

Low STYK1 expression indicates poor prognosis in gastric cancer

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Background: The expression of serine threonine tyrosine kinase 1 (STYK1), a member of the receptor protein tyrosine kinase (RPTK) family, is abnormal in several cancers. However, the molecular mechanism of STYK1 regulation of gastric cancer (GC) progression is unknown.

Materials and methods: We evaluated STYK1 expression in GC tissues and the corresponding normal tissues. Specimens from 93 patients with GC were examined with immunohistochemical staining. The relationship between STYK1 protein expression and the patients' clinicopathological features was assessed. Kaplan–Meier and Cox proportional regression analyses were used to evaluate the association between STYK1 expression and survival.

Results: STYK1 expression was decreased in GC tissues. Low STYK1 expression was significantly associated with poor tumor differentiation ($P=0.023$), advanced clinical stage ($P=0.021$), and poor overall survival (OS; $P=0.034$). Univariate and multivariate analyses revealed that STYK1 expression was an independent prognostic indicator (HR =0.53, 95% CI =0.29–0.95, $P=0.039$; HR =0.51, 95% CI =0.24–0.91, $P=0.030$, respectively).

Conclusion: Downregulated STYK1 expression correlated significantly with poor tumor differentiation, advanced clinical stage, and poor OS in GC. STYK1 might be a diagnostic and prognostic indicator in patients with GC.

Keywords: gastric cancer, STYK1, prognosis, overall survival

Introduction

Gastric cancer (GC) is a common malignancy of the human digestive system and the third leading cause of cancer-related death worldwide, caused an estimated 700,000 deaths in 2012.¹ Around the world, about 8% of all patients are newly diagnosed with GC each year; although the 1-year survival rate for GC is 80%, the 5-year survival rate is 50%, and the recurrence rate is over 60%.^{2,3} Invasion and distant metastasis are the most lethal biological characteristics of GC and an important cause of death in patients with GC.^{4,5} Therefore, it is critical to identify specific molecular markers and therapeutic targets for the early diagnosis and treatment of patients with GC.

Serine threonine tyrosine kinase 1 (STYK1), also known as novel oncogene with kinase domain (NOK), is a member of the receptor protein tyrosine kinase (RPTK) family.⁶ STYK1 expression is high in a wide range of cancers, including gallbladder carcinoma,⁷ lung cancer,⁸ colorectal cancer,⁹ nasopharyngeal carcinoma,¹⁰ ovarian cancer,¹¹ pancreatic cancer,¹² and hepatocellular carcinoma.¹³ These data indicate that increased levels of STYK1 expression are associated with progression in the aforementioned cancers. So far, however, the expression and possible role of STYK1

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in GC are unknown. In the present study, we sought to define the relationship between STYK1 expression and the clinical outcomes and clinicopathological features of GC.

Materials and methods

Data resources

GC tissues and the normal or adjacent tissue expression profiles of the GSE49051, GSE79973, and GSE54129 data sets were obtained from the Gene Expression Omnibus (GEO) database (www.ncbi.nlm.nih.gov/geo).¹⁴ The GSE49051 data set contains three GC tissue samples and three noncancerous samples; GSE79973 contains 10 pairs of GC tissues and adjacent noncancerous mucosa; GSE54129 contains 111 human GC tissues and 21 noncancerous gastric tissues.

Identification of differentially expressed genes (DEGs)

We used GeneSpring 13.1 software to screen the DEGs between the GC tissues and noncancerous samples. All raw data in the expression profiles were preprocessed via robust multi-array average (RMA) normalization.¹⁵ The cutoff values for DEG selection were expressed as fold change ≥ 2 and $P < 0.05$.

Bioinformatics analysis

STYK1 mRNA expression levels were analyzed using the databases of Oncomine (<http://www.Oncomine.com>) and The Cancer Genome Atlas (TCGA; <http://cancergenome.nih.gov>)¹⁶ downloaded from the University of California Santa Cruz (UCSC) Cancer Genomics Browser. Oncomine is an online cancer microarray database;¹⁷ the UCSC Cancer Genomics Browser is an online interactive genome browser hosted by the University of California, Santa Cruz, CA, USA, that offers access to genome sequence data (<https://genome-cancer.ucsc.edu>).¹⁸

Patients and tissue samples

We collected 93 samples of tumor tissues and the adjacent normal tissues at the Second People's Hospital of Wuxi, Jiangsu, China, between January 2012 and December 2015. All patients had been diagnosed and confirmed with GC by histology. The tumor stages had been determined according to the *Cancer Staging Manual* (seventh edition) of the American Joint Committee on Cancer (AJCC). The follow-up period was defined as the interval from the date of surgery to the date of death or last follow-up. Written informed consent was obtained from patients, and the School of the Second People's Hospital of Wuxi Ethics Committee approved this

study; this procedure was conducted in accordance with the Declaration of Helsinki.

Immunohistochemistry

Tissue blocks were fixed in 10% buffered formalin, embedded in paraffin, and serially sectioned at 5 μm thickness. Endogenous peroxidase activity was suppressed by 10-minute incubation with 3% hydrogen peroxide. The slides were then blocked with 5% BSA (Boster Bioengineering, Wuhan, China). The tissue sections were treated with primary antibodies against STYK1 (1:300) and then incubated overnight in a humidified chamber at 4°C. The sections were visualized with 3,3'-diaminobenzidine (DAB) and counterstained with hematoxylin for microscopic examination. The STYK1 protein expression levels in the tumor cell cytoplasm were scored as the staining intensity and the percentage of positive stained cells. The scoring system was as follows: 0, no positive cells; 1–3, positive cells with yellowish, light-brown, and dark-brown staining, respectively. The percentage of positive cells was scored as follows: 0, no positive cells; 1, $\leq 10\%$ positive cells; 2, 11–50% positive cells; 3, $> 50\%$ positive cells. STYK1 expression was scored by multiplying the percentage of positive tumor cells and the staining intensity and ranged from 0 to 9.

Western blotting

Whole-cell lysates were separated by SDS-PAGE (Bio-Rad Laboratories Inc., Hercules, CA, USA) and transferred to polyvinylidene difluoride (PVDF) membranes (EMD Millipore, Billerica, MA, USA). The membranes were blocked with 5% nonfat dry milk powder for 2 hours at room temperature and then incubated with primary antibodies against STYK1 and GAPDH (Santa Cruz Biotechnology Inc., Dallas, TX, USA) at 4°C overnight. The membranes were visualized using the appropriate secondary antibody (Abcam, Cambridge, MA, USA) at room temperature for 1 hour.

Statistical analyses

All statistical analyses were performed using SPSS 22.0 (IBM Corporation, Armonk, NY, USA). Significant differences among groups were measured by Student's *t*-test or one-way ANOVA. The correlation between STYK1 expression and clinicopathological factors was estimated using Fisher's exact test. Overall survival (OS) curves were plotted according to the Kaplan–Meier method, with the log-rank test applied for comparison estimated by Fisher's exact test. Cox proportional hazards regression analysis was used for univariate and

multivariate analyses of prognostic values. $P < 0.05$ was defined as statistically significant.

A total of 93 patients were enrolled in the survival analysis. Follow-up surveys were made by telephone to obtain updated patient information and survival data. The survey was completed in April 2015, with 5 years of follow-up records obtained for each patient. OS time was defined as the time from the date of surgery to death (for non-censored events) or to the end of the survey period (for censored events). OS was assessed using a Kaplan–Meier method and univariate and Cox regression analyses. We used Kaplan–Meier plotter (www.kmplot.com)¹⁹ to evaluate the prognostic value of STYK1 mRNA expression; the patients were divided into two groups based on the median STYK1 mRNA expression (high and low) and assessed using a Kaplan–Meier survival plot.

Results

Identification of STYK1 in GC

We analyzed DEGs in the GSE49051,²⁰ GSE79973,²¹ and GSE54129 data sets between GC tissues and noncancerous samples, which involved 10,433 genes (Figure 1A–C). Venn diagram analysis showed 252 DEGs, including *STYK1* (Figure 1D).

Correlation of STYK1 expression with prognosis in human cancer

We identified six studies^{7–10,12,13} from public databases (PubMed, Embase, Cochrane Library, Wiley Online Library, Medline). The meta-analysis results indicated that the pooled HR was 2.21 (95% CI = 1.81–2.71) for the high vs low STYK1 expression group ($P < 0.00001$; Figure 2). The result suggests that high STYK1 expression may predict poor prognosis among human cancers, except GC.

STYK1 expression was decreased in GC tissues

To determine STYK1 expression in GC, we analyzed STYK1 mRNA expression levels using Oncomine. We found that normal tissue had higher STYK1 mRNA expression levels than tumor tissues, which included diffuse gastric adenocarcinoma, gastric adenocarcinoma, gastric intestinal-type adenocarcinoma, gastric mixed adenocarcinoma, and gastrointestinal stromal tumor (Figure 3A–D). We also verified STYK1 expression with TCGA database: the result also showed that tumor tissues had decreased STYK1 expression compared to normal tissues (Figure 3E).

To verify the accuracy of the Oncomine and TCGA database results, STYK1 protein expression levels were analyzed

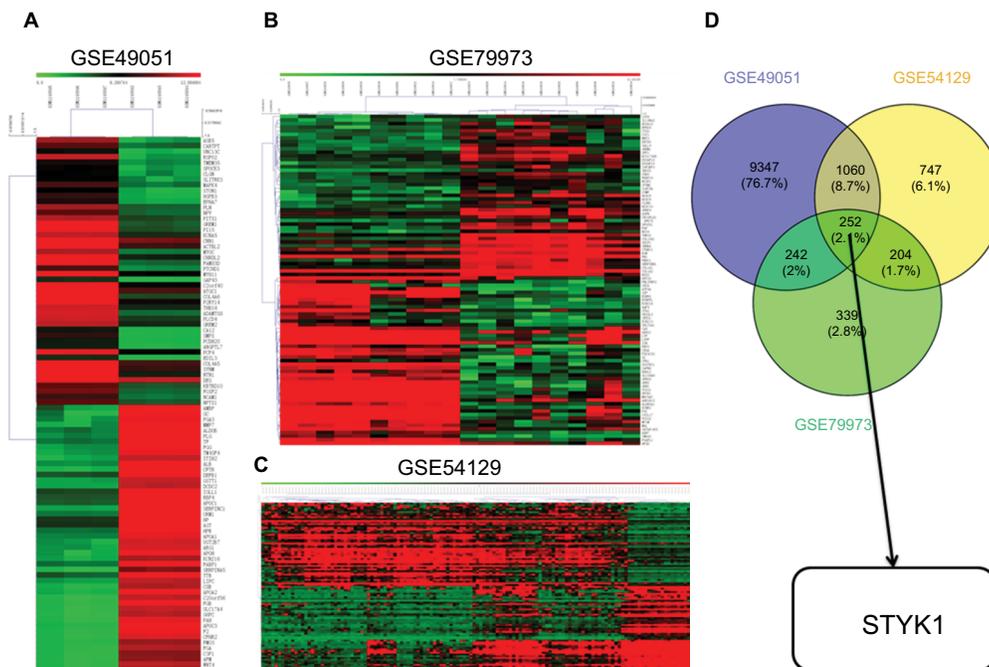


Figure 1 The results of hierarchical clustering analysis for DEGs between GC tissues and noncancerous tissues.

Notes: (A) GSE49051. (B) GSE79973. (C) GSE54129. (D) Venn diagram analyzing the intersection of the three data sets.

Abbreviations: DEGs, differentially expressed genes; GC, gastric cancer.

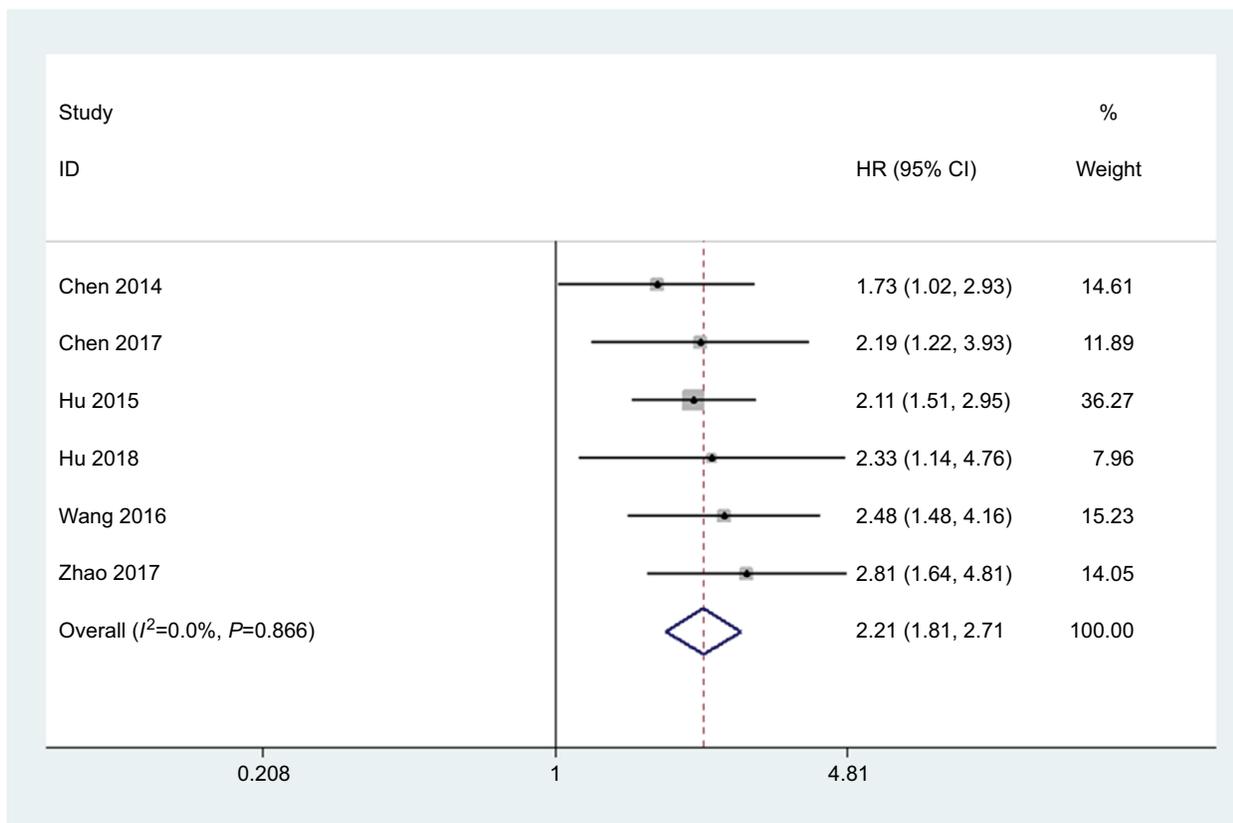


Figure 2 *STYK1* expression is increased in various cancers other than GC.

Note: The HR of *STYK1* expression in gallbladder carcinoma, lung cancer, colorectal cancer, nasopharyngeal carcinoma, pancreatic cancer, and hepatocellular carcinoma pooled by meta-analysis is shown.

Abbreviation: GC, gastric cancer.

by immunohistochemical staining in 93 paired tumor tissues and the corresponding normal tissues. The tumor tissues had decreased *STYK1* protein expression, which is consistent with the Oncomine database analysis (Figure 4A). Furthermore, we determined *STYK1* protein expression in the GC cell lines, HGC-27, SGC-7901, MKN-28, and BGC-823, and in the GES-1 normal gastric mucosal epithelial cell line by Western blotting. All the four GC cell lines had decreased *STYK1* protein expression levels compared to the GES-1 cell line (Figure 4B). These results suggest that *STYK1* protein expression is downregulated in GC tissues.

Relationship between *STYK1* protein expression and patients' clinicopathological features

To investigate the association between *STYK1* protein levels and the clinicopathological features of GC, we analyzed the correlation between *STYK1* protein expression and the clinicopathological features of 93 patients with GC. Table 1 summarizes that the patients could be classified into two

subgroups (low and high *STYK1* expression). Low *STYK1* expression was significantly associated with poor tumor differentiation ($P=0.023$) and advanced clinical stage ($P=0.021$). However, there were no obvious correlations between *STYK1* protein expression and sex ($P=0.709$), age ($P=0.219$), tumor size ($P=0.071$), and lymph node metastasis ($P=0.168$).

Prognostic value of *STYK1* protein expression

To elucidate the relationship between *STYK1* expression and the clinical outcome of patients with GC, we used Kaplan–Meier plotter (www.kmplot.com) to evaluate *STYK1* mRNA expression and the survival of patients with GC. The Kaplan–Meier analysis indicated that low *STYK1* mRNA expression correlated with poor OS and progression-free survival (PFS; Figure 5A and B). Then, we used Kaplan–Meier analysis to analyze the correlation between *STYK1* expression and the survival of the 93 patients and obtained similar results ($P=0.034$; Figure 5C). Finally, univariate and multivariate analyses revealed that *STYK1* expression was

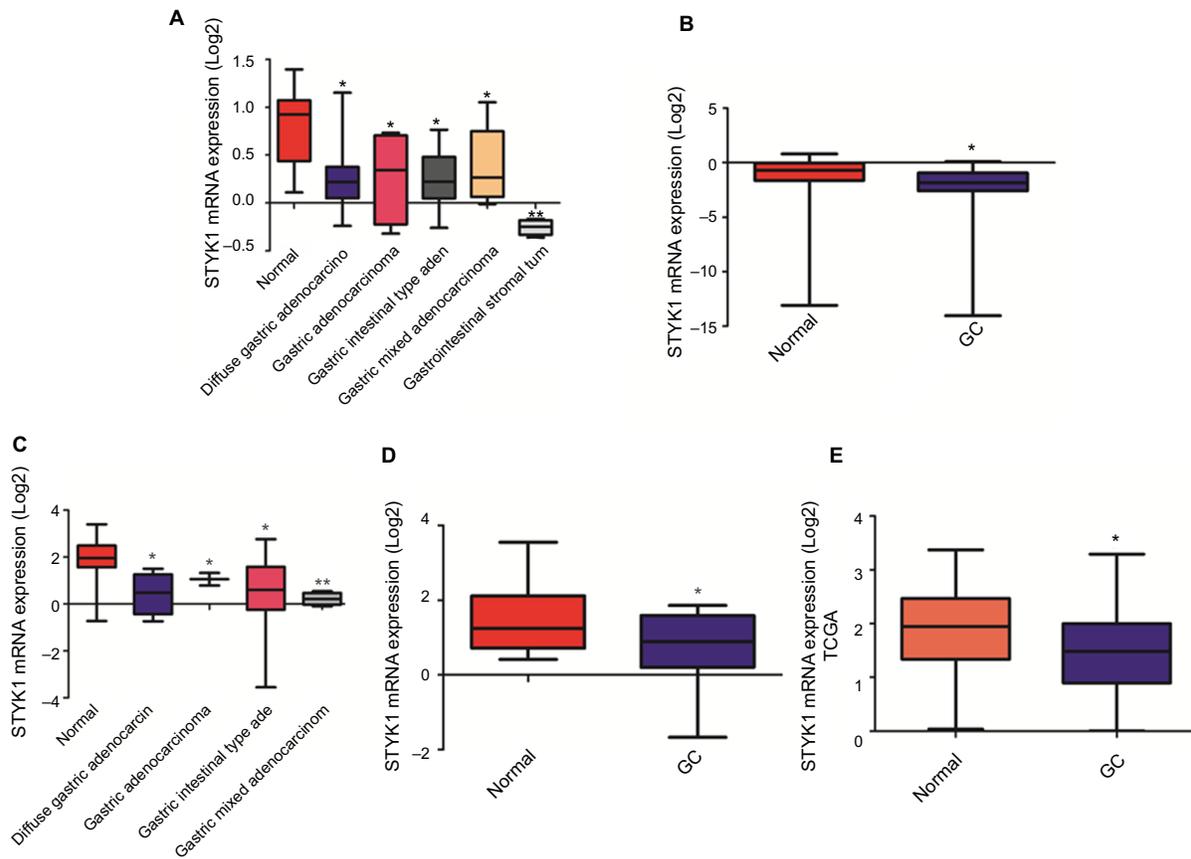


Figure 3 Analysis of *STYK1* mRNA expression in GC tissues and normal tissues based on public databases. **Notes:** (A) Cho gastric statistics, (B) Cui gastric statistics, (C) D'Errico gastric statistics, (D) Wang gastric statistics. All data are from Oncomine. (E) *STYK1* expression analysis based on TCGA database. * $P < 0.05$; ** $P < 0.01$. **Abbreviations:** GC, gastric cancer; TCGA, The Cancer Genome Atlas.

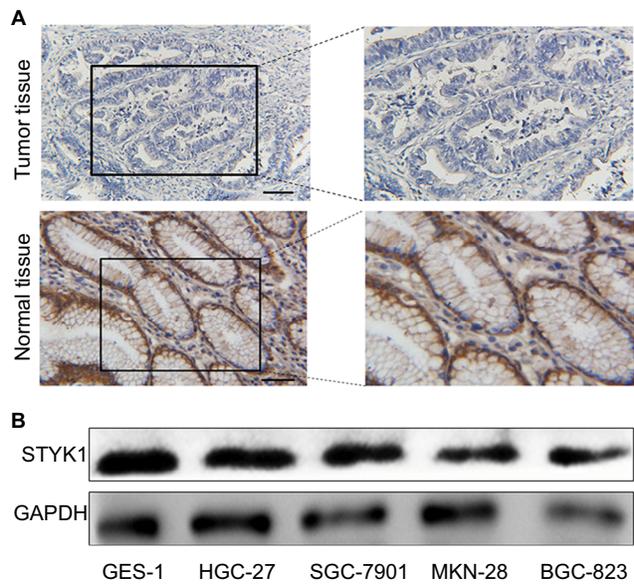


Figure 4 *STYK1* protein expression in primary GC tissues and GC cell lines. **Notes:** (A) Representative immunohistochemical staining of *STYK1* in GC tissues (0 points) and adjacent normal tissues (9 points). Scale bar: 100 μ m, magnification for left panel $\times 40$; right panel $\times 100$. (B) Western blot of *STYK1* protein expression in four GC cell lines and one normal GES-1. **Abbreviations:** GC, gastric cancer; GES-1, gastric mucosal epithelial cell line.

an independent prognostic indicator in patients with GC (HR =0.53, 95% CI =0.29–0.95, $P=0.039$; HR =0.51, 95% CI =0.24–0.91, $P=0.030$, respectively; Table 2).

Discussion

PTKs play a significant role in cellular regulation such as cell proliferation, migration, and invasion.²² *STYK1* is a member of the RPTK family. Previous studies have suggested that *STYK1* plays a different role in the progression of various cancers. Hu et al⁷ and Wang et al¹³ reported that *STYK1* promotes cancer cell epithelial–mesenchymal transition (EMT) and tumor metastasis by activating the PI3K–AKT pathway in gallbladder carcinoma and hepatocellular carcinoma. Chung et al²³ indicated that *STYK1* overexpression could have potential as a kinase in castration-resistant prostate cancer cells and could be a possible approach for the development of novel castration-resistant prostate cancer therapies. Chen et al¹² reported that *STYK1* repressed E-cadherin expression and promoted EMT, mediated by the p38 MAPK signaling pathway, and showed

Table 1 The correlation of *STYK1* expression with the clinical features of GC

Characteristics	No. of patients (n)	STYK1 expression		P-value
		Low (n=52)	High (n=41)	
Sex				0.709
Male	57	31	26	
Female	36	21	15	
Age (years)				0.219
>60	52	32	20	
≤60	41	20	21	
Differentiation				0.023
Poor	42	29	13	
Well and moderate	51	23	28	
Tumor size (cm)				0.071
≥5	32	22	10	
<5	61	30	31	
Clinical stage				0.021
I or II	42	18	24	
III or IV	51	34	17	
Lymph node metastasis				0.168
Positive	55	34	21	
Negative	38	18	20	

Notes: The significance of bold figures indicate $P < 0.05$.

Abbreviation: GC, gastric cancer.

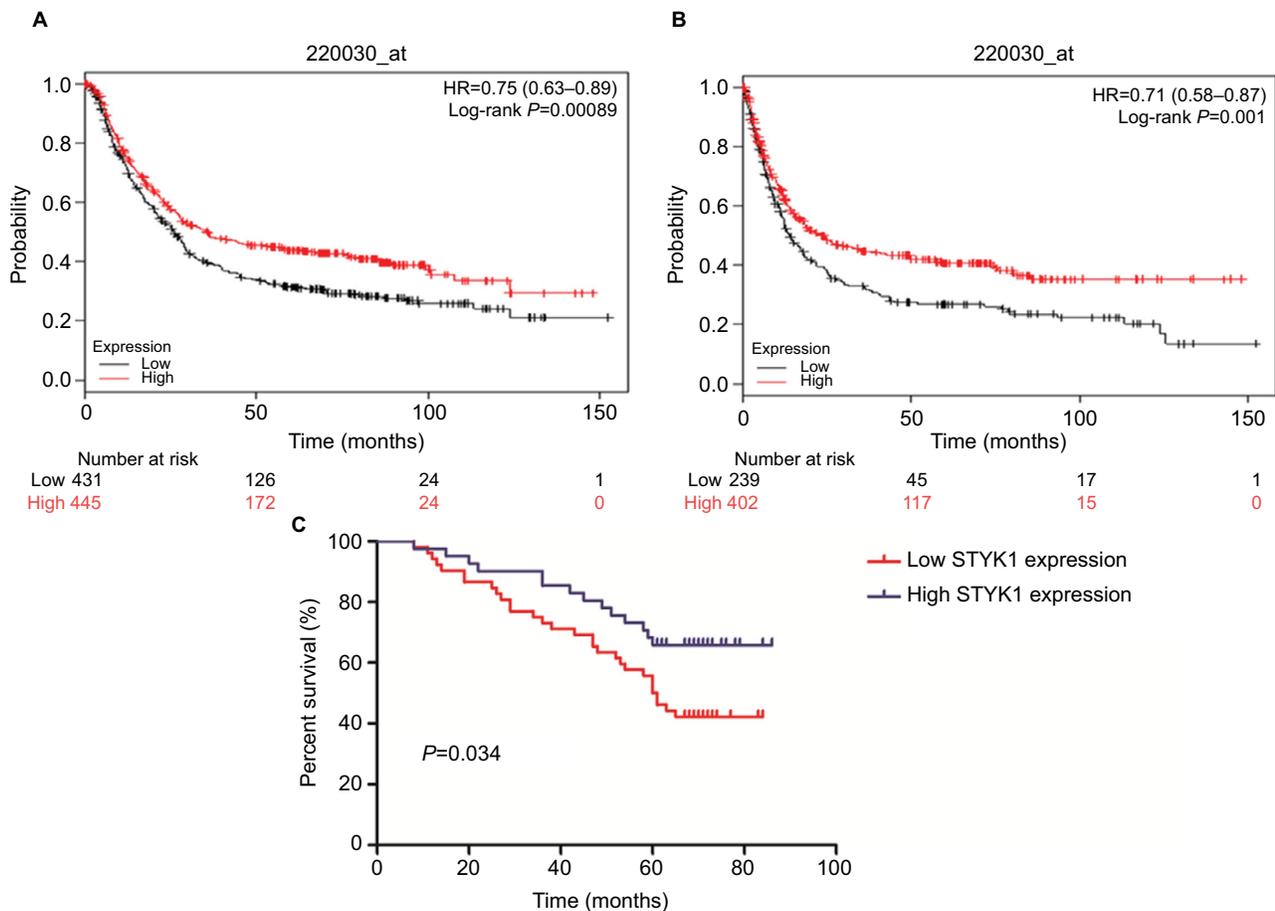


Figure 5 Prognostic significance of *STYK1* protein expression in patients with GC.

Notes: Kaplan–Meier plotter analysis of the effect of *STYK1* mRNA expression levels on the OS (A) and PFS (B) in patients with GC. (C) Kaplan–Meier analysis of the correlation between *STYK1* expression and survival in 93 patients with GC.

Abbreviations: GC, gastric cancer; PFS, progression-free survival.

Table 2 Univariate and multivariate analyses of prognostic factors for OS in GC

Parameters	Categories	Univariate analysis			Multivariate analysis		
		HR	95% CI	P-value	HR	95% CI	P-value
Differentiation	Poor vs well and moderate	0.74	0.43–1.27	0.272			
Clinical stage	I or II vs III or IV	0.44	0.11–1.81	0.259			
Lymph node metastasis	Positive vs negative	0.57	0.18–1.14	0.109			
STYK1 expression	High vs low	0.53	0.29–0.95	0.039	0.51	0.24–0.91	0.03

Notes: The significance of bold figures indicate $P < 0.05$.

Abbreviations: GC, gastric cancer; OS, overall survival.

that STYK1 might be a prognostic marker in patients with pancreatic cancer. In addition, Nirasawa et al²⁴ suggested that STYK1 is a novel drug resistance factor and could be a predictor of the therapeutic response in acute leukemia. These studies all demonstrate that high STYK1 expression plays important roles in various cancers and affects the prognosis of patients with cancer. In the present study, we first used three GEO data sets (GSE49051, GSE79973, GSE54129) to determine the differential expression of STYK1 in GC, and immunohistochemistry and Western blotting confirmed that STYK1 protein expression was decreased in GC tissues and cell lines, respectively. Furthermore, low STYK1 expression was associated with poor prognosis in patients with GC. To the best of our knowledge, this study is the first to suggest that STYK1 expression may be an independent prognostic factor in patients with GC. However, the reason for the low STYK1 expression in GC and the specific regulatory mechanism in GC progression remains unclear. STYK1 has been identified as a new member of the RPTK-like protein family.²⁵ RPTKs are key players in the regulation of numerous fundamental cellular processes such as growth, adhesion, differentiation, and migration.²⁶ In GC cells, the MAPK–ERK and PI3K–AKT signaling pathways affect RPTK activity.²⁷ Therefore, we speculated that the effect of other regulatory factors in GC leads to PI3K–AKT signaling pathway feedback acting on STYK1, thereby reducing its expression. Naturally, the detailed mechanism requires verification in further well-designed experiments.

Conclusion

Our study demonstrates that downregulated STYK1 correlates significantly with poor tumor differentiation, advanced clinical stage, and poor survival. The findings also demonstrate that STYK1 might be a diagnostic and prognostic indicator in patients with GC.

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Disclosure

The authors report no conflicts of interest in this work.

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