(38.46%)	0.2094
Gender	male	220(65.09%)	116(68.64%)	104(61.54%)	
Grade ^a	G2	123(36.39%)	48(28.4%)	75(44.38%)	
Grade	G3	198(58.58%)	113(66.86%)	85(50.3%)	
Grade	unknow	9(2.66%)	4(2.37%)	5(2.96%)	
M ^a	M0	301(89.05%)	144(85.21%)	157(92.9%)	0.2897
M	M1	19(5.62%)	12(7.1%)	7(4.14%)	
M	unknow	18(5.33%)	13(7.69%)	5(2.96%)	
N ^a	N0	103(30.47%)	52(30.77%)	51(30.18%)	0.9095
N	N1	87(25.74%)	41(24.26%)	46(27.22%)	
N	N2	71(21.01%)	37(21.89%)	34(20.12%)	
N	N3	69(20.41%)	36(21.3%)	33(19.53%)	
N	unknow	8(2.37%)	3(1.78%)	5(2.96%)	
T ^a	T1	18(5.33%)	6(3.55%)	12(7.1%)	0.0159
T	T2	67(19.82%)	30(17.75%)	37(21.89%)	
T	T3	160(47.34%)	79(46.75%)	81(47.93%)	
T	T4	93(27.51%)	54(31.95%)	39(23.08%)	
Stage ^a	Stage I	46(13.61%)	21(12.43%)	25(14.79%)	0.5991
Stage	Stage II	108(31.95%)	49(28.99%)	59(34.91%)	
Stage	Stage III	145(42.9%)	76(44.97%)	69(40.83%)	
Stage	Stage IV	29(8.58%)	16(9.47%)	13(7.69%)	

Note: a, Chi-squared test

		Total	High	Low	<i>P</i> -value
Stage	unknow	10(2.96%)	7(4.14%)	3(1.78%)	
Note: a, Chi-squared test					

Table 4
Correlation between SYT9 methylation levels and clinicopathologic characteristics
in TCGA database

		Total	High	Low	P-value
Age ^a	<=65	155(45.86%)	66(39.05%)	89(52.66%)	0.0208
Age	> 65	179(52.96%)	100(59.17%)	79(46.75%)	
Age	unknow	4(1.18%)	3(1.78%)	1(0.59%)	
Gender ^a	female	118(34.91%)	59(34.91%)	59(34.91%)	1
Gender	male	220(65.09%)	110(65.09%)	110(65.09%)	
Grade ^a	G1	8(2.37%)	5(2.96%)	3(1.78%)	0.5796
Grade	G2	123(36.39%)	66(39.05%)	57(33.73%)	
Grade	G3	198(58.58%)	97(57.4%)	101(59.76%)	
Grade	unknow	9(2.66%)	1(0.59%)	8(4.73%)	
M ^a	M0	301(89.05%)	161(95.27%)	140(82.84%)	0.0032
M	M1	19(5.62%)	3(1.78%)	16(9.47%)	
M	unknow	18(5.33%)	5(2.96%)	13(7.69%)	
N ^a	N0	103(30.47%)	60(35.5%)	43(25.44%)	0.2003
N	N1	87(25.74%)	38(22.49%)	49(28.99%)	
N	N2	71(21.01%)	37(21.89%)	34(20.12%)	
N	N3	69(20.41%)	32(18.93%)	37(21.89%)	
N	unknow	8(2.37%)	2(1.18%)	6(3.55%)	
T ^a	T1	18(5.33%)	11(6.51%)	7(4.14%)	0.3756
T	T2	67(19.82%)	28(16.57%)	39(23.08%)	
T	T3	160(47.34%)	84(49.7%)	76(44.97%)	
T	T4	93(27.51%)	46(27.22%)	47(27.81%)	
Stage ^a	Stage I	46(13.61%)	22(13.02%)	24(14.2%)	0.1083
Stage	Stage II	108(31.95%)	61(36.09%)	47(27.81%)	
Stage	Stage III	145(42.9%)	73(43.2%)	72(42.6%)	

Note: a, Chi-squared test

		Total	High	Low	<i>P</i> -value
Stage	Stage IV	29(8.58%)	9(5.33%)	20(11.83%)	
Stage	unknow	10(2.96%)	4(2.37%)	6(3.55%)	
Note: a, Chi-squared test					

Table 5
Correlation between SYT14 mRNA expression and clinicopathologic characteristics
in TCGA database

		Total	High	Low	P-value
Age ^a	<=65	155(45.86%)	81(47.93%)	74(43.79%)	0.6493
Age	> 65	179(52.96%)	88(52.07%)	91(53.85%)	
Age	unknow	4(1.18%)	0(0%)	4(2.37%)	
Gender ^a	female	118(34.91%)	56(33.14%)	62(36.69%)	0.5683
Gender	male	220(65.09%)	113(66.86%)	107(63.31%)	
Grade ^a	G1	8(2.37%)	4(2.37%)	4(2.37%)	0.4257
Grade	G2	123(36.39%)	56(33.14%)	67(39.64%)	
Grade	G3	198(58.58%)	105(62.13%)	93(55.03%)	
Grade	unknow	9(2.66%)	4(2.37%)	5(2.96%)	
M ^a	M0	301(89.05%)	151(89.35%)	150(88.76%)	1
M	M1	19(5.62%)	9(5.33%)	10(5.92%)	
M	unknow	18(5.33%)	9(5.33%)	9(5.33%)	
N ^a	N0	103(30.47%)	52(30.77%)	51(30.18%)	0.9068
N	N1	87(25.74%)	46(27.22%)	41(24.26%)	
N	N2	71(21.01%)	34(20.12%)	37(21.89%)	
N	N3	69(20.41%)	33(19.53%)	36(21.3%)	
N	unknow	8(2.37%)	4(2.37%)	4(2.37%)	
T ^a	T1	18(5.33%)	6(3.55%)	12(7.1%)	0.0129
T	T2	67(19.82%)	26(15.38%)	41(24.26%)	
T	T3	160(47.34%)	94(55.62%)	66(39.05%)	
T	T4	93(27.51%)	43(25.44%)	50(29.59%)	
Stage ^a	Stage I	46(13.61%)	18(10.65%)	28(16.57%)	0.2135
Stage	Stage II	108(31.95%)	57(33.73%)	51(30.18%)	
Stage	Stage III	145(42.9%)	76(44.97%)	69(40.83%)	

Note: a, Chi-squared test

		Total	High	Low	<i>P</i> -value
Stage	Stage IV	29(8.58%)	11(6.51%)	18(10.65%)	
Stage	unknow	10(2.96%)	7(4.14%)	3(1.78%)	
Note: a, Chi-squared test					

Table 6
Correlation between SYT14 methylation levels and clinicopathologic characteristics
in TCGA database

		Total	High	Low	P-value
Age ^a	<=65	155(45.86%)	66(39.05%)	89(52.66%)	0.0208
Age	> 65	179(52.96%)	100(59.17%)	79(46.75%)	
Age	unknow	4(1.18%)	3(1.78%)	1(0.59%)	
Gender ^a	female	118(34.91%)	56(33.14%)	62(36.69%)	0.5683
Gender	male	220(65.09%)	113(66.86%)	107(63.31%)	
Grade ^a	G1	8(2.37%)	2(1.18%)	6(3.55%)	0.3453
Grade	G2	123(36.39%)	63(37.28%)	60(35.5%)	
Grade	G3	198(58.58%)	101(59.76%)	97(57.4%)	
Grade	unknow	9(2.66%)	3(1.78%)	6(3.55%)	
M ^a	M0	301(89.05%)	157(92.9%)	144(85.21%)	0.0513
M	M1	19(5.62%)	5(2.96%)	14(8.28%)	
M	unknow	18(5.33%)	7(4.14%)	11(6.51%)	
N ^a	N0	103(30.47%)	60(35.5%)	43(25.44%)	0.0912
N	N1	87(25.74%)	40(23.67%)	47(27.81%)	
N	N2	71(21.01%)	39(23.08%)	32(18.93%)	
N	N3	69(20.41%)	28(16.57%)	41(24.26%)	
N	unknow	8(2.37%)	2(1.18%)	6(3.55%)	
T ^a	T1	18(5.33%)	11(6.51%)	7(4.14%)	0.5695
T	T2	67(19.82%)	31(18.34%)	36(21.3%)	
T	T3	160(47.34%)	77(45.56%)	83(49.11%)	
T	T4	93(27.51%)	50(29.59%)	43(25.44%)	
Stage ^a	Stage I	46(13.61%)	23(13.61%)	23(13.61%)	0.0273
Stage	Stage II	108(31.95%)	60(35.5%)	48(28.4%)	
Stage	Stage III	145(42.9%)	74(43.79%)	71(42.01%)	

Note: a, Chi-squared test

		Total	High	Low	P-value
Stage	Stage IV	29(8.58%)	7(4.14%)	22(13.02%)	
Stage	unknow	10(2.96%)	5(2.96%)	5(2.96%)	
Note: a, Chi-squared test					

Association of SYTs family members expression with immune cells infiltration

According to increasing evidence on the correlations between immune cells infiltration and prognosis in cancer, we utilized the TIMER database to evaluate the association of SYT4, SYT9 and SYT14 expression with immune cells infiltration. As shown in Fig. 9A, 9B, 9C, 9D and 9E, the expression of SYT4 was positively correlated with CD4⁺T cells, CD8⁺T cells, DC, macrophage cells and neutrophil cells (all $P < 0.001$), whereas which had no correlation with B cells ($P = 0.881$) (Fig. 9F). As shown in Fig. 9G, 9H, 9I, 9J, 9K and 9L, the expression of SYT9 had positive association with B cells, CD4⁺T cells, CD8⁺T cells, DC, macrophage cells and neutrophil cells (all $P < 0.001$). As shown in Fig. 9M and 9N, the expression of SYT14 was positively associated with CD4⁺T cells and macrophage cells (all $p < 0.001$), whereas which was not associated with B cells, CD8⁺T cells, DC and neutrophil cells ($P = 0.52$, $P = 0.51$, $P = 0.96$ and $P = 0.81$, respectively) (Fig. 9O, 9P, 9Q and 9R).

Discussion

SYTs family members are abundant, evolutionarily conserved integral membrane proteins, which play crucial roles in regulated exocytosis in nervous and endocrine systems⁸. However, several studies have recently reported that SYTs family members had vital roles in the pathogenesis of human cancer. It was discovered that SYT13 was up-regulated in the lung adenocarcinoma as well as in colorectal cancer, which promoted the lung adenocarcinoma cells and colorectal cancer cells proliferation and migration, and contributed to unsatisfactory prognosis^{10,11}. Sung HY *et al.* demonstrated that SYT2 was up-regulated in ovarian cancer, promoted the ovarian carcinoma cells migration and invasiveness, and associated with poor survival for patients with ovarian cancer¹². SYT7 was overexpressed in lung cancer as well as in colorectal cancer and glioma, which promoted cells proliferation and inhibited cells apoptosis, and resulted in unfavorable prognosis^{13–15}. SYT4 was up-regulated in triple-negative breast cancer, conferred paclitaxei resistance and indicated poor prognosis¹⁶. However, few studies evaluated the significance of SYTs family members for patients with GC, especially for prognosis. In this study, we first used the online database to comprehensively explore the mRNA expression and methylation levels of SYTs family members and their significance in prognosis and underlying mechanisms in GC. We discovered that mRNA expression of SYT4, SYT9 and SYT14 were significantly up-regulated in GC tissues compared to normal control, and which contributed to unsatisfactory OS and PFS for patients with GC. Moreover, we discovered that SYT14 can well predict 5-year OS for patients with GC. Kanda M. *et*

al. discovered that SYT8 was elevated in GC patients with peritoneal recurrence or metastasis, which was consistent with our results¹⁷. However, we further discovered that SYT8 was not associated with OS for the patients with GC. Kanda M *et al.* demonstrated that overexpression of SYT7 was detected in GC tissues, and significantly correlated with hepatic recurrence, metastasis, and poor prognosis¹⁸. Nevertheless, we found that the expression of SYT7 was similar between GC samples and normal controls in this study. The reason may be small samples in above-mentioned studies.

It had reported that the methylation profile was crucial to evaluate the functional status of the genes, because of their expressions depending on the DNA CpG island methylation status^{19,20}. Foschini MP *et al.* discovered that MAGEA family members were hypomethylation in male breast cancer (MBC) leading to their overexpression, which enhanced the androgen receptor (AR) activity and AR therapy response²¹. Meanwhile, gene methylation has constituted an attractive research field in oncology, especially useful to predict prognostic and therapeutical significance of cancer profile²². However, no study has been published on methylation levels of SYTs family members in GC. In this study, the Pearson's correlation analysis demonstrated that the inverse correlation between mRNA expressions and methylation levels of SYT4, SYT9 and SYT14 in GC. Then, we further explored the association between mRNA expression and methylation cg site levels in GC, among SYT4, SYT9 and SYT14. The results showed that 2 methylation cg sites (cg12053284 and cg16222762) negatively correlated with the expression of SYT4, 9 methylation cg sites (cg02269161, cg03226737, cg08185661, cg14243481, cg16437728, cg18560328, cg22723056, cg24678137 and cg26945996) inversely correlated with the expression of SYT9, and 6 methylation cg sites (cg02795029, cg07581146, cg15149095, cg19922137, cg25371503 and cg26158959) were negative association with the expression of SYT14. Subsequently, we found that the methylation cg sites levels (cg12053284 and cg16222762) of SYT4 were not associated with OS and PFS for patients with GC. These results indicated that the regulation of SYT4 promoter methylation was not the main route to regulate the expression of SYT4 in GC. Moreover, we demonstrated that low methylation cg sites levels (cg02269161, cg03226737, cg08185661, cg16437728, cg22723056 and cg24678137) of SYT9 were associated with unfavorable OS and PFS for patients with GC. These results showed that these methylation cg sites might be potent prognostic indicators and therapeutic targets for GC. Furthermore, we demonstrated that the low methylation cg sites level (cg07581146) of SYT14 were correlated with unsatisfactory OS and PFS for the patients with GC. This result indicated that cg07581146 methylation cg site of SYT14 also might be potent prognostic indicator and therapeutic target for GC. In addition, we discovered that SYT4, SYT9 and SYT14 mRNA expression and their methylation levels were closely correlated with T stage and pathological stage. Therefore, these results uncovered that SYT4, SYT9 and SYT14 expression, and their methylation might play crucial roles in the development and progression of the patients with GC.

Several studies have pointed out that tumor immune microenvironment plays pivotal roles in development and prognosis of GC²³⁻²⁶. In this study, we first explored that the SYTs family members expression correlated with formation of tumor immune microenvironment in GC. We discovered that the SYT4 expression was positively correlated with CD4⁺T cells, CD8⁺T cells, DC, macrophage cells and

neutrophil cells, whereas the SYT9 expression had positive association with B cells, CD4⁺T cells, CD8⁺T cells, DC, macrophage cells and neutrophil cells, indeed, the SYT14 expression was positively associated with CD4⁺T cells and macrophage cells. These results demonstrated that SYTs family members were mainly implicated in the immune microenvironment of GC through the regulation of these immune cells, which might be promising immunotherapy targets in the future.

Obviously, it is undeniable that there were some limitations to this study. First, all the data were based on the online databases, further experiments are required to confirmed our findings. Second, further experiments are needed to verify the underlying mechanism between SYTs family members and GC *in vivo* and *iv intro*.

In conclusion, we have demonstrated that SYT4, SYT9 and SYT14 was up-regulated and negatively correlated their methylation levels in GC. Meanwhile, the overexpression of SYT4, SYT9 and SYT14 was significantly associated with unsatisfactory OS and PFS. Moreover, methylation cg sites of SYT9 and SYT14 which significantly correlated with OS and PFS for the patents with GC, were identified. Furthermore, the expression of SYT4, SYT9 and SYT14 played a pivotal role in immune cells infiltration. Therefore, SYT4, SYT9 and SYT14 might be potent prognostic indictors and promising immunotherapy targets for the patients with GC.

Declarations

Ethics approval and consent to participate

Not Applicable.

Consent for publication

Not applicable.

Availability of data and materials

The high-throughput sequencing (HTSeq) Fragments Per Kilobase of transcript per Million mapped reads (FPKM) data, methylation450 profile and clinical data with GC were downloaded from TCGA database *via* UCSC Xena (<https://xena.ucsc.edu/>). All data generated during this study are included in this published article.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

De-feng Li, Ben-hua Wu, Li-sheng Wang and Jun Yao were responsible for design of the study and reviewed the manuscript. Mei-feng Yang, Xing-xing Long and Hong-sai Hu drafted the manuscript. Yu-ling Bin, Xuan-ming Chen, Ben-hua Wu and De-feng Li obtained and calculated the data. De-feng Li, Jun Yao and Li-sheng Wang was responsible for revising manuscript. All authors have read and approved the final manuscript.

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Figures

Figure 1

The SYTs family members mRNA expression in patients with GC compared to normal control from TCGA database.

Figure 2

Overall survival (OS) value of SYTs members family mRNA expression in patients with GC. Kaplan-Meier curves comparing the high and low expression of SYTs family members in patients with GC from TCGA database.

Figure 3

Progression free survival (PFS) value of SYTs members family mRNA expression in patients with GC. Kaplan-Meier curves comparing the high and low expression of SYTs family members in patients with GC from TCGA database.

Figure 4

Evaluate the capabilities of SYT4, SYT9 and SYT14 predicting OS for patients with GC. A, The receiver operating characteristic (ROC) of SYT4 to predict 1-year, 3-year and 5-year OS for the patients with GC; B, The ROC of SYT9 to predict 1-year, 3-year and 5-year OS for the patients with GC; C, The ROC of SYT14 to predict 1-year, 3-year and 5-year OS for the patients with GC.

Figure 5

Correlation of SYTs family members expression and methylation in GC. A, The distribution of SYT4 DNA promoter CpG sites; B, The distribution of SYT9 DNA promoter CpG sites; C, The distribution of SYT14 DNA promoter CpG sites; D, SYT4 mRNA expression was negatively correlated with its methylation levels; E, SYT9 mRNA expression was negatively correlated with its methylation levels; F, SYT14 mRNA expression was negatively correlated with its methylation levels.

Figure 6

Correlation of SYTs family members expression and their methylation cg sites in GC.

Figure 7

OS value of SYTs members methylation cg sites in patients with GC. Kaplan-Meier curves comparing the high and low expression of methylation cg sites in patients with GC from TCGA database.

Figure 8

PFS value of SYTs members methylation cg sites in patients with GC. Kaplan-Meier curves comparing the high and low expression of methylation cg sites in patients with GC from TCGA database.

Figure 9

Association between the SYTs family members expression and tumor infiltration immune cells (B cells, CD4+T cells, CD8+T cells, DC, macrophage cells and neutrophil cells) in the patients with GC using the TIMER database. Tumor purity is shown in the panels on the left.