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Identification of biomarkers for the prediction of relapse-free survival in pediatric B-precursor acute lymphoblastic leukemia

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Abstract. B-precursor acute lymphoblastic leukemia (B-ALL) is the most common cancer diagnosed in children and adolescents. Despite the fact that the 5-year survival rate has increased from 60 to 90%, approximately a quarter of children suffer from relapse with poor outcome. To improve the clinical management of B-ALL, there is an urgent need for prognostic biomarkers for the prediction of B-ALL outcomes. In the present study, we performed a comprehensive analysis of the gene expression data of 456 samples from five independent cohorts. We first sought to identify B-ALL-associated genes by differential gene expression analysis by applying linear models. Then, the statistical modelling was applied to identify candidates related to relapse-free survival. We identified a total of 1,273 B-ALL-associated genes that have functions relevant to chemokine signaling. From these genes, 59 genes were identified as prognostic biomarkers. Based on expression patterns of these genes, we successfully distinguished high- and low-risk groups of B-ALL patients (log-rank test, P-value=0.025). We further investigated the 59-gene expression levels in ALL chemotherapy-treated cohorts and identified 4 genes as potential drug targets associated with drug sensitivity. Our results provided a novel biomarker panel. By leveraging the large scale of data and statistical modelling, we believe this 59-gene biomarker could help to unveil the mechanisms underlying B-ALL progression and become potential drug targets.

Introduction

B-precursor acute lymphoblastic leukemia (B-ALL) is the most common cancer diagnosed in children and adolescents (1,2). One of the major causes of mortality is relapse despite intensive multi-agent chemotherapy (3). For the past two decades, several studies have reported that molecular abnormalities including TP53 mutations (4), deletion of INK4A/ARF (5) and TEL deletion (6) contribute to B-ALL relapse. However, the pathogenesis and biological mechanisms underlying relapsed ALL remain largely unknown. Thus, we sought to provide novel insights by identifying prognostic biomarkers from genome-wide expression profiling data generated by DNA microarrays.

Microarray technology has been developed more than a decade ago and is widely used in biomedical and clinical research. This high-throughput strategy enables profiling genome-wide expression simultaneously. Previously, based on committee neural networks, the leukemia gene expression data can be subcategorized into B-cell acute lymphoblastic leukemia, T-cell acute lymphoblastic leukemia and acute myeloid leukemia (7). Unsupervised hierarchal clustering of ALL gene expression could be used to reveal unique clusters with distinct cytogenetic, genomic and transcriptomic characterizations (8). By comparing gene expression profiles of specimens at the time of diagnosis vs. at relapse, or early- vs. late-relapse, several biological pathways such as cell cycle regulation, WNT and mitogen-activated protein kinase pathways (9,10) were identified to contribute to ALL relapse.

However, these findings lack connections to clinical practice, as the prediction of prognosis plays a crucial role in facilitating clinical decision-making. The purpose of this study was to develop a prognostic biomarker from gene expression profiles. Unlike the previously published study (11), we integrated data sets from multiple cohorts and implemented a comprehensive computational pipeline to identify a 59-gene biomarker that could serve as a B-ALL prognostic biomarker in practical applications.

Materials and methods

Gene expression data and preprocessing. We collected raw microarray data from two cohorts including TARGET-ALL study (11) and Microarray Innovations in LEukemia (MILE) (12,13). For the TAGET-ALL study, 207 high-risk B-precursor ALL patients between March 15, 2000 and April 25, 2003 were recruited from the Children's Oncology Group (COG) Clinical Trial P9906. RNA was first purified from samples with >80% blasts (131 bone marrow, 76 peripheral

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Key words: pediatrics, acute lymphoblastic leukemia, relapse-free survival, prognosis, gene expression, drug sensitivity

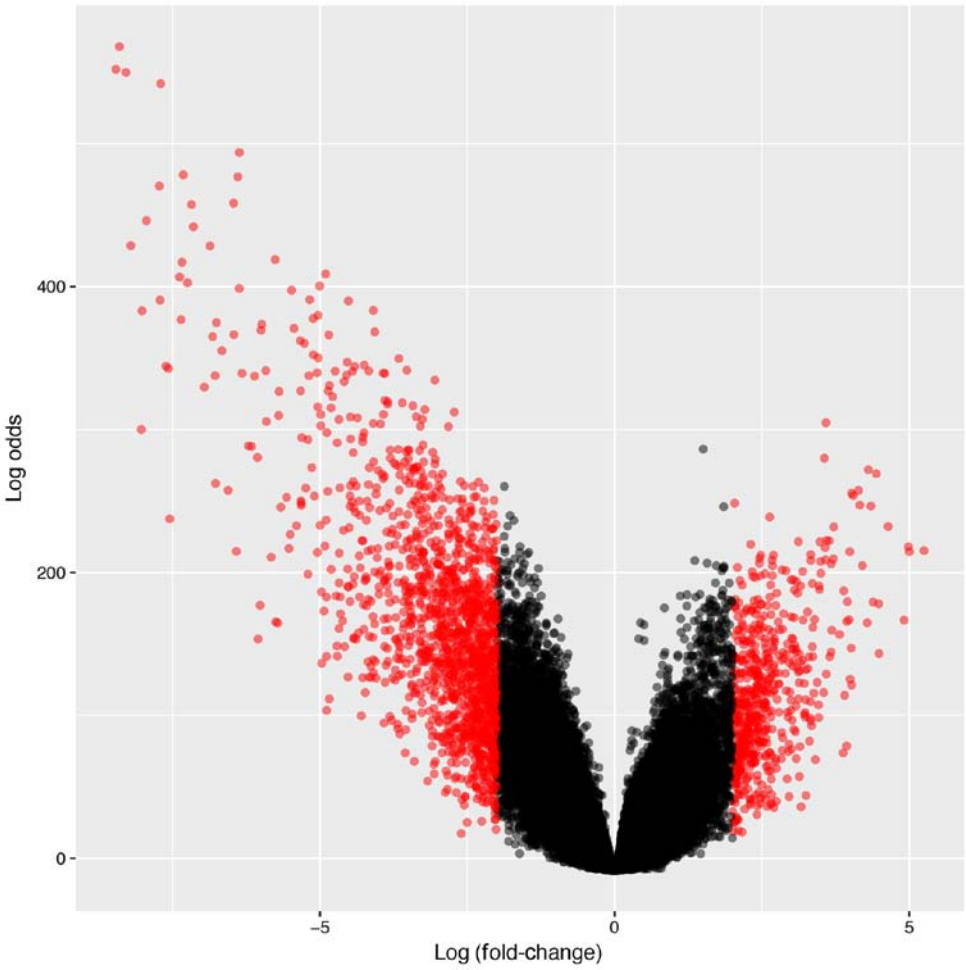

Figure 1. Volcano plot of results from the differential gene expression analysis. Red dots denote identified B-ALL-associated genes. B-ALL, B-precursor acute lymphoblastic leukemia.

blood) and then hybridized to Affymetrix Human Genome U133 Plus 2.0 Array. The raw intensity *.CEL files were retrieved from NCBI Gene Expression Omnibus (http://www.ncbi.nih.gov/geo) under the accession number GSE11877. For the MILE study, there were in total 2,096 blood or bone marrow samples of acute and chronic leukemia patients from 11 participant centers. In this study, we restricted to take 74 non-leukemia healthy samples as controls. Consistent with the TARGET-ALL study, Affymetrix Human Genome U133 Plus 2.0 Array was also used for the gene expression profiling.

For the discovery of prognostic biomarkers, we used the gene expression data set from one previously published study (14), where 80 samples collected at the time of diagnosis were considered in this study. The RNA was hybridized to Affymetrix HG-U133A oligonucleotide microarrays. The raw intensity *CEL files were retrieved from EBI ArrayExpress (https://www.ebi.ac.uk/arrayexpress/) under the accession number E-MTAB-1216.

The raw intensity *.CEL files were preprocessed using the *frma* (15) package in R 3.4.1 (https://www.r-project.org) environment. Briefly, the *frma* package converted raw probe-level intensities into background-corrected gene-level intensities. The data was then normalized based on the estimation of probe-specific effects, which allowed us to combine data from various cohorts without batch effects.

Identification of B-ALL-associated genes. We sought to identify B-ALL-associated genes by performing differential gene expression analysis between B-ALL (n=207) and healthy samples (n=74). The R package *limma* (16) package was used to conduct the differential gene expression analysis. The expression data was first Log2 transformed and then fitted into linear models with Empirical Bayesian methods for the analysis. We filtered the results at Log2 fold-change ≥2, or ≤-2 and B-statistics ≥4.6, which indicates that the probabilities of the genes were differentially expressed were >99%. After filtering, 1,273 genes were identified. The gene set enrichment analysis was then conducted using the R package *clusterProfiler* (17). The enrichment P-value was Benjamini & Hochberg adjusted.

Prognostic biomarker identification for relapse-free survival (RFS). For the development of prognostic gene signature, the 1,273 B-ALL-associated genes were first fitted with the Cox proportional hazards model in 80 samples. The prognostic significance of each gene test was assessed by log-rank test. We selected 59 at the cut-off P-value ≤0.05. The hierarchal clustering algorithm was then applied to the 59-gene expression profiles and the patients were divided into high- and low-risk groups. The Kaplan Meier-plot and log-rank test were used to test the prognostic significance of the two groups.

Table I. Gene Ontology enrichment results of ALL-related genes.

Gene Ontology	Biological process	P-value
GO:0009617	Response to bacterium	$1.39x10^{-14}$
GO:0050900	Leukocyte migration	$2.31x10^{-10}$
GO:0002274	Myeloid leukocyte activation	$7.56x10^{-10}$
GO:0006778	Porphyrin-containing compound metabolic process	$7.71x10^{-10}$
GO:0042168	Heme metabolic process	$8.40x10^{-10}$
GO:0002237	Response to molecule of bacterial origin	$2.55x10^{-9}$
GO:0006783	Heme biosynthetic process	$3.53x10^{-9}$
GO:0032496	Response to lipopolysaccharide	$3.53x10^{-9}$
GO:0046501	Protoporphyrinogen IX metabolic process	$3.53x10^{-9}$
GO:0042742	Defense response to bacterium	$4.61x10^{-9}$
Gene Ontology	**Cellular component**	**P-value**
GO:0030141	Secretory granule	$1.95x10^{-6}$
GO:0099503	Secretory vesicle	$5.15x10^{-5}$
GO:0098857	Membrane microdomain	$5.15x10^{-5}$
GO:0030667	Secretory granule membrane	$5.15x10^{-5}$
GO:0031091	Platelet alpha granule	$6.73x10^{-5}$
GO:0045121	Membrane raft	$6.73x10^{-5}$
GO:0098589	Membrane region	$7.00x10^{-4}$
GO:0031092	Platelet alpha granule membrane	$9.60x10^{-4}$
GO:0098552	Side of membrane	$9.60x10^{-4}$
GO:0009897	External side of plasma membrane	$1.30x10^{-3}$
Gene Ontology	**Molecular function**	**P-value**
GO:0030246	Carbohydrate binding	$3.98x10^{-5}$
GO:0008329	Signaling pattern recognition receptor activity	$2.94x10^{-4}$
GO:0038187	Pattern recognition receptor activity	$2.94x10^{-4}$
GO:0017171	Serine hydrolase activity	$2.94x10^{-4}$
GO:0008236	Serine-type peptidase activity	$4.53x10^{-4}$
GO:0042379	Chemokine receptor binding	$6.71x10^{-4}$
GO:0004252	Serine-type endopeptidase activity	$1.14x10^{-3}$
GO:0019865	Immunoglobulin binding	$1.14x10^{-3}$
GO:0004601	Peroxidase activity	$2.59x10^{-3}$
GO:0016684	Oxidoreductase activity, acting on peroxide as acceptor	$4.99x10^{-3}$

Top 10 significantly enriched Gene Ontology (Biological process, Cellular component, Molecular function) terms of the identified B-ALL-associated genes. P-values were Benjamini & Hochberg adjusted. B-ALL, B-precursor acute lymphoblastic leukemia.

Results

In the present study, we sought to identify B-ALL RFS biomarkers using transcriptome data. We hypothesized that the biomarkers may be involved in B-ALL pathogenesis, thus the gene differential expression analysis was conducted by comparing 207 B-ALL samples with 74 healthy normal blood, or bone marrow samples. After applying the linear models and stringent cut-off [Log fold-change ≥2, ≤-2, and false discovery rate (FDR) ≤0.01], a total of 1,273 genes were identified as ALL-associated genes as they were significantly upregulated or downregulated in the ALL samples (Fig. 1).

The Gene Ontology (GO) enrichment analysis revealed that the B-ALL-associated genes were involved in various biological processes (Table I). Surprisingly, we found that the B-ALL-associated genes were most significantly enriched in the biological processes that respond to bacterium (GO:0009617). We further categorized these genes and found that they were involved in cytokine-cytokine receptor interaction pathway (Fig. 2). These genes include chemokine ligands (CCL5, CXCL1, CXCL16, CXCL2, CXCL3, CXCL8), tumor

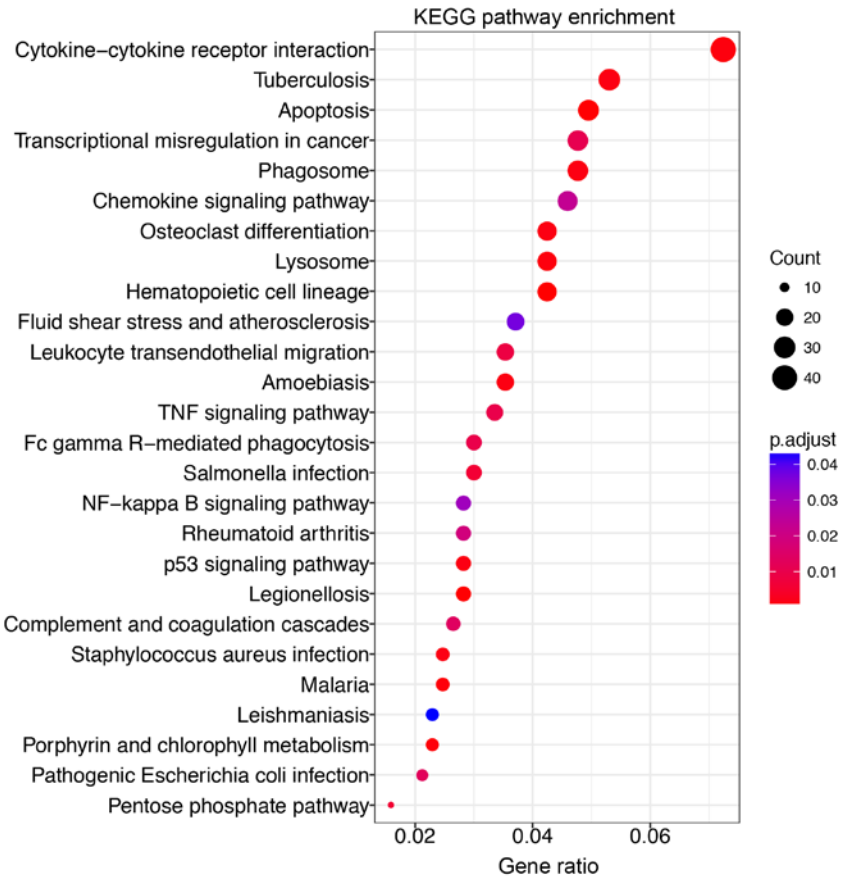

Figure 2. KEGG pathway enrichment analysis results of the identified B-ALL-associated genes. B-ALL, B-precursor acute lymphoblastic leukemia.

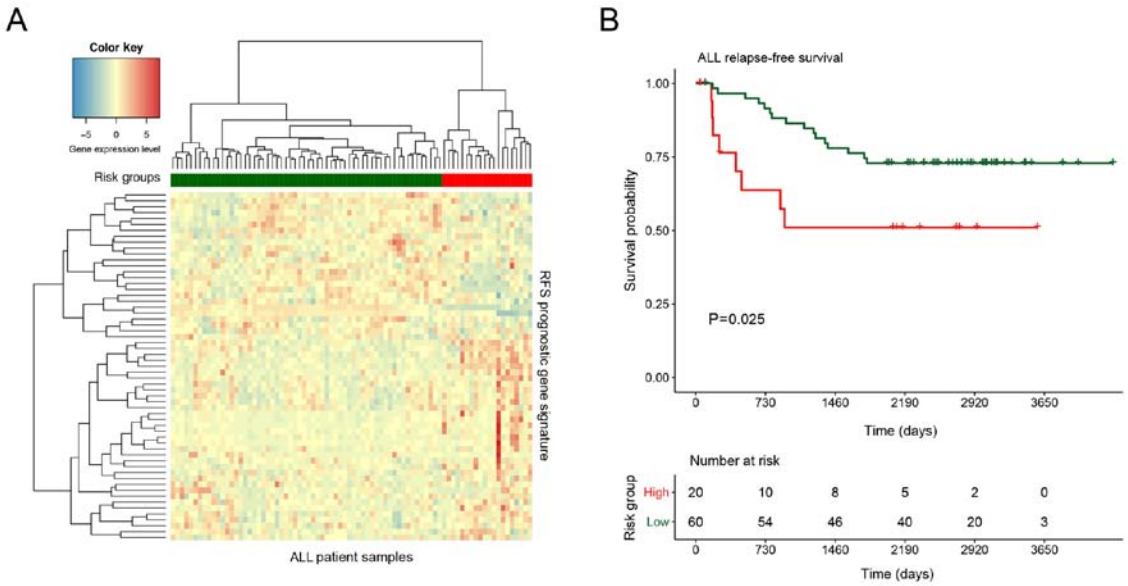

Figure 3. Identification of the B-ALL relapse-free survival 59-gene biomarker. (A) Hierarchal clustering of the 59-gene expression profile from B-ALL specimens. Two groups (red and green) were determined by cutting the branch of the dendrogram. (B) Kaplan-Meier plot of two groups, high (red)- and low (green)-risk B-ALL samples. The P-value was calculated using the log-rank test. B-ALL, B-precursor acute lymphoblastic leukemia.

necrosis factor receptor superfamily (FAS, TNFRSF10C, TNFRSF1B, TNFSF8), interleukins (IL-18, CXCL8) and interleukin receptors (IL-10RA, IL-6R). These findings agreed with previous studies on B-ALL pathogenesis. Chemokines and their receptors are vital in many cellular activities such as migrations, targeting developing and mature leukocytes (18). It was previously reported that the CXCR5-CXCL13 axis plays a vital role in chronic lymphocytic leukemia (CLL) (19). There are also other chemokines such as CCR7, CXCR4 and CXCR5 (20), CXCR3 (21), CCL25 (22)

Table II. List of 59 prognostic genes for RFS in ALL.

Gene symbol	Gene ID	Chromosome location	Gene name	HR (95% CI)	P-value
ADAM9	8754	8p11.22	ADAM metallopeptidase domain 9	0.51 (0.27-0.94)	$2.83x10^{-2}$
AOAH	313	7p14.2	Acyloxyacyl hydrolase	2.06 (1.12-3.78)	$1.63x10^{-2}$
APP	351	21q21.3	Amyloid β precursor protein	0.53 (0.34-0.84)	$5.49x10^{-3}$
B4GALT5	9334	20q13.13	β-1,4-galactosyltransferase 5	0.43 (0.20-0.95)	$4.53x10^{-2}$
BSG	682	19p13.3	Basigin (Ok blood group)	2.05 (1.05-4.00)	$3.37x10^{-2}$
C1GALT1	56913	7p22.1-p21.3	Core 1 synthase, glycoprotein-N-acetylgalactosamine 3-β-galactosyltransferase 1	7.40 (1.68-32.52)	$7.47x10^{-3}$
CDK6	1021	7q21.2	Cyclin-dependent kinase 6	3.06 (1.14-8.24)	$2.71x10^{-2}$
CHPT1	56994	12q23.2	Choline phosphotransferase 1	0.41 (0.24-0.69)	$5.35x10^{-4}$
CKAP2	26586	13q14.3	Cytoskeleton associated protein 2	0.42 (0.19-0.97)	$4.20x10^{-2}$
CLC	1178	19q13.2	Charcot-Leyden crystal galectin	1.52 (1.02-2.29)	$3.43x10^{-2}$
CLINT1	9685	5q33.3	Clathrin interactor 1	0.28 (0.09-0.88)	$2.72x10^{-2}$
CLU	1191	8p21.1	Clusterin	12.51 (1.36-115.07)	$2.42x10^{-2}$
COL17A1	1308	10q25.1	Collagen type XVII α 1 chain	18.63 (1.35-256.93)	$2.69x10^{-2}$
CRTAP	10491	3p22.3	Cartilage associated protein	0.20 (0.05-0.82)	$2.71x10^{-2}$
CTSA	5476	20q13.12	Cathepsin A	2.55 (1.22-5.30)	$1.33x10^{-2}$
DACH1	1602	13q21.33	Dachshund family transcription factor 1	2.04 (1.26-3.31)	$1.68x10^{-3}$
DNAJB9	4189	7q31.1;14q24.2-q24.3	DnaJ heat shock protein family (Hsp40) member B9	0.40 (0.18-0.91)	$3.24x10^{-2}$
DNTT	1791	10q24.1	DNA nucleotidylexotransferase	0.82 (0.67-0.99)	$3.74x10^{-2}$
ECRP	643332	14q11.2	Ribonuclease A family member 2 pseudogene	2.77 (1.20-6.40)	$1.27x10^{-2}$
FCGR1B	2210	1p11.2	Fc fragment of IgG receptor Ib	1.79 (1.18-2.70)	$1.96x10^{-3}$
GALNT10	55568	5q33.2	Polypeptide N-acetylgalactosaminyl transferase 10	4.01 (1.46-11.02)	$6.34x10^{-3}$
GPI	2821	19q13.11	Glucose-6-phosphate isomerase	2.37 (1.03-5.43)	$4.16x10^{-2}$
JCHAIN	3512	4q13.3	Joining chain of multimeric IgA and IgM	1.39 (1.13-1.71)	$1.10x10^{-3}$
KYNU	8942	2q22.2	Kynureninase	2.33 (1.10-4.93)	$1.88x10^{-2}$
LPP	4026	3q27.3-q28	LIM domain containing preferred translocation partner in lipoma	6.42 (1.19-34.48)	$2.92x10^{-2}$
MICALL2	79778	7p22.3	MICAL-like 2	19.04 (3.85-94.09)	$2.70x10^{-4}$
MME	4311	3q25.2	Membrane metalloendopeptidase	0.83 (0.69-1.00)	$4.42x10^{-2}$
MTMR11	10903	1q21.2	Myotubularin related protein 11	1.65 (1.01-2.70)	$3.78x10^{-2}$
MZT2B	80097	2q21.1	Mitotic spindle organizing protein 2B	14.10 (3.05-65.23)	$5.59x10^{-4}$
NR3C1	2908	5q31.3	Nuclear receptor subfamily 3 group C member 1	0.56 (0.36-0.86)	$8.11x10^{-3}$
NRBF2	29982	10q21.3	Nuclear receptor binding factor 2	0.28 (0.10-0.74)	$1.00x10^{-2}$
PDZD8	118987	10q25.3-q26.11	PDZ domain containing 8	0.42 (0.21-0.85)	$1.49x10^{-2}$
PGRMC2	10424	4q28.2	Progesterone receptor membrane component 2	0.37 (0.15-0.92)	$3.05x10^{-2}$
PIP4K2C	79837	12q13.3	Phosphatidylinositol-5-phosphate 4-kinase type 2 γ	2.36 (1.01-5.48)	$4.44x10^{-2}$
PLAGL2	5326	20q11.21	PLAG1 like zinc finger 2	0.13 (0.02-0.84)	$2.93x10^{-2}$
PLD1	5337	3q26.31	Phospholipase D1	49.97 (3.91-637.92)	$2.28x10^{-3}$
PRR11	55771	17q22	Proline rich 11	8.79 (1.49-51.87)	$1.56x10^{-2}$
PRSS21	10942	16p13.3	Protease, serine 21	1.88 (1.18-2.99)	$5.29x10^{-3}$
PTAFR	5724	1p35.3	Platelet activating factor receptor	4.12 (1.20-14.20)	$2.46x10^{-2}$

Table II. Continued.

Gene symbol	Gene ID	Chromosome location	Gene name	HR (95% CI)	P-value
QKI	9444	6q26	QKI, KH domain containing RNA binding	0.50 (0.26-0.98)	$4.47x10^{-2}$
RAB27B	5874	18q21.2	RAB27B, member RAS oncogene family	112.03 (3.02-4156.89)	$1.05x10^{-2}$
RAB7A	7879	3q21.3	RAB7A, member RAS oncogene family	0.02 (0.00-0.39)	$8.24x10^{-3}$
RBM6	10180	3p21.31	RNA binding motif protein 6	3.35 (1.08-10.37)	$3.49x10^{-2}$
RNASE3	6037	14q11.2	Ribonuclease A family member 3	2.49 (1.58-3.92)	$1.89x10^{-6}$
RORA	6095	15q22.2	RAR-related orphan receptor A	2.29 (1.12-4.66)	$2.06x10^{-2}$
RRAGD	58528	6q15	Ras-related GTP binding D	0.41 (0.23-0.73)	$1.96x10^{-3}$
S100A10	6281	1q21.3	S100 calcium binding protein A10	1.57 (1.10 to 2.24)	$1.18x10^{-2}$
SAR1B	51128	5q31.1	Secretion associated Ras related GTPase 1B	0.42 (0.18 to 0.97)	$4.49x10^{-2}$
CAVIN2	8436	2q32.3	Caveolae associated protein 2	13.41 (1.33 to 135.33)	$2.71x10^{-2}$
SLAMF7	57823	1q23.3	SLAM family member 7	5.91 (1.01 to 34.70)	$4.79x10^{-2}$
SLC25A16	8034	10q21.3	Solute carrier family 25 member 16	93.59 (1.42 to 6174.64)	$3.47x10^{-2}$
SOCS3	9021	17q25.3	Suppressor of cytokine signaling 3	6.70 (1.35 to 33.25)	$2.00x10^{-2}$
SORT1	6272	1p13.3;1p21.3-p13.1	Sortilin 1	5.74 (1.24 to 26.54)	$2.28x10^{-2}$
SP1	6667	12q13.13	Sp1 transcription factor	0.10 (0.01 to 0.65)	$1.60x10^{-2}$
STEAP3	55240	2q14.2	STEAP3 metalloreductase	3.08 (1.00 to 9.43)	$4.95x10^{-2}$
TOB2	10766	22q13.2	Transducer of ERBB2, 2	0.38 (0.17 to 0.85)	$2.01x10^{-2}$
U2SURP	23350	3q23	U2 snRNP associated SURP domain containing	0.31 (0.13 to 0.77)	$1.17x10^{-2}$
UBE2D1	7321	10q21.1	Ubiquitin conjugating enzyme E2 D1	0.15 (0.02 to 0.95)	$4.14x10^{-2}$
ZNF91	7644	19p12	Zinc finger protein 91	0.59 (0.35 to 0.99)	$4.80x10^{-2}$

Fifty-nine genes associated with B-ALL relapse-free survival. HR, hazard ratio; CI, confidence interval; B-ALL, B-precursor acute lymphoblastic leukemia; RFS, recurrence-free survival.

and the CXCR4-CXCL12 axis (23) that have been identified as potential targets in leukemia treatment.

Then, we applied Cox proportional hazards modelling to the B-ALL-associated genes and selected 59 top ranked genes based on log-rank test P-values as B-ALL RFS genes (Table II). After applying the hierarchal clustering to this 59-gene profile, the high- (n=20), and low-risk (n=60) samples were identified from the B-ALL cohort with significant (log-rank test P=0.025) different relapse-free survival outcome (Fig. 3). The B-ALL RFS biomarkers included genes from various families, such as glycosyltransferases (C1GALT1 and B4GALT5), RAS oncogene GTPases (RAB27B and RAB7A), nuclear hormone receptors (NR3C1 and RORA), RNA binding proteins (RBM6 and U2SURP) and Zinc finger proteins (PLAGL2, SP1 and ZNF91). We also found that among these biomarkers, 4 genes, B4GALT5, CDK6, PDZD8 and RAB7A, were candidates that were associated with chemotherapy response (Fig. 4) in two independent ALL cohorts, GSE19143 (n=52) (24) and GSE13280 (n=44) (25) where patients were treated with prednisolone. Indeed, the B4GALT family is involved in mediating drug resistance in human leukemia cells by regulating the Hedgehog pathway (26). This suggest that the 59-gene biomarker could also indicate drug sensitivity in ALL treatment.

Discussion

The major challenge in clinical treatments of B-precursor acute lymphoblastic leukemia (B-ALL) is relapse after chemotherapy; thus or the development of alternative treatments is critical. Here, we demonstrated the ability of gene expression profiling to reveal not only biological mechanisms but also clinical diagnostic markers.

Among the 59 genes, several genes have been characterized as being involved in the progression of leukemia and solid tumors. For instance, MME, which is also known as CD10, or CALLA, encodes a type II transmembrane glycoprotein and a common acute lymphocytic leukemia antigen. It is an important cell surface marker in the diagnosis of human acute lymphocytic leukemia (27) has been well-documented in many leukemia-related studies (28-32). SOCS3 is a member of the suppressor of cytokine signaling (SOCS) family and negatively regulates JAK2 kinase. Its altered expression is associated with leukemia (33,34) and solid tumors including melanoma (35-38), cervical cancer (39-41), renal cell carcinoma (42-45), prostate cancer (46,47) and gastric cancer (48). CDK6 is a serine/threonine protein that is important for cell cycle G_1 phase progression and G1/S transition. CDK6 is dysregulated or disrupted in many types of cancer (49-54), and it is previously

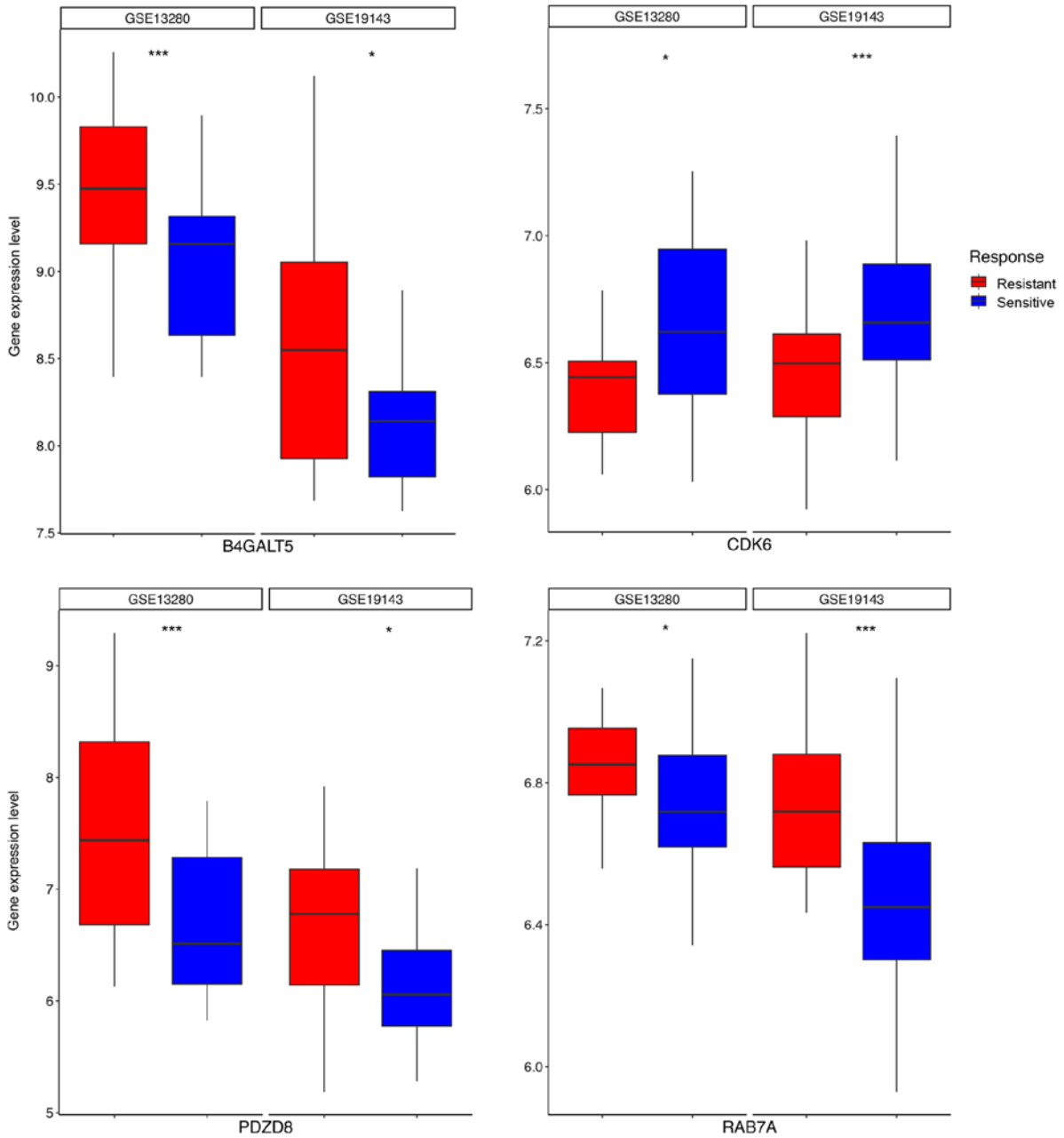

Figure 4. Box plot indicating gene expression levels of *B4GALT5, CDK6, PDZD8* and *RAB7A* in the drug-sensitive and -resistant groups from two cohorts (*P<0.05, ***0.0001<P<0.01). P-values were computed using Wilcoxon rank sum and signed tests.

reported to be in a three-way rearrangement including other two elements: MLL and AF-4 in a case of infant ALL (55). This indicates the biological relevance of the 59-gene biomarker to B-ALL and could propose new directions for investigations.

However, we do realize that there is merely a one gene (JCHAIN known as IGJ) overlap of our 59-gene biomarker with a previously published 38-gene classifier (11). We reasoned that this discordance is due to differences in the analysis strategy. While in the previous study, the biomarker selection was implemented directly on all of the microarray probe sets, we pre-filtered the candidate list to B-ALL-associated genes by differential gene expression analysis. We also optimized the process of preprocessing the microarray data by using up-to-date algorithms and software, which could lead to higher confidence in producing final results.

In conclusion, our systematic approach provided an intriguing guideline for the identification of B-ALL prognostic biomarkers and revealed their potential roles in chemosensitivity. Further investigations are expected to validate the performances of these biomarkers before being applied to clinical management. As the RNA-seq technologies are trending in transcriptome profiling, we will also collect RNA-seq data to re-train and optimize our model. We will also try to reduce the number of biomarkers while maintaining the predictive power, so that the application in clinical management is more feasible.

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Availability of data and materials

The datasets used during the present study are available from NCBI GEO Database with corresponding accession numbers.

Authors' contributions

WJ and JL conceived and designed the study. WJ collected the data and performed the data analysis. WJ and JL wrote and edited the manuscript. Both authors read and approved the manuscript and agreed to be accountable for all aspects of the research in ensuring that the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors do not have any competing interests.

References

1. Locatelli F, Schrappe M, Bernardo ME and Rutella S: How I treat relapsed childhood acute lymphoblastic leukemia. Blood 120: 2807-2816, 2012.
2. Bhojwani D and Pui CH: Relapsed childhood acute lymphoblastic leukaemia. Lancet Oncol 14: e205-e217, 2013.
3. Hunger SP, Lu X, Devidas M, Camitta BM, Gaynon PS, Winick NJ, Reaman GH and Carroll WL: Improved survival for children and adolescents with acute lymphoblastic leukemia between 1990 and 2005: A report from the children's oncology group. J Clin Oncol 30: 1663-1669, 2012.
4. Zhu YM, Foroni L, McQuaker IG, Papaioannou M, Haynes A and Russell HH: Mechanisms of relapse in acute leukaemia: Involvement of p53 mutated subclones in disease progression in acute lymphoblastic leukaemia. Br J Cancer 79: 1151-1157, 1999.
5. Maloney KW, McGavran L, Odom LF and Hunger SP: Acquisition of *p16*^{INK4A} and *p15*^{INK4B} gene abnormalities between initial diagnosis and relapse in children with acute lymphoblastic leukemia. Blood 93: 2380-2385, 1999.
6. Zuna J: *TEL* deletion analysis supports a novel view of relapse in childhood acute lymphoblastic leukemia. Clin Cancer Res 10: 5355-5360, 2004.
7. Sewak MS, Reddy NP and Duan ZH: Gene expression based leukemia sub-classification using committee neural networks. Bioinform Biol Insights 3: 89-98, 2009.
8. Harvey RC, Mullighan CG, Wang X, Dobbin KK, Davidson GS, Bedrick EJ, Chen IM, Atlas SR, Kang H, Ar K, *et al*: Identification of novel cluster groups in pediatric high-risk B-precursor acute lymphoblastic leukemia with gene expression profiling: Correlation with genome-wide DNA copy number alterations, clinical characteristics, and outcome. Blood 116: 4874-4884, 2010.
9. Hogan LE, Meyer JA, Yang J, Wang J, Wong N, Yang W, Condos G, Hunger SP, Raetz E, Saffery R, *et al*: Integrated genomic analysis of relapsed childhood acute lymphoblastic leukemia reveals therapeutic strategies. Blood 118: 5218-5226, 2011.
10. Staal FJ, van der Burg M, Wessels LF, Barendregt BH, Baert MR, van den Burg CM, van Huffel C, Langerak AW, van der Velden VH, Reinders MJ, *et al*: DNA microarrays for comparison of gene expression profiles between diagnosis and relapse in precursor-B acute lymphoblastic leukemia: Choice of technique and purification influence the identification of potential diagnostic markers. Leukemia 17: 1324-1332, 2003.
11. Kang H, Chen IM, Wilson CS, Bedrick EJ, Harvey RC, Atlas SR, Devidas M, Mullighan CG, Wang X, Murphy M, *et al*: Gene expression classifiers for relapse-free survival and minimal residual disease improve risk classification and outcome prediction in pediatric B-precursor acute lymphoblastic leukemia. Blood 115: 1394-1405, 2010.
12. Kohlmann A, Kipps TJ, Rassenti LZ, Downing JR, Shurtleff SA, Mills KI, Gilkes AF, Hofmann WK, Basso G, Dell'orto MC, *et al*: An international standardization programme towards the application of gene expression profiling in routine leukaemia diagnostics: The microarray innovations in LEukemia study prephase. Br J Haematol 142: 802-807, 2008.
13. Haferlach T, Kohlmann A, Wieczorek L, Basso G, Kronnie GT, Béné MC, De Vos J, Hernández JM, Hofmann WK, Mills KI, *et al*: Clinical utility of microarray-based gene expression profiling in the diagnosis and subclassification of leukemia: Report from the international microarray innovations in leukemia Study Group. J Clin Oncol 28: 2529-2537, 2010.
14. Beesley AH, Cummings AJ, Freitas JR, Hoffmann K, Firth MJ, Ford J, de Klerk NH and Kees UR: The gene expression signature of relapse in paediatric acute lymphoblastic leukaemia: Implications for mechanisms of therapy failure. Br J Haematol 131: 447-456, 2005.
15. McCall MN, Jaffee HA and Irizarry RA: fRMA ST: Frozen robust multiarray analysis for Affymetrix Exon and Gene ST arrays. Bioinformatics 28: 3153-3154, 2012.
16. Ritchie ME, Phipson B, Wu D, Hu Y, Law CW, Shi W and Smyth GK: Limma powers differential expression analyses for RNA-sequencing and microarray studies. Nucleic Acids Res 43: e47, 2015.
17. Yu G, Wang LG, Han Y and He QY: clusterProfiler: An R package for comparing biological themes among gene clusters. Omi A J Integr Biol 16: 284-287, 2012.
18. Campbell DJ, Kim CH and Butcher EC: Chemokines in the systemic organization of immunity. Immunol Rev 195: 58-71, 2003.
19. Burkle A, Niedermeier M, Schmitt-Graff A, Wierda WG, Keating MJ and Burger JA: Overexpression of the CXCR5 chemokine receptor, and its ligand, CXCL13 in B-cell chronic lymphocytic leukemia. Blood 110: 3316-3325, 2007.
20. López-Giral S, Quintana NE, Cabrerizo M, Alfonso-Pérez M, Sala-Valdés M, De Soria VG, Fernández-Rañada JM, Fernández-Ruiz E and Muñoz C: Chemokine receptors that mediate B cell homing to secondary lymphoid tissues are highly expressed in B cell chronic lymphocytic leukemia and non-Hodgkin lymphomas with widespread nodular dissemination. J Leukoc Biol 76: 462-471, 2004.
21. Jones D, Benjamin RJ, Shahsafaei A and Dorfman DM: The chemokine receptor CXCR3 is expressed in a subset of B-cell lymphomas and is a marker of B-cell chronic lymphocytic leukemia. Blood 95: 627-32, 2000.
22. Qiuping Z, Jei X, Youxin J, Wei J, Chun L, Jin W, Qun W, Yan L, Chunsong H, Mingzhen Y, *et al*: CC chemokine ligand 25 enhances resistance to apoptosis in $CD4^+$ T cells from patients with T-cell lineage acute and chronic lymphocytic leukemia by means of livin activation. Cancer Res 64: 7579-7587, 2004.
23. Burger JA and Peled A: CXCR4 antagonists: Targeting the microenvironment in leukemia and other cancers. Leukemia 23: 43-52, 2009.
24. Stam RW, Den Boer ML, Schneider P, de Boer J, Hagelstein J, Valsecchi MG, de Lorenzo P, Sallan SE, Brady HJ, Armstrong SA, *et al*: Association of high-level *MCL-1* expression with in vitro and in vivo prednisone resistance in *MLL*-rearranged infant acute lymphoblastic leukemia. Blood 115: 1018-1125, 2010.
25. Spijkers-Hagelstein JA, Schneider P, Hulleman E, de Boer J, Williams O, Pieters R and Stam RW: Elevated *S100A8*/*S100A9* expression causes glucocorticoid resistance in *MLL*-rearranged infant acute lymphoblastic leukemia. Leukemia 26: 1255-1165, 2012.
26. Zhou H, Ma H, Wei W, Ji D, Song X, Sun J, Zhang J and Jia L: B4GALT family mediates the multidrug resistance of human leukemia cells by regulating the hedgehog pathway and the expression of p-glycoprotein and multidrug resistance-associated protein 1. Cell Death Dis 4: e654, 2013.

27. Epstein J, Xiao HQ and He XY: Markers of multiple hematopoietic-cell lineages in multiple myeloma. N Engl J Med 322: 664-668, 1990.
28. Dunphy CH, Gardner LJ, Evans HL and Javadi N: CD15+ acute lymphoblastic leukemia and subsequent monoblastic leukemia: Persistence of t(4;11) abnormality and B-cell gene rearrangement. Arch Pathol Lab Med 125: 1227-1230, 2001.
29. Rozovskaia T, Feinstein E, Mor O, Foa R, Blechman J, Nakamura T, Croce CM, Cimino G and Canaani E: Upregulation of *Meis1* and *HoxA9* in acute lymphocytic leukemias with the t(4:11) abnormality. Oncogene 20: 874-878, 2001.
30. Burmeister T, Meyer C, Schwartz S, Hofmann J, Molkentin M, Kowarz E, Schneider B, Raff T, Reinhardt R, Gökbuget N, *et al*: The *MLL* recombinome of adult CD10-negative B-cell precursor acute lymphoblastic leukemia: Results from the GMALL study group. Blood 113: 4011-4015, 2009.
31. Gleissner B, Goekbuget N, Rieder H, Arnold R, Schwartz S, Diedrich H, Schoch C, Heinze B, Fonatsch C, Bartram CR, *et al*: CD10- pre-B acute lymphoblastic leukemia (ALL) is a distinct high-risk subgroup of adult ALL associated with a high frequency of *MLL* aberrations: Results of the german multicenter trials for adult ALL (GMALL). Blood 106: 4054-4056, 2005.
32. Ha K, Hozumi N, Hrincu A and Gelfand EW: Lineage specific classification of leukaemia: Results of the analysis of sixty cases of childhood leukaemia. Br J Haematol 61: 237-249, 1985.
33. Suzuki R, Sakamoto H, Yasukawa H, Masuhara M, Wakioka T, Sasaki A, Yuge K, Komiya S, Inoue A and Yoshimura A: CIS3 and JAB have different regulatory roles in interleukin-6 mediated differentiation and STAT3 activation in M1 leukemia cells. Oncogene 17: 2271-2278, 1998.
34. Chen C, Yu K, Yan QX, Xing CY, Chen Y, Yan Z, Shi YF, Zhao KW and Gao SM: Pure curcumin increases the expression of SOCS1 and SOCS3 in myeloproliferative neoplasms through suppressing class I histone deacetylases. Carcinogenesis 34: 1442-1449, 2013.
35. Komyod W, Böhm M, Metze D, Heinrich PC and Behrmann I: Constitutive suppressor of cytokine signaling 3 expression confers a growth advantage to a human melanoma cell line. Mol Cancer Res 5: 271-281, 2007.
36. Lesinski GB, Zimmerer JM, Kreiner M, Trefry J, Bill MA, Young GS, Becknell B and Carson WE III: Modulation of SOCS protein expression influences the interferon responsiveness of human melanoma cells. BMC Cancer 10: 142, 2010.
37. Tokita T, Maesawa C, Kimura T, Kotani K, Takahashi K, Akasaka T and Masuda T: Methylation status of the SOCS3 gene in human malignant melanomas. Int J Oncol 30: 689-694, 2007.
38. Fojtova M, Boudny V, Kovarik A, Lauerova L, Adamkova L, Souckova K, Jarkovsky J and Kovarik J: Development of IFN-gamma resistance is associated with attenuation of SOCS genes induction and constitutive expression of SOCS 3 in melanoma cells. Br J Cancer 97: 231-237, 2007.
39. Shivapurkar N, Sherman ME, Stastny V, Echebiri C, Rader JS, Nayar R, Bonfiglio TA, Gazdar AF and Wang SS: Evaluation of candidate methylation markers to detect cervical neoplasia. Gynecol Oncol 107: 549-553, 2007.
40. Zhang P, Yang B, Yao YY, Zhong LX, Chen XY, Kong QY, Wu ML, Li C, Li H and Liu J: PIAS3, SHP2 and SOCS3 Expression patterns in Cervical Cancers: Relevance with activation and resveratrol-caused inactivation of STAT3 signaling. Gynecol Oncol 139: 529-535, 2015.
41. Kim MH, Kim MS, Kim W, Kang MA, Cacalano NA, Kang SB, Shin YJ and Jeong JH: Suppressor of cytokine signaling (SOCS) genes are silenced by DNA hypermethylation and histone deacetylation and regulate response to radiotherapy in cervical cancer cells. PLoS One 10: e0123133, 2015.
42. Urbschat A, Stumpf S, Hänze J, Paulus P, Maier TJ, Weipert C, Hofmann R and Hegele A: Expression of the anti-inflammatory suppressor of cytokine signaling 3 (SOCS3) in human clear cell renal cell carcinoma. Tumour Biol 37: 9649-9656, 2016.
43. Stofas A, Levidou G, Piperi C, Adamopoulos C, Dalagiorgou G, Bamias A, Karadimou A, Lainakis GA, Papadoukakis S, Stravodimos K, *et al*: The role of CXC-chemokine receptor CXCR2 and suppressor of cytokine signaling-3 (SOCS-3) in renal cell carcinoma. BMC Cancer 14: 149, 2014.
44. Tomita S, Ishibashi K, Hashimoto K, Sugino T, Yanagida T, Kushida N, Shishido K, Aikawa K, Sato Y, Suzutani T, *et al*: Suppression of SOCS3 increases susceptibility of renal cell carcinoma to interferon-α. Cancer Sci 102: 57-63, 2011.
45. Oguro T, Ishibashi K, Sugino T, Hashimoto K, Tomita S, Takahashi N, Yanagida T, Haga N, Aikawa K, Suzutani T, *et al*: Humanised antihuman IL-6R antibody with interferon inhibits renal cell carcinoma cell growth in vitro and in vivo through suppressed SOCS3 expression. Eur J Cancer 49: 1715-1724, 2013.
46. Neuwirt H, Puhr M, Cavarretta IT, Mitterberger M, Hobisch A and Culig Z: Suppressor of cytokine signalling-3 is up-regulated by androgen in prostate cancer cell lines and inhibits androgen-mediated proliferation and secretion. Endocr Relat Cancer 14: 1007-1019, 2007.
47. Kneitz B, Krebs M, Kalogirou C, Schubert M, Joniau S, van Poppel H, Lerut E, Kneitz S, Scholz CJ, Ströbel P, *et al*: Survival in patients with high-risk prostate cancer is predicted by miR-221, which regulates proliferation, apoptosis, and invasion of prostate cancer cells by inhibiting IRF2 and SOCS3. Cancer Res 74: 2591-2603, 2014.
48. Li G, Xu J, Wang Z, Yuan Y, Li Y, Cai S and He Y: Low expression of SOCS-1 and SOCS-3 is a poor prognostic indicator for gastric cancer patients. J Cancer Res Clin Oncol 141: 443-452, 2015.
49. Lien HC, Lin CW, Huang PH, Chang ML and Hsu SM: Expression of cyclin-dependent kinase 6 (cdk6) and frequent loss of CD44 in nasal-nasopharyngeal NK/T-cell lymphomas: Comparison with CD56-negative peripheral T-cell lymphomas. Lab Invest 80: 893-900, 2000.
50. Lee YH, Judge AD, Seo D, Kitade M, Gómez-Quiroz LE, Ishikawa T, Andersen JB, Kim BK, Marquardt JU, Raggi C, *et al*: Molecular targeting of CSN5 in human hepatocellular carcinoma: A mechanism of therapeutic response. Oncogene 30: 4175-4184, 2011.
51. Ouyang Q, Chen G, Zhou J, Li L, Dong Z, Yang R, Xu L, Cui H, Xu M and Yi L: Neurotensin signaling stimulates glioblastoma cell proliferation by upregulating c-Myc and inhibiting miR-29b-1 and miR-129-3p. Neuro Oncol 18: 216-226, 2016.
52. Easton J, Wei T, Lahti JM and Kidd VJ: Disruption of the cyclin D/cyclin-dependent kinase/INK4/retinoblastoma protein regulatory pathway in human neuroblastoma. Cancer Res 58: 2624-2632, 1998.
53. Brandi G, Paiardini M, Cervasi B, Fiorucci C, Filippone P, De Marco C, Zaffaroni N and Magnani M: A new indole-3-carbinol tetrameric derivative inhibits cyclin-dependent kinase 6 expression, and induces G1 cell cycle arrest in both estrogen-dependent and estrogen-independent breast cancer cell lines. Cancer Res 63: 4028-4036, 2003.
54. Ye Y, Yang H, Grossman HB, Dinney C, Wu X and Gu J: Genetic variants in cell cycle control pathway confer susceptibility to bladder cancer. Cancer 112: 2467-2474, 2008.
55. Raffini LJ, Slater DJ, Rappaport EF, Lo Nigro L, Cheung NK, Biegel JA, Nowell PC, Lange BJ and Felix CA: Panhandle and reverse-panhandle PCR enable cloning of der(11) and der(other) genomic breakpoint junctions of *MLL* translocations and identify complex translocation of *MLL*, *AF-4*, and *CDK6*. Proc Natl Acad Sci USA 99: 4568-4573, 2002.