Aberrant expression of SPRING1 is involved in the progression of B-cell acute lymphoblastic leukemia (B-ALL)

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Abstract

Background: Precursor B-cell acute lymphoblastic leukemia (B-ALL) is one of the most common types of leukemias in children. The majority of B-ALL patients are distinguished by chromosomal rearrangements; however, alternative splicing and epigenetic deregulations can also change the expression level of transcripts correlated with B-ALL. Therefore, the identification of prognostic and predictive biomarkers as well as the use of individualized treatments can help in B-ALL therapy. In this study, we performed an RNA-seq analysis to determine differentially expressed RNA transcripts in B-ALL. **Methods:** The RNA-seq data of 79 B-ALL and 14 non-malignant ITP (immune thrombocytopenic purpura) samples were obtained from the Gene Expression Omnibus (GEO) database. Moreover, RNA-seq was performed for Iranian patients with B-ALL to identify differentially expressed genes (DEGs). In order to experimentally validate the findings, the mRNA expression of *SPRING1* (or C12orf49) was evaluated in bone marrow aspiration samples of B-ALL patients using quantitative reverse transcription-PCR. **Results:** Differential expression analysis revealed 920 downregulated and 1216 upregulated genes in B-ALL compared to ITP samples. Quantitative RT-PCR revealed the significant upregulation of *SPRING1* (80%) in B-ALL patients. Functional enrichment analysis exhibited that *SPRING1* was principally associated with lipopolysaccharide-mediated signaling pathways. **Conclusion:** Our results provided evidence for the involvement of *SPRING1* in the B-ALL pathogenesis. However, further functional and clinical research is needed to understand its role in dysregulation of lipopolysaccharide-mediated signaling pathways in B-ALL.

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Abstract

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Methods: The RNA-seq data of 79 B-ALL and 14 non-malignant ITP (immune thrombocytopenic purpura) samples were obtained from the Gene Expression Omnibus (GEO) database. Moreover, RNA-seq was performed for Iranian patients with B-ALL to identify differentially expressed genes (DEGs). In order to experimentally validate the findings, the mRNA expression of *SPRING1* (or C12orf49) was evaluated in bone marrow aspiration samples of B-ALL patients using quantitative reverse transcription-PCR.

Results: Differential expression analysis revealed 920 downregulated and 1216 upregulated genes in B-ALL compared to ITP samples. Quantitative RT-PCR revealed the significant upregulation of*SPRING1* (80%) in B-ALL patients. Functional enrichment analysis exhibited that *SPRING1* was principally associated with lipopolysaccharide-mediated signaling pathways.

Conclusion: Our results provided evidence for the involvement of *SPRING1* in the B-ALL pathogenesis. However, further functional and clinical research is needed to understand its role in dysregulation of lipopolysaccharide-mediated signaling pathways in B-ALL.

Keywords: B-cell acute lymphoblastic leukemia, B-ALL, Biomarker, RNA-seq, SPRING1**Introduction**

Acute lymphoblastic leukemia (ALL) is the most common cancer in children under five years of age, with an annual incidence rate of nearly 60% (36.2 per million person-year) and a 5-year survival rate of 90% worldwide . Moreover, it accounts for nearly 25% of all acute leukemias in adults with a 5-year survival rate of 75-85% . Accumulating evidence suggests that excessive proliferation of B (B-ALL) and T (T-ALL) lineage lymphoid progenitors in the bone marrow, blood, and extramedullary sites leads to the formation of ALL . Many genetic alterations are associated with the onset of ALL, such as chromosomal translocations, mutations, and aneuploidies that occur in genes responsible for controlling cell cycle regulation and lymphoid cell development . B cell precursor ALL (B-ALL) is associated with an infectious and in hereditary etiology distinguished by chromosomal changes, resulting in protein dysregulation via the formation of chimeric genes . B-ALL patients carry a chromosomal translocation and acquire secondary genetic alterations, which lead to the differentiation and proliferation of B cells . The genetic heterogeneity in B-ALL is a promising source of biomarkers used for early detection, monitoring tools, and new chemotherapy targets . Despite the advances in the treatment of B-ALL, this disease still plays a key role in the mortality rate of patients . Consequently, the identification of specific biomarkers, pathways, and gene networks related to B-ALL is crucial for developing new strategies to improve prognostic capabilities, accelerate early diagnosis, and provide therapeutic strategies at early stages of the disease . A comprehensive understanding of the possible causes of B-ALL can be based on integrated bioinformatic analysis of high-throughput sequencing, identification of differential expression profiles, and prediction of possible biomarker function . Next-generation sequencing (NGS) methods, such as transcriptome sequencing have revealed the enormous genetic diversity of B-ALL and could be a promising diagnostic platform .

In this study, we aimed to identify a novel B-ALL-related marker that could serve as a diagnostic, prognostic, and therapeutic target as well as provide new insights into the underlying molecular mechanisms of this disease. To facilitate this, we have performed transcriptome analysis to identify a genetic biomarker for B-ALL. Moreover, we conducted differentially expressed genes (DEGs) analysis, weighted gene co-expression network analysis (WGCNA), and gene ontology (GO) enrichment analysis on a B-ALL clinical cohort, in parallel with other samples from the GEO data bank, to identify genes and pathways associated with B-ALL. Here, we identified *SPRING1* (or C12orf49) as a novel marker in B-ALL using RNA-seq analysis and validated its mRNA expression level with quantitative real-time PCR.

Materials and Methods

Data resource

RNA-seq data profiles were obtained from the Gene Expression Omnibus (GEO) database (https://www.ncbi.nlm.nih.gov/geo/). These data profiles have included 69 B-ALL and 12 ITP samples from three different projects, including PRJNA397402, PRJNA475264, and PRJNA549254 . Moreover, we performed RNA-seq analysis for 10 Iranian B-ALL and 2 ITP samples, and the raw RNA-seq data is available under accession number PRJNA589314. Data profiles and the number of samples are indicated in Table 1. FASTQ raw data were trimmed by Trimmomatic software and mapped to the human reference genome (GRCh38/hg38) using STAR (version: 2.7.3a). Then, HTSeq-count (version: 1.99.2) was utilized for gene quantification and DEGs were identified by the DESeq2 package in the R environment (version 4.0.5). A —log2 fold change (FC)— > 2 with P -adjusted values < 0.05 and baseMean > 100 was considered significant for validating DEGs .

Network analysis and module detection

Network analysis identifies gene sets among different subtypes that interact together and are concordantly expressed. The Weighted gene co-expression network analysis (WGCNA) package constructed the gene network and determined modules to identify the function of candidate genes using all 93 samples . After highly co-expressed genes were clustered into co-expressed modules, the module-trait association for all genes was used by topological analysis to generate gene dendrograms to identify modules . Then, the top 10 GO enrichment pathways for each module were identified using the GO enrichment analysis function.

Finally, network visualization and functional analysis were performed using Cytoscape software version 3.9.1 (http://www.cytoscape.org) .

Patients and ethics statement

Bone marrow mononuclear cells (BMMNCs) samples were obtained from 20 B-ALL patients and 20 non-malignant ITP (immune thrombocytopenic purpura) patients who had not received any chemoradiotherapy before aspiration and had no history of any cancer type, respectively. Specimens were obtained from Dr. Sheikh Children's Hospital of Mashhad University of Medical Sciences (MUMS), Mashhad, Iran. All individual participants signed informed consent forms prior to their inclusion in the study. The ethics committee of the Ferdowsi University of Mashhad, Mashhad, Iran approved the research protocol (ethical code: IR.UM.REC.1400.058) and it was performed following the Helsinki Declaration.

RNA extraction, RNA-sequencing library, and comparative real-time PCR analysis

Total RNA was isolated from BMMNCs using the TriPure reagent (QIAGEN, Netherlands) according to the manufacturer's instructions. RNA-seq libraries were manufactured by the KAPA HyperPrep kit with RiboErase and the RNA-seq was performed by 100 million paired-end reads on an Illumina NovaSeq 6000 platform (CeGaT company, Tubingen, Germany). Additionally, total RNA was treated with *DNase I* (Thermo Fisher Scientific, Waltham, MA) to eliminate genomic DNA, and the first strand cDNA was synthesized using the First Strand cDNA Synthesis Kit (AddScript cDNA Synthesis kit) according to the manufacturer's protocol. The mRNA expression level of *SPRING1* (or C12orf49) was determined by comparative real-time PCR using SYBR Green Master Mix (AMPLIQON, Denmark) on a CFX96 BioRad System thermocycler (Roche, Germany) with specific primers (forward primer 5'-CCCAACAAGCAACTTCTC-3' and reverse primer 5'-TCTCCATAGCAATACTTTGC-3') through calculating the*SPRING1* mRNA expression level using the $2^{-\Delta\Delta\hat{\tau}}$method and beta-glucuronidase (*GUSB*) as an endogenous control.

Statistical analysis and data mining using GEPIA

Gene expression profile interactive analysis (GEPIA) (http://gepia.cancer-pku.cn/detail.php) was performed to indicate the expression level, stage-specific expression of *SPRING1* , and the effect of *SPRING1* on survival in diffuse large B-cell lymphoma as a lymphoid disorder. Statistical analysis and visualization were performed using IBM SPSS 26 statistical software (La Jolla, CA, USA) and GraphPad Prism (version 9.0.0.121). All P -values < 0.05 were considered as significant. The measurement of the predictive efficacy of *SPRING1* was carried out via the use of receiver operating characteristic (ROC) curve analysis and the area under the ROC curve (AUC) for B-ALL . The AUC value is between 0 to 1, which indicates the diagnostic power based on: 0.9-1.0 (favorable), 0.8-0.9 (appropriate), 0.7-0.8 (acceptable), 0.6-0.7 (weak), and 0.5-0.6 (less) .

Results

Transcriptome profiling and WGCNA analysis correlated to B-ALL phenotypes

Considering the importance of early diagnosis of B-ALL which can lead to effective treatment of patients, we evaluated the expression profile of B-ALL compared to ITP samples. RNA-seq data related to Iranian samples were deposited in the SRA BioProject database with accession number PRJNA589314. A total of 2136 dysregulated genes were recognized (adjusted P -value < 0.05), including 920 downregulated genes and 1216 upregulated genes, which are demonstrated as a volcano plot in Fig. 1.

WGCNA was applied to identify the association between the gene expression patterns of . Our analysis identified 18 separate gene co-expression modules, each containing a group of genes that tended to be co-expressed across samples. Based on WGCNA results, blue and turquoise modules were positively correlated with B-ALL status, while yellow and magenta modules were negatively correlated (Fig. 2A). Key modules were identified by evaluating the association between these 18 modules and clinical traits, and the results are demonstrated as a heatmap in Fig. 2B. Accordingly, 12 models were identified, whose key genes included *ADPRHL1, SPRING1, LINC01343, FCN1, UBASH3B, FHL1, LINC00692, BCL7A, MYSM1, NRIP1, GAB1, H2AC20* . Moreover, the expression level of key genes was significantly correlated to some biological pro-

cesses (BP), such as the lipopolysaccharide-mediated signaling pathway via GO enrichment analysis. The *in-silico* data showed significant upregulation of *SPRING1* in B-ALL samples. Accordingly, we selected *SPRING1* as a novel marker to investigate its effects in B-ALL tumorigenesis.

Study population

The general mean age of the B-ALL patients (60% males and 40% females) and ITP subjects (55% males and 45% females) was 6.05 ± 3.77 and 5.05 ± 3.05 years, respectively. The immunophenotyping and cytogenetics data of patients are demonstrated in Table 2. Most of the patients expressed CD3/10/13/19/33/34/79a/ and 117, while the expression of CD2/7/41/ and 61 markers was indicated in fewer patients.

The mRNA expression of *SPRING1* in B-ALL patients

We examined the mRNA expression level of *SPRING1* in 20 B-ALL patients and 20 non-malignant ITP cases by comparative qRT-PCR. The qRT-PCR data are demonstrated as a box plot in Fig. 3A based on the log2 FC of mRNA expression level. A significant upregulation of*SPRING1* (80%) was detected in B-ALL patients. The mean FC of*SPRING1* was 3.21 in patients. Moreover, RNA-seq data revealed the upregulation of *SPRING1* in B-ALL patients compared to ITP samples, as shown in Fig. 3B. Interestingly, the *SPRING1*expression level is increased in B-ALL patients compared to ITP based on both *in-silico* and qRT-PCR data.

Diagnostic value of *SPRING1* for B-ALL patients

The diagnostic value of the *SPRING1* mRNA expression was quantified by ROC curve and AUC measurement (Fig. 4). The area under the ROC curve of *SPRING1* ($P < 0.0001$) was 0.89 ± 0.04 (95% CI: 0.792-0.987), which is consistent with the appropriate accuracy (Sensitivity=80%, Specificity=75%). Accordingly, the ROC curve of *SPRING1* can be applied for auxiliary diagnosis of B-ALL patients.

Validating the mRNA expression of *SPRING1*

We examined the mRNA expression level of *SPRING1* in diffuse large B-cell lymphoma as a lymphoid disorder via the GEPIA database. The findings indicated an increase in the *SPRING1* expression level, as demonstrated in Fig. 5A. The overexpression of *SPRING1* did not differ in the stage-specific status (Fig. 5B). Moreover, there was a correlation between the overexpression of *SPRING1* and overall survival of patients based on GEPIA database analysis (Fig. 5C).

Discussion

Molecular mechanisms involved in leukemogenesis, such as structural chromosomal rearrangements, alternative splicing, and epigenetic deregulation can alter the expression of transcripts that are associated with the B-ALL development. Identifying these mechanisms through molecular diagnostic technologies will provide a better understanding of the disease pathogenesis, leading to the rapid expansion of molecular biomarkers related to B-ALL. Identification of these novel biomarkers can improve the precision of risk diagnosis, personalized treatment, better monitoring of treatment response, development of new risk-adapted targeted therapies, and patient outcome .

This study aimed to evaluate the transcriptome profile of B-ALL patients to find a novel diagnostic or prognostic biomarker through RNA-seq data analysis. The differential expression analysis, WGCNA, and GO analysis was performed to identify patterns of gene expression alterations and biological pathways related to DEGs. Our first findings demonstrated the *SPRING1* upregulation in B-ALL patients based on WGCNA analysis and qRT-PCR. Moreover, there was a positive correlation between the lipopolysaccharide-mediated signaling pathway and*SPRING1* activity based on GO enrichment analysis. These data propose that *SPRING1* has an imminent role in B-ALL tumorigenesis and can be a potential diagnostic marker for this disease. This integrated approach which includes a combination of bioinformatics and experimental validation, could be a powerful strategy to identify potential biomarkers and increase our understanding of the underlying biology of B-ALL.

Lipid rafts serve as a principal modality for signaling in cancer cells, where cholesterol assumes a pivotal function in this context. Accordingly, targeting cholesterol metabolism may be used as a viable therapeutic approach for the treatment of B-ALL . It is noteworthy that the disruption of cholesterol metabolism may lead to the formation of malignancy, such as breast, prostate, and colorectal cancers; consequently, there is a positive correlation between the cholesterol levels and cancer incidence . SPRING1 protein (or C12orf49) is localized in the Golgi and plays a crucial role in regulation of fatty acid metabolism and tumor pathogenesis (18,19). SPRING1 binds to the peptidase-site membrane-bound transcription factor 1 (MBTPS1) and triggers the cleavage of its substrate, specifically sterol regulatory element binding protein (SREBP) transcription factors (19,21,22).*SPRING1* plays a key role in the activation and cleavage of the SREBP precursor protein through the protease activity and maturation of S1P during its transport from the endoplasmic reticulum to the Golgi . Therefore, *SPRING1* is essential for maintaining both precursor and mature SREBP protein levels . SREBPs, as a large class of transcription factors, regulate lipid homeostasis by modulating the expression of enzymes necessary for endogenous cholesterol, fatty acid (FA), triacylglycerol, and phospholipid synthesis . Activation of SREBP occurs through its transport from the endoplasmic reticulum to the Golgi, where the S1P enzyme cleaves the endoplasmic reticulum loop of SREBP . It has been reported that S1P cleavage activity depends on its interaction with *SPRING1* . Moreover, SREBPs transcription factors regulate cholesterol biosynthesis and uptake for cellular function . Cholesterol is a major component of membrane lipids, which plays a critical role in cancer development as the primary platform in the membrane for signaling regulation in cancer . Consequently, regulation of membrane cholesterol balance is essential to maintain cellular homeostasis. Dysregulation of *SPRING1* leads to unbalanced cholesterol metabolism and the possibility of some disorders, such as tumor formation, cardiovascular, and neurodegenerative diseases .

It has been reported that the function of *SPRING1* is associated with divergent prognostic outcomes across various tumor types, including kidney, breast, liver, and sarcoma by The Cancer Genome Atlas (TCGA) database analysis . The upregulation of *SPRING1* was indicated in CRC tissues compared to non-cancerous tissue samples that correlated to tumor growth. Therefore, *SPRING1* was identified as a potential biomarker that provides therapeutic benefits for CRC patients . Moreover, the overexpression of *SPRING1* in breast cancer tissues was associated with significantly reduced survival rates of the patients as demonstrated by TCGA database analysis . Additionally, the significant upregulation of *SPRING1* was confirmed in clear cell renal cell carcinoma-small renal masses (ccRCC-SRMs) compared to renal oncocytoma, which was associated with decreased overall survival rate of patients based on proteome analysis . Consistent with previous studies, our data showed significant overexpression of *SPRING1* in B-ALL patients. Taken together, our data propound that the *SPRING1*dysregulation can impact the lipopolysaccharide-mediated signaling pathway and the downstream genes, which can correlate to B-ALL formation and development.

Conclusion

In summary, differential expression analysis, WGCNA, and GO analysis highlighted several transcripts in B-ALL. Here, we indicated the upregulation of *SPRING1* in B-ALL patients compared to ITP samples for the first time. The findings propose that *SPRING1* directly affects cholesterol metabolism. However, further functional and clinical analysis are needed to fully understand the role of *SPRING1* in B-ALL.

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Consent for publication

Not applicable.

Availability of data and materials

The data used and/or analyzed during the current study are available from the corresponding authors upon reasonable request.

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Ethics approval and consent to participate

All procedures were approved by Mashhad University of Medical Sciences, and Ferdowsi University of Mashhad and performed according to 1964 Helsinki Declaration.

Informed consent

Informed consent was obtained from all individual participants included in the study.

Declaration of competing interest

The authors declare that they have no competing interests.

Data availability

All raw data are available upon request.

References

Abbreviations	
AUC	area under the curve
B-ALL	B-cell acute lymphoblastic leukemia
BMMNCs	bone marrow mononuclear cells
BP	biological process
ccRCC-SRMs	clear cell renal cell carcinoma-small renal masses
CRC	colorectal cancer
DEGs	differentially expressed genes
FA	fatty acid
GEPIA	Gene expression profiling interactive analysis
GO	gene ontology
GEO	gene expression omnibus
ITP	immune thrombocytopenic purpura
Log2 FC	log2 fold change
MBTPS1	membrane-bound transcription factor peptidase site 1
MUMS	Mashhad University of Medical Sciences
NGS	next-generation sequencing
NCBI	National Center for Biotechnology Information
qRT-PCR	quantitative reverse transcription-polymerase chain reaction
RNA-seq	RNA sequencing
ROC	receiver operating characteristic
RPKM/FPKM	reads/ fragments per kilobase of transcript per million mapped reads
SPRING1	SREBF pathway regulator in Golgi 1
SRA	sequence read archive
SREBP	sterol regulatory element binding protein
S1P	site-1 protease
T-ALL	T-cell acute lymphoblastic leukemia
TCGA	The Cancer Genome Atlas
WGCNA	weighted gene co-expression network analysis

Figure legends

Figure 1. Volcano plot of DEGs between B-ALL patients and normal samples. Statistically significant DEGs were defined with adjusted P -value < 0.05 and absolute log2 FC > 2.0 as the cut-off threshold.

Figure 2. Weighted Gene Co-Expression Network Analysis (WGCNA) to identify Gene Modules. **(A)** The clustering dendrogram of differentially expressed genes in B-ALL. A total of 18 co-expression modules were identified, each leaf (vertical line) in the dendrogram was assigned to one co-expression gene, and the color row below the dendrogram represents the modules. The *SPRING1* was clustered in the turquoise module, **(B)** Heatmap plot of the adjacencies of modules. Red represents a positive correlation, and green represents a negative correlation. The amount of correlation and the calculated$P$ -values are also shown in each cell. The module assignment is shown on the left. The yellow, blue, turquoise, and magenta modules have the highest correlation with B-ALL cancer. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Figure 3. (A) Box plot representing a descriptive analysis of relative gene expression of *SPRING1* in B-ALL patients and ITP samples; (B) Mean of log2 fold change for the *SPRING1* mRNA expression.

Figure 4. ROC curve of *SPRING1* for diagnosis of B-ALL patients. The AUC value differs from 0 to 1, which indicates the diagnostic power based on favorable (0.9-1.0), appropriate (0.8-0.9), acceptable (0.7-0.8), weak (0.6-0.7), and less (0.5-0.6). ROC: receiver operating characteristic; AUC: area under the ROC curve.

Figure 5. Validating the role of *SPRING1* in lymphoid disorder by the GEPIA dataset. (A) Box plot shows expression of*SPRING1* in cancer (left) and normal (right) samples; (B) Stage plot shows SPRING1 expression in 4 stages; (C) Overall survival between patients with the higher (red) and lower (blue) *SPRING1*expression.

Table legends

Table 1. The characteristics of different datasets used in this study

Table 2. The immunophenotype data of 20 B-ALL patients

Figure 1

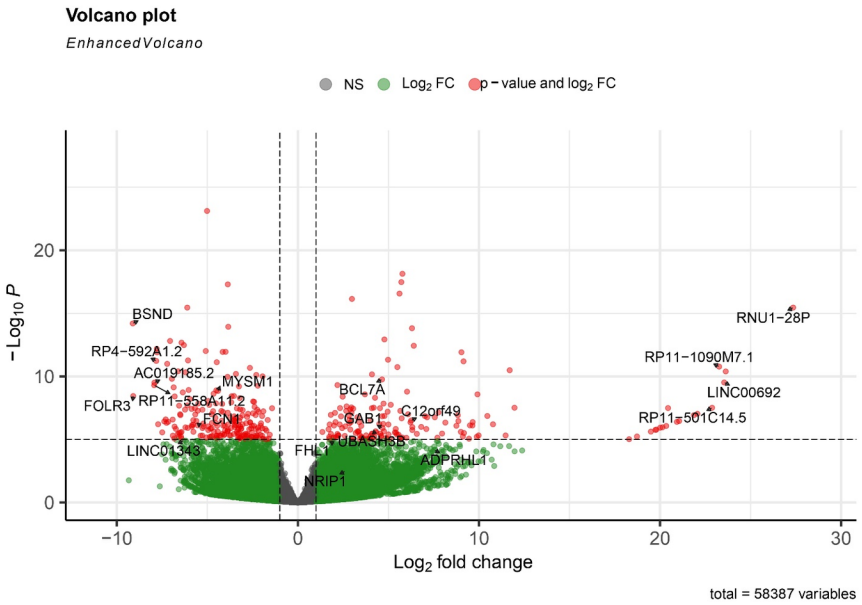
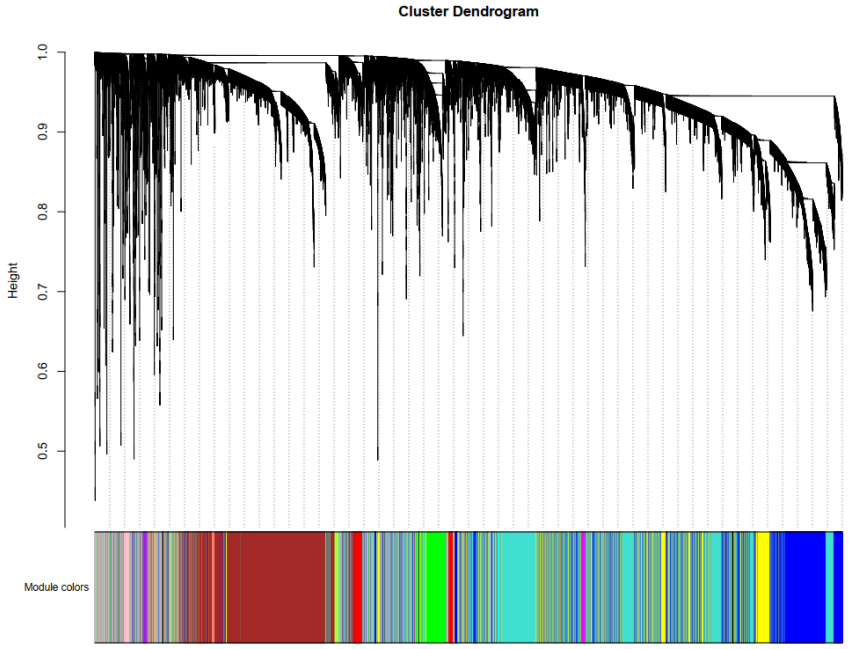
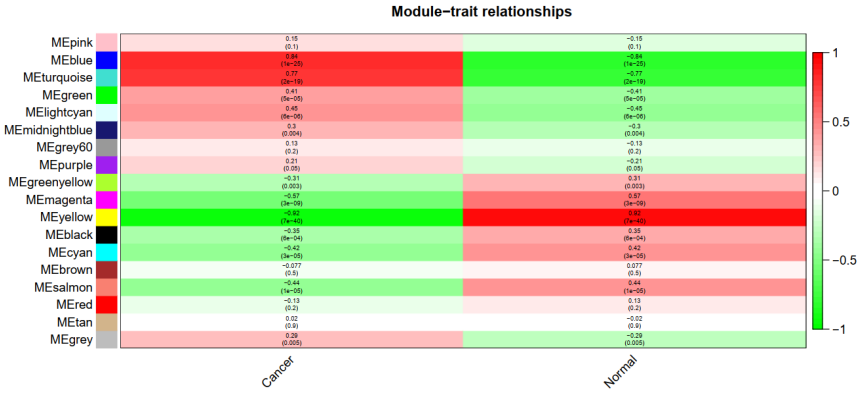


Figure 2



A

B



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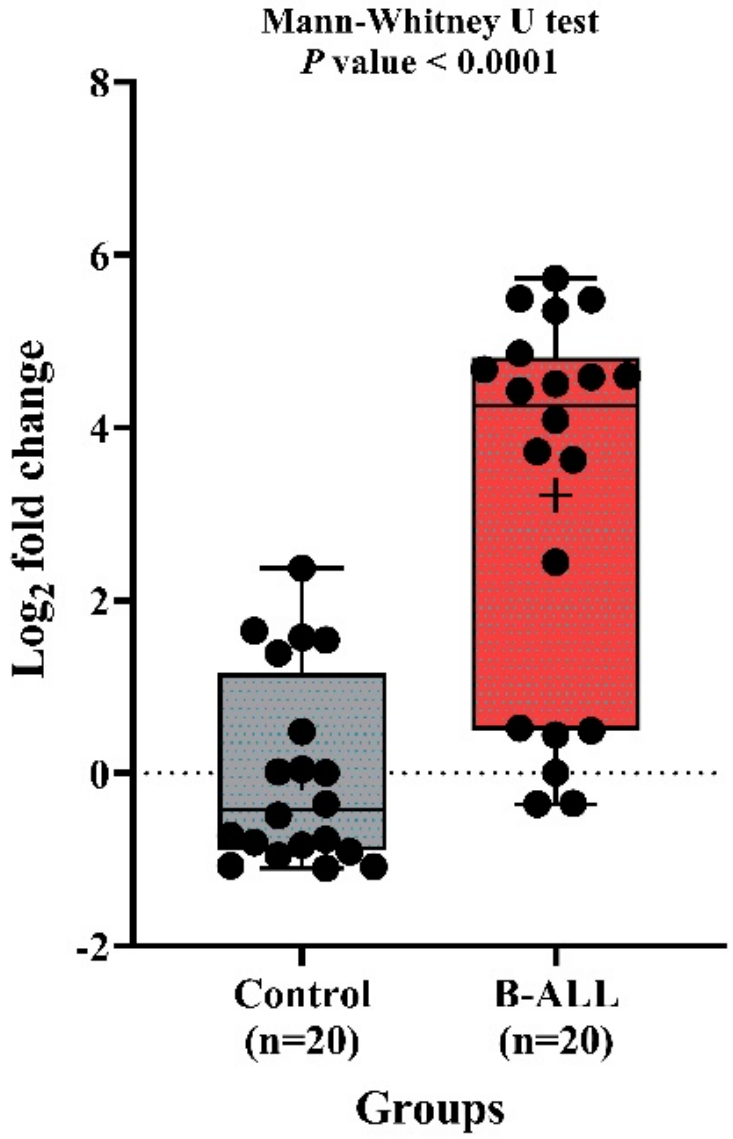

Expression of *SPRING1* in B-ALL patients compared to the ITP group based on RNA-seq analysis and qRT-PCR

Log2 Fold change *SPRING1*	Log2 Fold change *SPRING1*	Log2 Fold change *SPRING1*
RNA-seq data analysis	Iranian patients	6.25 (P-adjusted < 0.0001)
	Non-Iranian patients	7.06 (P-adjusted < 0.0001)
Real-time PCR	Iranian patients	3.21 ($P < 0.0001$)

Figure 4

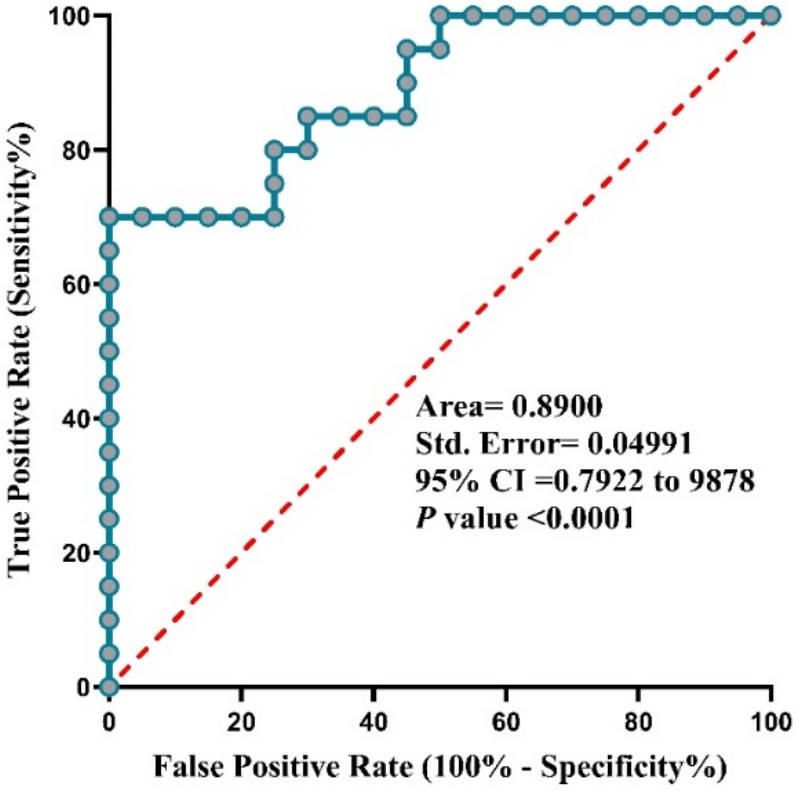

Figure 5

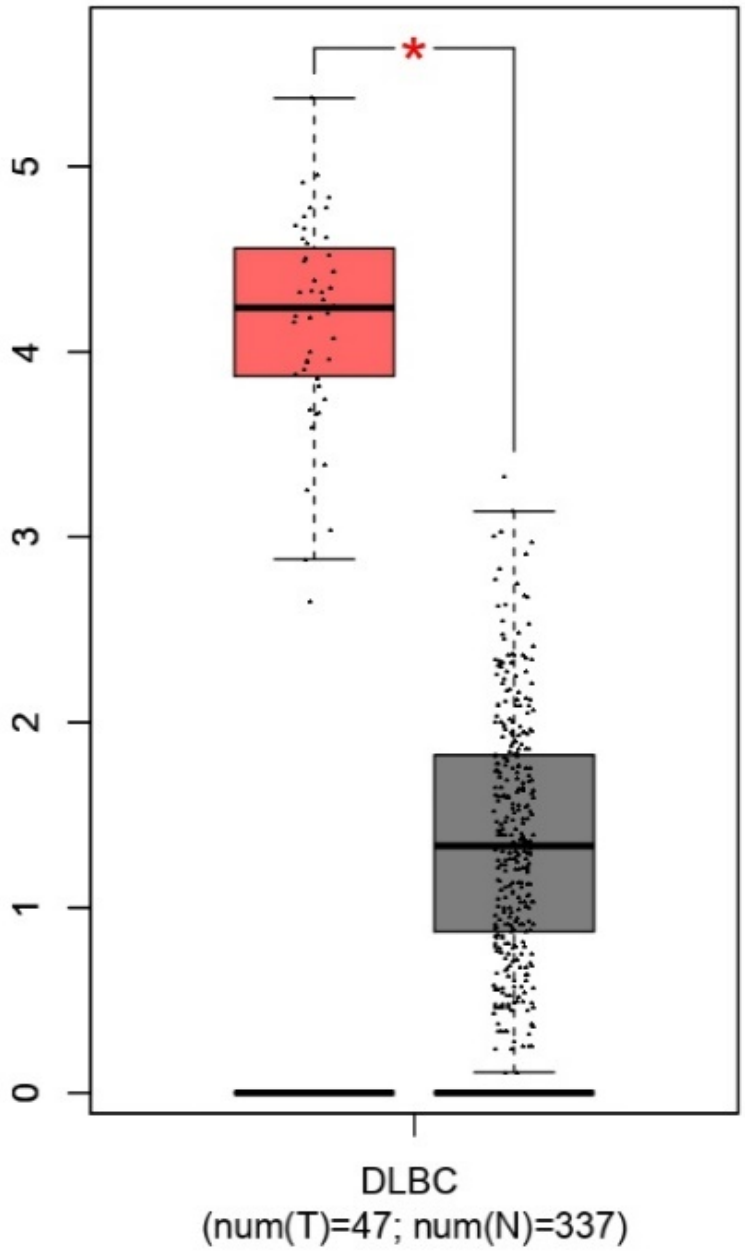

Table 1. Dataset's characteristics	Table 1. Dataset's characteristics	Table 1. Dataset's characteristics	Table 1. Datase
	Bio Project	**Type**	**Number of sa**
1	PRJNA589314	B-ALL	10
		ITP	2
2	PRJNA397402	ITP	7
3	PRJNA475264	B-ALL	69
4	PRJNA549254	ITP	5

Total				Total				Total				93		
Table 2. Immunophenotype data of 20 B-ALL patients	Table 2. Immunophenotype data of 20 B-ALL patients	Table 2. Immunophenotype data of 20 B-ALL patients	Table 2. Immunophenotype data of 20 B-ALL patients	Table 2. Immunophenotype data of 20 B-ALL patients	Table 2. Immunophenotype data of 20 B-ALL patients	Table 2. Immunophenotype data of 20 B-ALL patients	Table 2. Immunophenotype data of 20 B-ALL patients	Table 2. Immunophenotype data of 20 B-ALL patients	Table 2. Immunophenotype data of 20 B-ALL patients	Table 2. Immunophenotype data of 20 B-ALL patients	Table 2. Immunophenotype data of 20 B-ALL patients	Table 2. Immunophenotype data of 20 B-ALL patients	Table 2. Immunophenotype data of 20 B-ALL patients	Tabl 2. Immunophenotype data of 20 B-ALL patients
	CD2	**CD3**	**CD7**	**CD10**	**CD10+/ CD19+**	**CD13**	**CD19**	**CD33**	**CD34**	**CD41**	**CD61**	**CD79a**	**Cyt CD7**
Patient Yes No	5 15	20 -	9 11	20 -	13 7	17 3	20 -	13 7	17 3	1 19	4 16	14 6	1 19