Original Article

Systematic Analysis of Spleen Tyrosine Kinase Expression and its Clinical Outcomes in Various Cancers

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Abstract

Background: Spleen tyrosine kinase (SYK) is an important enzyme in the proliferation and differentiation of all hematopoietic tissues. Its role as a cancer driver is well documented in liquid tumors; however, cumulative evidence has suggested an opposite role in other tumor types.

Objectives: To systematically assess the expression of *SYK*, its prognostic value and epigenetic status in different cancers using bioinformatics tools.

Methods: In this bioinformatics study, Oncomine database and cBioPortal were used to study the *SYK* gene expression, Kaplan–Meier plotter to study its prognostic value and MethHC to assess the *SYK* gene methylation in various cancers.

Results: From 542 unique analyses of the *SYK* gene, it was found to be overexpressed in bladder, breast and colon cancers but downregulated in leukemia, lymphoma and myeloma. Compared with normal tissues, breast and brain tumors showed an overexpression of the *SYK* gene, whereas lymphoma and leukemia had lower expression. The Kaplan–Meier survival analysis revealed that *SYK* expression in pancreatic, gastric, liver and lung patients were correlated with better overall survival. Using cBioPortal, prostate cancer was found to have the highest *SYK* gene mutation frequency, and the mean expression was highest in diffuse large B-cell lymphoma, acute myeloid leukemia and thymoma. Using the MethHC database, *SYK* promoter hypermethylation was found to be significantly higher in breast, renal, liver, lung, pancreatic, prostatic, skin and stomach cancers compared with the normal tissue ($P < 0.005$).

Conclusion: The results of this study indicate the potential use of SYK as a diagnostic and therapeutic target for different type of cancers. However, further experimental data are required to validate these results before use of SYK in clinical settings.

Keywords: Cancer, expression, methylation, mutation, spleen tyrosine kinase, survival rate

INTRODUCTION

Spleen tyrosine kinase (SYK) is a cytosolic nonreceptor protein tyrosine kinase that is highly expressed in all hematopoietic tissues and plays a role in the development and growth of B-cells.[1] Stimulation of B-cell receptors activates downstream pathways that involve SYK

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stimulation, and consequently, B-cell activation, resulting in B-cell development and growth. SYK activation has been shown to play a role in allergy and autoimmunity responses, in B-cell transformation[2] and as a viral oncogene.[3]

Kinase inhibitors have been developed to target and inhibit the abnormal stimulation of SYK. For example, R112, a selective inhibitor of SYK, has been found to have therapeutic effects on allergic rhinitis patients,[4] while fostamatinib, also a SYK inhibitor, has been shown to reduce the onset and severity of arthritis.[5] SYK targeting has also been shown to be a therapeutic candidate for lymphoma and leukemia. Entospletinib, for example, is a selective inhibitor of SYK that has shown to have effects on patients with relapsed chronic lymphoblastic leukemia.[6]

Although *SYK* is considered as an oncogene in some hematological malignancies,[7] cumulative data have suggested that it plays a tumor suppressive role in various cancers.[8] This role has been documented in the epithelial cells of solid cancers such as breast cancer. Low expression of *SYK* in breast cancer is correlated with low survival rates and a more invasive cancer.[9] In addition, reactivation of *SYK* has been shown to inhibit tumor growth of breast cancer cells, whereas inhibition of *SYK* activation decreases the p53-dependent apoptosis of epithelial cancer cells.[8]

Based on the current evidence, *SYK* has been demonstrated to have a dual role in cancer progression and suppression. Therefore, there is a need to better understand the role *SYK* plays in different cancers. Accordingly, in this study, a systematic analysis of *SYK* was conducted using online databases to assess its expression in different cancer types, prognostic value and epigenetic status in different cancers.

METHODS

This systematic bioinformatics study used various databases for analyzing *SYK* gene expression and methylation as well as its prognostic value in various cancers.

Spleen tyrosine kinase expression levels in different cancers

Oncomine™ database (https://www.oncomine.org/resource/login.html) is an open-access, online tool containing data from numerous published cancer microarray studies. In this study, the level of *SYK* gene expression in the normal and tumor tissues was identified in different cancer types using the Oncomine database in July 2019.[10] The threshold of *SYK* mRNA fold was determined according to the following parameters: $P <$

1×10^{-4}, fold change >2 and gene ranking in the top 10%.

Relationship between spleen tyrosine kinase expression and overall survival

Kaplan–Meier plotter (http://kmplot.com/analysis/) is an online database of published microarray datasets that assess the effect of 54,675 genes on survival using 18,674 samples from different cancers. In the current study, this tool was used in July 2019 to assess the prognosis of *SYK* expression, which was only found in patients with pancreatic, gastric, liver and lung cancer. The hazard ratio with 95% confidence intervals and log-rank P value were computed.[11]

Spleen tyrosine kinase expression and mutations frequencies

The cBioPortal for cancer genomics is an open-access tool (http://www.cbioportal.org/), wherein about 55,833 tumor samples from >210 cancer studies in The Cancer Genome Atlas (TCGA) can be visualized and analyzed. The search page of the tool helps extract customized data that allow exploring genetic mutations in different samples of the gene of interest. In this study, data from TCGA[12] were retrieved using cBioPortal in July 2019 for analyzing the expression and mutations frequency in *SYK* gene, and Kaplan–Meier (log rank) test was used to calculate the P value for the differences between patients with/or without alteration in *SYK*.[13]

Spleen tyrosine kinase promoter methylation in different cancers

To illustrate other possible mechanisms by which *SYK* gene is compromised, the DNA methylation status of *SYK* promoter in different cancer types was assessed using the MethHC database in July 2019,[14] an online tool that provides data on DNA methylation of different human cancers. MethHC integrates data such as DNA methylation, gene expression and correlation of methylation and gene expression from TCGA. *SYK* promoter methylation was analyzed in the following tumor types: bladder urothelial carcinoma; breast invasive carcinoma; cervical squamous cell carcinoma and endocervical adenocarcinoma; colon adenocarcinoma; head-and-neck squamous cell carcinoma; kidney renal clear cell carcinoma; kidney renal papillary cell carcinoma; liver hepatocellular carcinoma; lung adenocarcinoma; lung squamous cell carcinoma; pancreatic adenocarcinoma; prostate adenocarcinoma; rectum adenocarcinoma; sarcoma; skin cutaneous melanoma; stomach adenocarcinoma; thyroid carcinoma; and uterine corpus endometrial carcinoma.

In the boxplot, *t*-test was used to test the difference between two groups, i.e., tumor and normal samples, and *P* value is the probability of obtaining a statistically significant result.

RESULTS

Oncomine database analyses for gene expression
From the Oncomine data analysis of *SYK* expression in normal and tumor tissues of various cancers, it was found that the *SYK* gene was overexpressed in bladder, breast and colon cancers, whereas it was underexpressed in leukemia, lymphoma and myeloma [Table 1]. Figure 1 shows the results of 542 unique analyses of *SYK* gene. In total, 25 studies of various cancers showed

a statistical difference in the *SYK* expression between normal and tumor tissues, while 19 studies each found overexpression of *SYK* gene in normal and tumor tissues. Remarkably, breast and brain tumors showed an overexpression of *SYK* gene in relation to normal tissues. On the contrary, hematological malignancies such as lymphoma and leukemia showed that *SYK* expression was significantly higher in normal cells than in tumor cells.

Data also showed that *SYK* expression was significantly downregulated in lung carcinoid tumors in comparison with normal tissues [Figure 2a and f]. However, in cancer types such as invasive breast and ductal breast carcinoma, *SYK* was overexpressed in tumor cells by 2.5- and 4.8-fold, respectively, in relation to normal tissue [Figure 2b and c]. Furthermore, in colorectal carcinoma and brain glioblastoma, *SYK* gene was upregulated by 2.4- and 2.1-fold, respectively [Figure 2d and e].

Kaplan–Meier survival analysis
The Kaplan–Meier plotter data analyses revealed that overexpression of *SYK* gene was significantly associated with better overall survival in pancreatic, gastric and lung cancer patients, while this association was nonsignificant in liver cancer patients [Figure 3].

cBioPortal mutation site and frequency analyses
From the 55,833 samples of 210 studies that were retrieved from TCGA pipeline using cBioPortal, a total of 562 *SYK* gene mutation sites were detected and found to be located between amino acids 0 and 635 [Figure 4]. Prostate cancer had the highest *SYK* gene mutation frequency, occurring at 14 different sites, and was mostly found in SH2 and kinase domains.

After showing the mutation sites and the frequency of *SYK* mutation in different cancers, the mean expression of mutated- and wild-type *SYK* in different cancer types was compared. As shown in Figure 5a, the mean *SYK* expression was high in diffuse large B-cell lymphoma, acute myeloid leukemia and thymoma compared with other cancer types. Nonmutated form of SYK represented the majority of the expressed gene. In addition, there is no prognostic value between mutated- and wild-type *SYK* in different cancer types, as shown in Figure 5b.

MethHC promoter methylation analysis
The *SYK* promoter hypermethylated in tumor samples of different cancer types compared with the normal
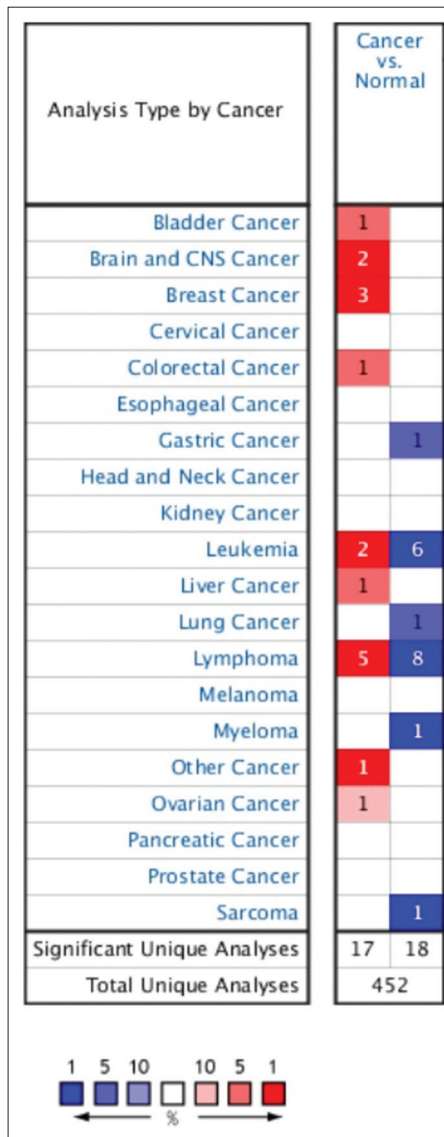

Analysis Type by Cancer	Cancer vs. Normal	
Bladder Cancer	1	
Brain and CNS Cancer	2	
Breast Cancer	3	
Cervical Cancer		
Colorectal Cancer	1	
Esophageal Cancer		
Gastric Cancer		1
Head and Neck Cancer		
Kidney Cancer		
Leukemia	2	6
Liver Cancer	1	
Lung Cancer		1
Lymphoma	5	8
Melanoma		
Myeloma		1
Other Cancer	1	
Ovarian Cancer	1	
Pancreatic Cancer		
Prostate Cancer		
Sarcoma		1
Significant Unique Analyses	17	18
Total Unique Analyses	452	

Figure 1: Overexpression (red) or underexpression (blue) of spleen tyrosine kinase mRNA in tumor tissues versus normal tissues
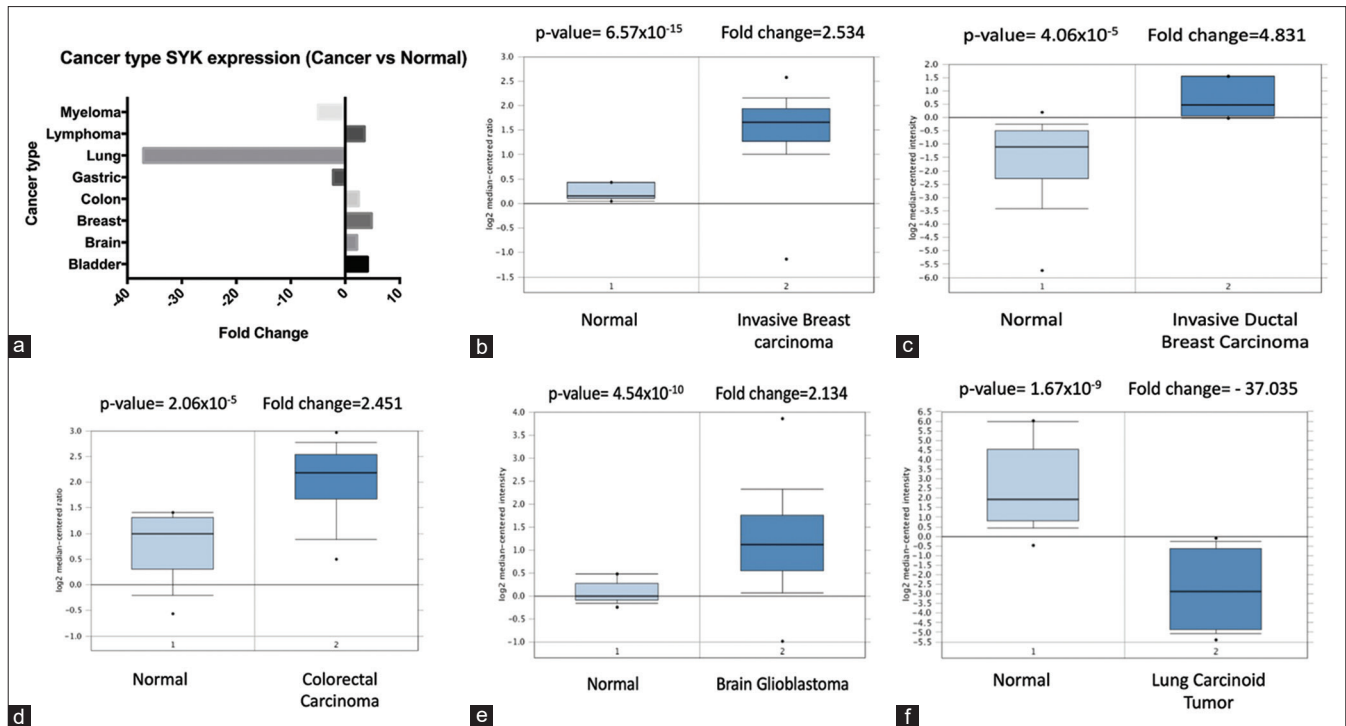
Figure 2: Spleen tyrosine kinase analysis in different cancer types using the Oncomine database. (a) Fold change of spleen tyrosine kinase in various identified cancers. The box plot illustrates the expression of spleen tyrosine kinase gene in normal tissue (left) versus tumor tissue (right). Spleen tyrosine kinase expression in (b and c) breast carcinoma, (d and e) colorectal carcinoma and brain glioblastoma and (f) lung carcinoid tumor

Table 1: Spleen tyrosine kinase expression in different cancer types

Cancer	Cancer subtype	P	Fold change	Rank (%)	Sample	Study
Bladder	Infiltrating bladder urothelial carcinoma	1.47×10^{12}	4.072	2	157	Sanchez-Carbayo et al.[15]
Brain	Brain glioblastoma	4.54×10^{10}	2.134	4	557	Levine and The Cancer Genome Atlas Research Network[16]
Breast	Invasive ductal breast carcinoma	4.06×10^{5}	4.831	1	22	Karnoub et al.[17]
	Invasive breast carcinoma	6.57×10^{15}	2.534	6	59	Finak et al.[18]
	Medullary breast carcinoma	6.09×10^{8}	2.093	9	176	Curtis et al.[19]
Colon	Colorectal carcinoma	2.06×10^{5}	2.451	5	30	Graudens et al.[20]
Gastric	Gastric intestinal type adenocarcinoma	3.17×10^{5}	−2.203	4	57	D'Errico et al.[21]
Leukemia	Chronic lymphocytic leukemia	1.59×10^{9}	4.529	1	50	Rosenwald et al.[22]
	T-cell childhood acute lymphoblastic leukemia	2.02×10^{20}	−31.556	1	50	Coustan-Smith et al.[23]
	B-cell childhood acute lymphoblastic leukemia	1.65×10^{7}	−11.069	2	242	Maia et al.[24]
	B-cell acute lymphoblastic leukemia	7.37×10^{9}	−3.856	1	22	Haferlach et al.[25]
	T-cell acute lymphoblastic leukemia	1.57×10^{28}	−2.263	6	248	Piccaluga et al.[26]
Lung	Lung carcinoid tumor	1.67×10^{9}	−37.035	3	37	Alizadeh et al.[27]
Lymphoma	Follicular lymphoma	1.02×10^{7}	3.515	1	40	Bhattacharjee et al.[28]
	T-cell lymphoma	1.05×10^{9}	3.096	7	48	Brune et al.[29]
	Hodgkin's lymphoma	1.44×10^{13}	−5.647	1	37	
	Burkitt lymphoma	2.79×10^{9}	−5.598	1	30	
	Diffuse large B-cell lymphoma	2.74×10^{7}	−2.856	2	36	
Myeloma	Multiple myeloma	4.20×10^{13}	−4.975	1	118	Zhan et al.[30]
Ovarian	Ovarian serious adenocarcinoma	7.11×10^{6}	2.695	6	53	Yoshihara et al.[31]
Sarcoma	Synovial sarcoma	6.94×10^{7}	−12.82	1	19	Detwiller et al.[32]

tissue is shown in Figure 6. Compared with normal tissues, *SYK* promoter hypermethylation was significantly higher (P < 0.005) in breast, renal, liver, lung, pancreatic, prostatic, skin and stomach cancers, but there was no differences in bladder, colon, head-and-neck and rectal cancers.

DISCUSSION

SYK is involved in many hematopoietic cell responses, especially immune signaling, that control cellular proliferation and differentiation. *SYK* has also been reported to be expressed in nonhematopoietic cells,
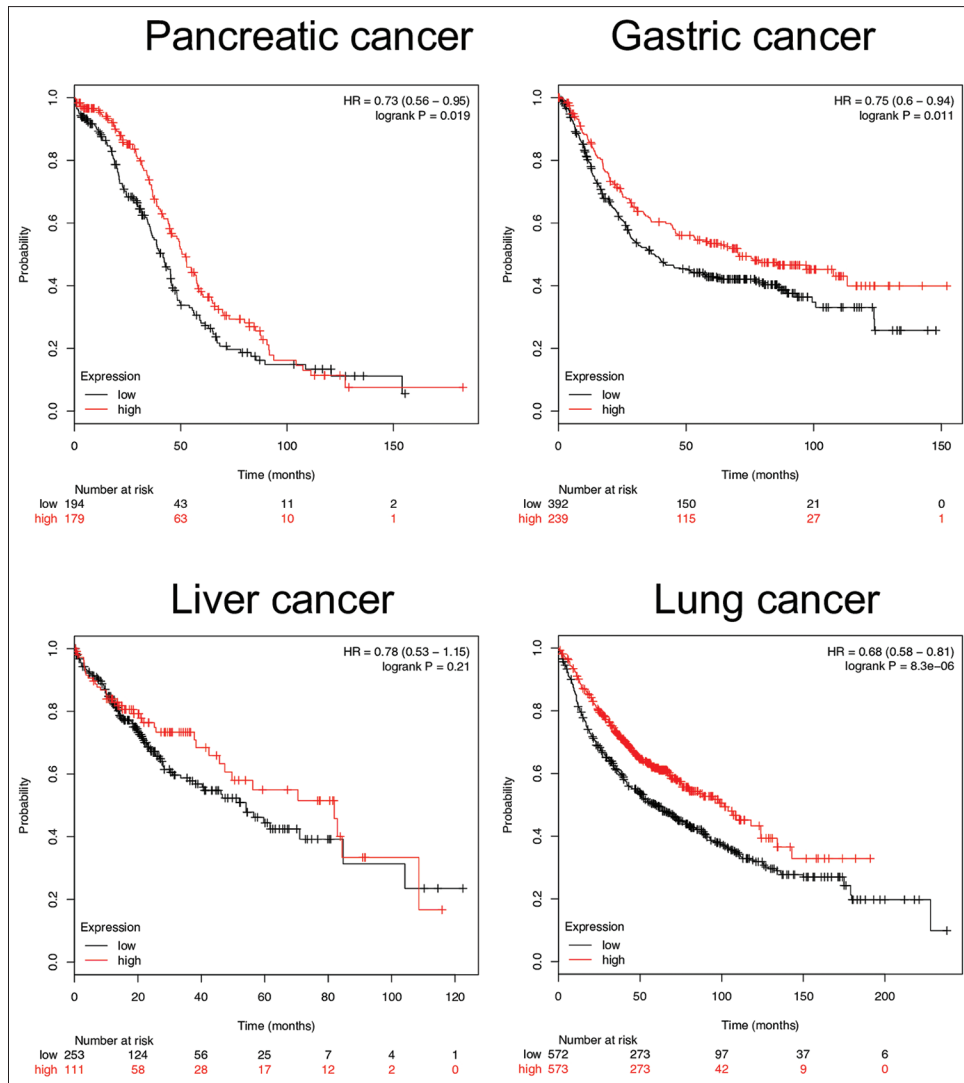
Figure 3: Correlations between spleen tyrosine kinase expression and survival rates of patients with different cancer types using Kaplan–Meier plotter database. Survival curves of patients with pancreatic, liver, gastric and lung cancers, segregated according to high (red) or low (black) expression of spleen tyrosine kinase

including epithelial cells, fibroblast cells and endothelial cells.[33] It is functionally important in many cell types and plays a central role in a wide variety of cells. In cancers, numerous studies have shown the dual role of *SYK*: as a tumor promoter[34-36] and as a malignant cell growth suppressor.[37] The current study found that the mRNA expression of the *SYK* gene varied among different solid cancer types; *SYK* was overexpressed in breast, brain and pancreatic tumors as compared with normal cells. These findings suggest that *SYK* does play a role in the tumorigenesis of certain types of cancer. However, in liquid tumors, *SYK* seems to drive cell transformation.[7]

SYK undergo autophosphorylation at the Tyr-518 site. After ligand binding to receptors, tyrosine residues are phosphorylated by Lyn, which is a Src-family nonreceptor.

The phosphorylation of tyrosine residues of SYK produces binding sites for CBL, VAV1 and PLC-γ and controls B-cell receptor cascades.[38] These result in increasing the IP3 concentration, which in turn increases calcium ion internalization. In addition, SYK can activate several molecules such as a Bruton's tyrosine kinase (BTK) during the B-cell receptor-signaling pathway.[39] BTK has been proposed to play a tumor-suppressive action in the epithelial cancer cells through p53-dependent mechanisms.[40,41] Therefore, *SYK* could play a role as an antitumor gene in the epithelial cells though BTK regulation.

This study found that compared with normal cells, *SYK* is overexpressed in chronic lymphoblastic leukemia but underexpressed in acute childhood lymphoblastic leukemia. In line with this observation, the high expression of *SYK* in chronic lymphoblastic leukemia has been reported in a
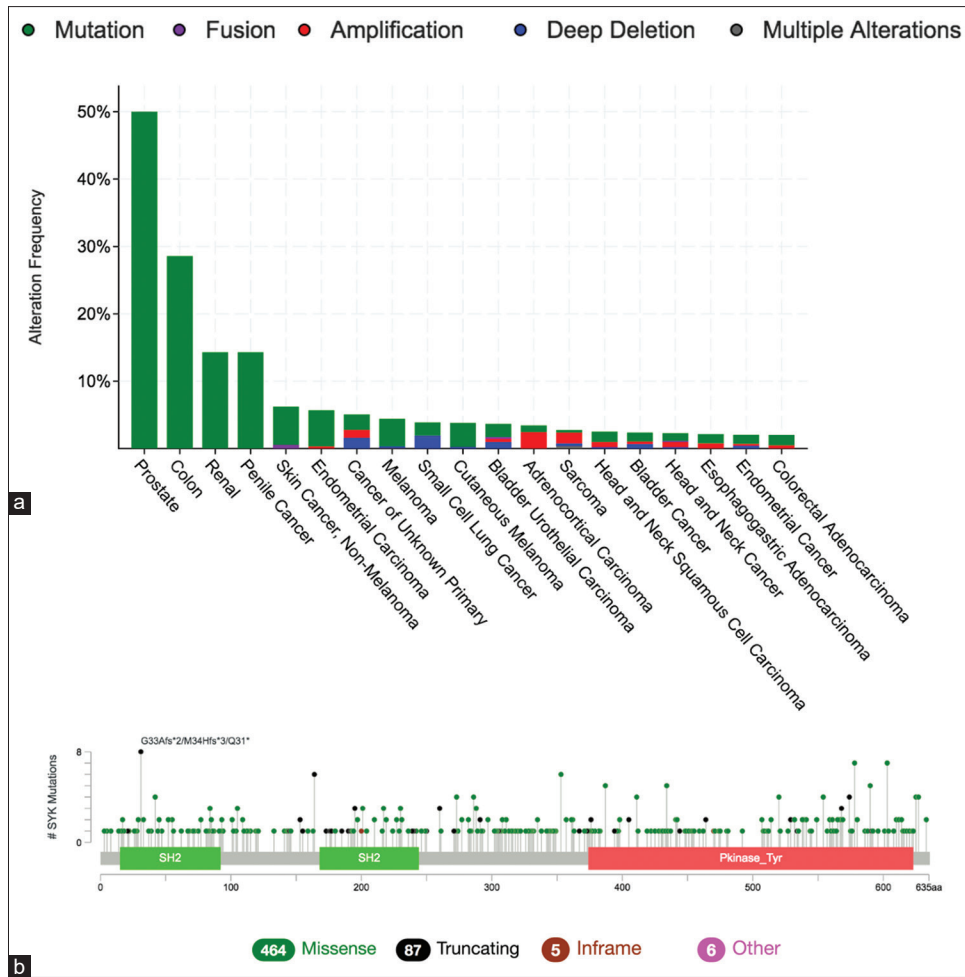
Figure 4: Mutation diagram of spleen tyrosine kinase in different cancer types across protein domains showing that spleen tyrosine kinase mutation mainly occurred in prostate cancer (a) and existed in different protein sites (b)

number of studies.[34,35] However, there is no consensus in the literature regarding the *SYK* expression levels in acute leukemia: Some studies have shown elevated levels of *SYK* in this cancer,[42,43] while others have shown downregulation of *SYK* expression in childhood acute leukemia.[7] The reason for such underexpression of *SYK* is yet to be explained.

This study found that *SYK* expression is highly downregulated in lung carcinoid tumors compared with those in normal tissues, which is in contrast with the findings of several studies, where *SYK* expression was found to be higher in cancer cells. For instance, Fotheringham *et al.*[44] showed that *SYK* is elevated in lung tumor. Similarly, *SYK* expression was significantly upregulated in the primary samples of neuroendocrine tumors compared with in the normal alveolar epithelium samples.[36,45] However, given that lung carcinoids account for only 1%–2% of lung cancer cases, it would be difficult to conclude that the status of *SYK* expression differ across tumors in different organs.

The current study found a positive correlation between high expression of *SYK* and survival of patients in different solid tumors. This is consistent with the findings of other clinical studies. For instance, Nakashima *et al.*[46] found a strong association between *SYK* expression and absence of lymph node metastasis in gastric cancer. Moreover, Toyama *et al.*[47] showed that low mRNA expression of *SYK* in patients diagnosed with breast cancer was significantly associated with distant metastasis and poor prognosis.[47] Taken together, these findings clearly indicate a relationship between low *SYK* expression and poor prognosis of many types of cancer.

The analysis in this study revealed that, in general, there are low number of mutations in the *SYK* gene in many cancers such as breast, colon, pancreatic and ovarian cancers. In addition, these alterations in the *SYK* gene did not appear to play a pivotal role in the overall survival. Although a growing body of evidence has shown that the loss of *SYK* clearly contributes to malignant phenotypes, no gene alterations such as mutations, deletions or translocation in
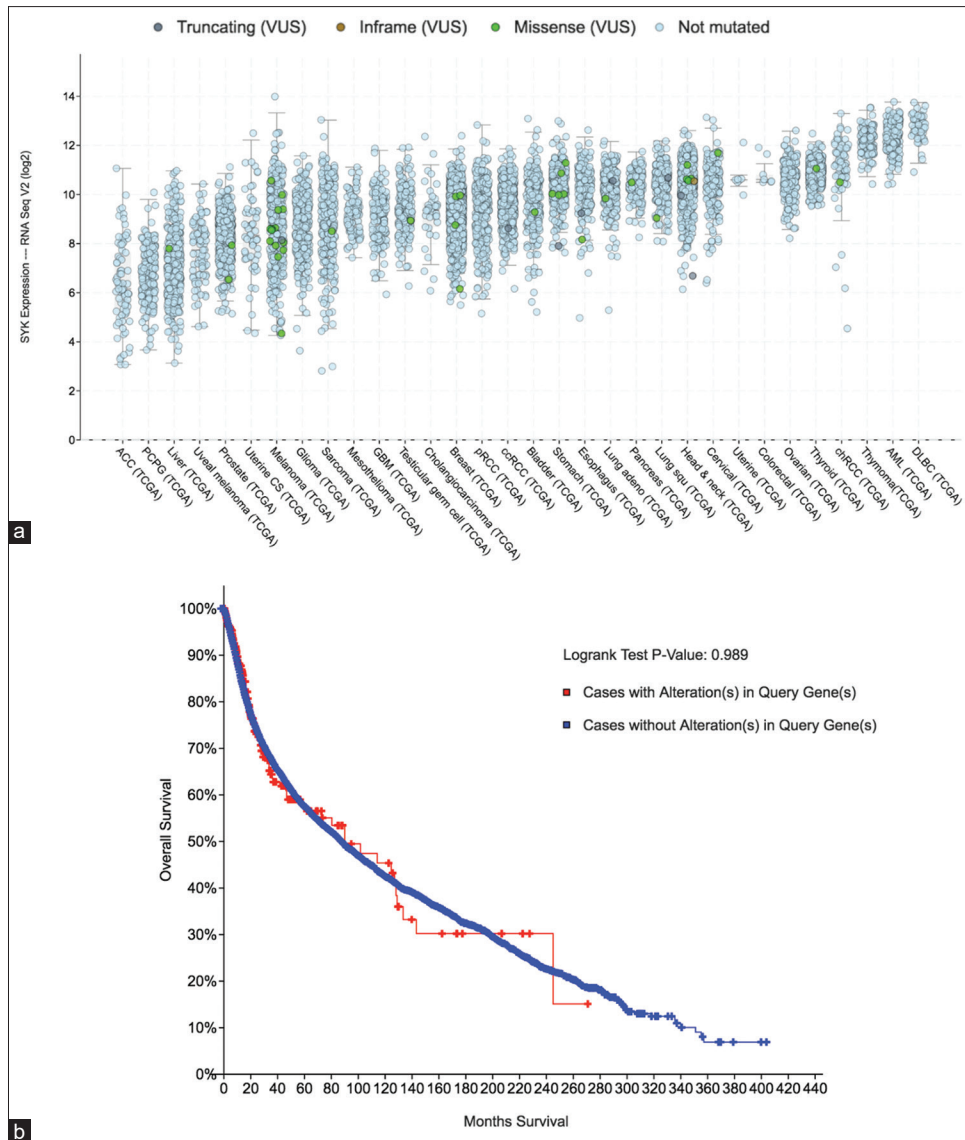
Figure 5: Wild-type and mutated-type spleen tyrosine kinase expression in different cancer types. (a) Mean spleen tyrosine kinase expression in different cancers. (b) The prognostic value of mutated- and/or wild-type spleen tyrosine kinase in different cancer type

the *SYK* gene have been found to be involved in naturally occurring tumor. For instance, Coopman and Mueller[48] reported that in invasive breast cancer, the loss of SYK protein is associated with reduction in its mRNA levels, clearly explaining that the loss of *SYK* expression occurs at the transcription level. Moreover, mutation frequencies vary in different tumor stages.[49,50]

Methylation is considered as a major mechanism for silencing tumor-suppressor genes.[51] DNA hypermethylation, increasing of methyltransferase activity and global hypomethylation have been observed in human cancers. For instance, one of the most important tumor-suppressing genes in colorectal cancer is *CDKN2A*.[52] *CDKN2A* has been found to be silenced by hypermethylation, and a recent meta-analysis suggested that *CDKN2A* promoter

hypermethylation is associated with unfavorable prognosis in colorectal cancer patients.[53]

DNA methylation has also been used to predict and discover cancer origin from biological samples.[54] Brock *et al*.[55] discovered that four hypermethylated genes are associated with early tumor recurrence in lung cancer patients. These DNA methylation candidates can be targeted by certain demethylating agents to treat lung cancer. Indeed, Juergens *et al*.[56] tested the efficacy of a combination of epigenetic inhibitors for lung cancer patients who had hypermethylated genes, and significant progression-free survival and overall survival were reported in the clinical trial. The study highlighted the possible role of methylation of SYK promoter in solid cancer, but no data were provided
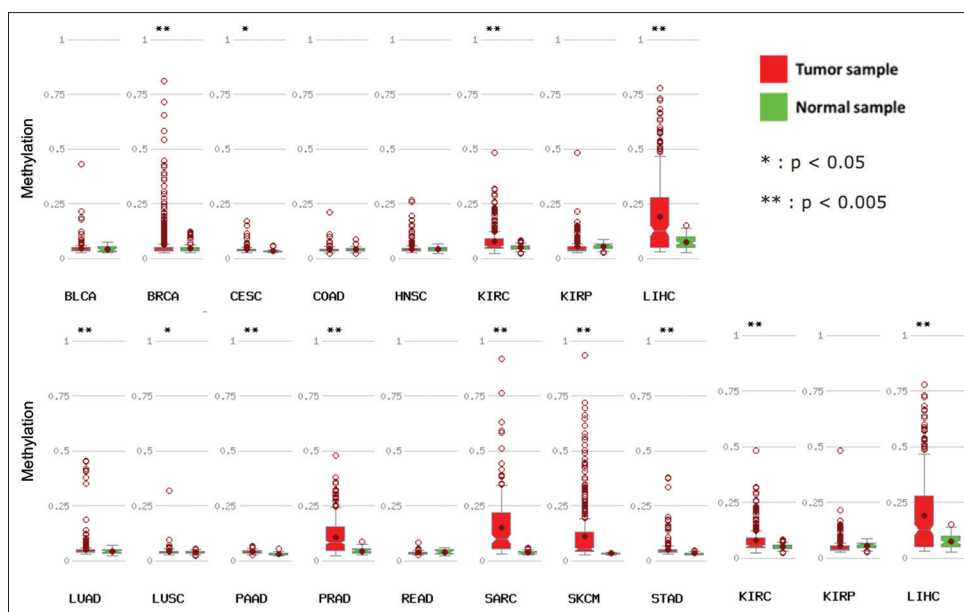
Figure 6: Average beta value of methylation of spleen tyrosine kinase promoter in different cancer types. The level of CpG methylation across the spleen tyrosine kinase proximal promoter (from – 1500 bp to TSS +1) upstream of the first exon analyzed using an *in silico* bioinformatics software MethHC49. Methylation across the spleen tyrosine kinase proximal promoter was determined in different tumors type (red) and the paired normal tissue (green)

regarding its status in blood cells. Previous work in monomorphic epitheliotropic intestinal T-cell lymphoma showed that *SYK* overexpression was associated with promoter hypomethylation.[57] Therefore, *in vitro* and *in vivo* epigenetic studies should be conducted to validate the bioinformatics data of this study before *SYK* demethylation is used as a therapy in clinical trials.

Limitations

Although the systematic analysis revealed the dual role of SYK in different cancer types, it has some limitations that should be highlighted. First, analyses were conducted using different databases; therefore, there are likely to be differences in ethnicity, tumor stage and other cancer genetic factors in these genomic databases. In addition, there are multiple CpG sites in the promoter region of *SYK*, but the MethHC software does not provide the exact CpG methylation site of *SYK*.

CONCLUSION

In this analytical study, *SYK* was found to be overexpressed in bladder, colon and breast cancers and downregulated in leukemia and lymphoma. High expression of *SYK* was correlated with better prognosis of pancreatic, gastric, liver and lung patients. It was also found that in different cancers types, there was hypermethylation in the *SYK* promoter compared with the normal tissue. Therefore, using demethylating agents for *SYK* promoter could be exploited for increasing the sensitivity of

conventional cancer therapy. However, further analysis should be conducted to analyze the mutation patterns in each cancer and to validate the results before initiating the use of *SYK* in clinical settings.

Peer review

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Conflicts of interest

There are no conflicts of interest.

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