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Research paper

High RASD1 transcript levels at diagnosis predicted poor survival in adult B-cell acute lymphoblastic leukemia patients

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ABSTRACT

B-cell acute lymphoblastic leukemia (B-ALL) in adults remains a highly challenging disease. Identifying new prognostic biomarkers is necessary to help select the best therapeutic schedules and to improve prognosis. We performed bioinformatics analyses of transcriptomic data to identify aberrantly-expressed mRNA transcripts in B-ALL and focused on RASD1 (Ras-related dexamethasone-induced 1). To date, no information is available on the prognostic value of RASD1 in B-ALL. Fifty-three consecutive adults with de novo B-ALL were enrolled in this study. Our data suggested that RASD1 was abnormally overexpressed in B-ALL. High RASD1 transcript levels at diagnosis were associated with lower survival probabilities (44% [20%–61%] vs. 79% [60%–97%]; P = 0.037) and were also an independent prognostic factor in adult B-ALL (HR = 4.9 [1.5–15.9]; P = 0.008). Functional in vitro analyses and bioinformatic analyses indicated that RASD1 promoted cell proliferation, cell cycle progression and chemotherapy resistance and inhibited cell apoptosis. These data demonstrated that RASD1 might serve as a novel prognostic biomarker for adult B-ALL and as a potential therapeutic target in adult B-ALL patients.

1. Introduction

Despite significant improvements in the management of B-cell acute lymphoblastic leukemia (B-ALL), the prognosis of adult patients remains poor with long-term survival rates in the 30–40% range in the best cases [1–3]. Currently, prognostic models for B-ALL include age, sex, white blood cell count (WBC) at the time of the initial diagnosis, immunophenotype, cytogenetics, mutational landscape, response to induction therapy and measurable residual disease (MRD) after induction and/or consolidation therapy [4]. However, adult B-ALL patients with none of the adverse prognostic variables remain clinically heterogeneous with varying outcomes. Therefore, identifying new prognostic markers is necessary to help select the best therapeutic schedules.

We used publicly available genome-wide mRNA expression data from B-ALL patients to identify genes that were differentially expressed in B-ALL patients compared with their expression levels in normal control patients [5]. We identified 9 candidate genes (7 of which we validated) and then focused our attention on RASD1 (Ras-related dexamethasone-induced 1), which belongs to the Rap subfamily of the Ras family. RASD1 was discovered in murine AtT-20 cells by Kemppainen et al. in 1998 [6]. The gene maps to 17p11.2 and encodes a monomeric small G protein that acts as a molecular switch in signal transduction [7]. RASD1 is involved in many human physiological processes, including the secretion of various hormones and diet biorhythmicity and is associated with pathological processes such as cardiovascular disease, Huntington's disease, and neurotransmitter-mediated behavioral diseases [7]. RASD1 also plays an important role in tumor growth and expansion. In a variety of tumors, RASD1 can inhibit tumor cell growth and migration and promote tumor cell apoptosis [8–11]. However, it has also been reported that RASD1 plays a role as an oncogene [12–15]. Currently, there are no studies on RASD1 expression or function in B-ALL.

In this study, we studied the prognostic value of the RASD1 expression level in adults with B-ALL and found that high RASD1 transcript levels were independently associated with poor survival in adult B-ALL patients. We further demonstrated that RASD1 promoted cell proliferation, cell cycle progression and chemotherapy resistance and inhibited cell apoptosis in a B-ALL cell line.

2. Materials and methods

2.1. Patients and treatment regimen

Fifty-three newly-diagnosed B-ALL patients ≥ 14 years old were

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enrolled from June 2011 to

January 2017 in the Hematology Department of the First Affiliated Hospital of Zhengzhou University, Zhengzhou, China. The subjects were followed until death, loss to follow-up or until January 2019. The study was approved by the Ethics Committee of the First Affiliated Hospital of Zhengzhou University, and informed consent was obtained according to the Declaration of Helsinki. The diagnosis, response criteria and risk-stratification were as previously reported [16]. All of the patients lacking BCR-ABL fusions received 1–2 cycles of induction chemotherapy (CODPL, including cyclophosphamide, vincristine, daunorubicin, prednisone/dexamethasone and L-asparaginase). The nineteen patients with BCR-ABL fusions received induction chemotherapy with the same regimen but without L-asparaginase and imatinib (400 mg/d) beginning at diagnosis; 2 of these patients were later switched to dasatinib because of resistant mutations. Patients who achieved complete remission received 2 cycles of consolidation chemotherapy with hyper-CVAD and then continued to receive chemotherapy until they completed 6 additional cycles or received an allotransplant. Post-consolidation therapy included 6-mercaptopurine and methotrexate. All of the subjects received CNS prophylaxis with intrathecal methotrexate, cytarabine and dexamethasone for ≥ 6 doses during the induction and consolidation therapy phases. Twenty-nine patients received allogeneic hematopoietic stem cell transplantation (allo-HSCT): 10 from an HLA-identical sibling and 19 from an HLA-haplotype-mismatched related donor. The conditioning regimens used for the allogeneic stem cell transplants and graft-versus-host disease prophylaxis were as previously reported [17]. The therapy recommendations were based on risk stratification and the results of MRD testing after 1–2 cycles of consolidation chemotherapy. The actual treatment selection was based on both the physician's recommendation and the patient's preference.

2.2. Immune phenotype, cytogenetic and molecular analyses, and measurable residual disease (MRD)

Immune phenotypes were identified as reported previously [16]. Cytogenetic analyses were performed using G-banding. BCR-ABL fusions and MLL-translocations were detected as previously described [17]. MRD was quantified by analyzing leukemia-associated aberrant immune phenotypes (LAIPs) using flow cytometry as previously described [18]. A positive MRD-test was defined as cases where ≥0.1% of the cells displayed an LAIP phenotype [5].

2.3. RNA extraction, cDNA synthesis and RQ-PCR

Mononuclear cells were obtained via Ficoll-Hypaque™ density gradient centrifugation, and total RNA was extracted using TRIzol Reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions; the cDNA was then synthesized using a High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA) as previously described [19]. The 10-μl PCR reaction mixture contained 5 μl 1× TaqMan® Universal PCR Master Mix (Applied Biosystems, Foster City, CA, USA), 250 nM fluorescent probes, 400 nM primers, and 150–500 ng cDNA. The real-time quantitative PCR (RQ-PCR) was performed with an ABI PRISM 7500 Sequence Detector (Applied Biosystems, Foster City, CA, USA) at 50 °C for 2 min, 95 °C for 10 min, and followed by 50 cycles at 95 °C for 15 s and 60 °C for 1 min. The RASD1 transcript levels were calculated as the ratio of the RASD1 transcript copy number/Abelson (ABL) copy number as previously described [5]. The RASD1 and ABL copy numbers were calculated from standard curves using the C_t values. Samples were assayed in duplicate to evaluate data reproducibility, and average threshold C_t values were calculated for the expression analyses. Serial dilutions of plasmids expressing ABL and RASD1 were amplified to construct standard quantification curves [20]. A linear correlation was observed between the C_t values and plasmid copy number, with a correlation coefficient of > 0.99 for all curves. These curves indicated similar amplification efficiency for ABL and RASD1. The detection sensitivity was approximately 1–10 copies in the plasmid DNA standards. For each measurement, the curve threshold amplification was set at 0.08 for ABL and RASD1. The primers and probe sequences are shown in Table 1.

Table 1
Sequences of primers and probe used in this study.

Name	Sequence (5'-3')
RASD1-Forward primer	CGACTCGGAGCTGAGTATCC
RASD1-Reverse primer	GGTGGAAGTCCTCGATGGTA
RASD1-Probe	FAM-CAAGAACTGCTATCGCATGGTCATCCT-BHQ
ABL-Forward primer	CCGCTGACCATCAATAAGGAA
ABL-Reverse primer	GATGTAGTTGCTTGGGACCCA
ABL-Probe	FAM-CCATTTTTGGTTTGGGCTTCACACCATT-TAMARA

2.4. Cell lines

The BALL-1 human B-ALL cell line was obtained from Guangzhou Jennio Biotech Co. Ltd. (Guangzhou, China). The cells were cultured in Roswell Park Memorial Institute (RPMI) 1640 medium (Gibco, Billings, MT, USA) containing 10% fetal bovine serum (Gibco), penicillin (100 U/ml, Gibco) and streptomycin (100 μg/ml, Gibco). The cells were grown at 37 °C in a humidified 5% CO_2 atmosphere.

2.5. Preparation of lentivirus-mediated RASD1-overexpressing cells

A human RASD1 lentiviral construct was generated by inserting the full-length human RASD1 cDNA into the Ubi-MCS-3FLAG-SV40-EGFP-IRES-puromycin lentiviral vector (GeneChem, Shanghai, China). Either the human RASD1 lentiviral expression plasmid or a GFP-puromycin-LV vector was co-transfected into 293Ta cells with the Lenti-Pac HIV Packaging Mix (GeneChem, Shanghai, China). Lentivirus-containing supernatant was harvested 48 h after transfection. To establish stable RASD1-overexpressing cell lines and control cell lines, BALL-1 cells were transduced with serial dilutions of RASD1 lentiviral or control lentiviral supernatant at an MOI (multiplicity of infection) of 100 in the presence of 5 μg/ml polybrene, and transduced cells were selected using 1 mg/ml puromycin. After 3 weeks of antibiotic selection, stable RASD1-overexpressing cells and control cells were obtained. The RASD1 expression levels were confirmed by RQ-PCR and western blot analyses.

2.6. Western blot analyses

Western blotting was done as previously described [21]. The primary antibodies were anti-RASD1 (rabbit monoclonal, 1:1000; Cell Signaling, Danvers, MA, USA) and anti-GAPDH (rabbit monoclonal, 1:1000; Cell Signaling, Danvers, MA, USA), and the second antibody was horseradish peroxidase-conjugated goat anti-rabbit IgG (1:10,000; Santa Cruz Biotechnology, Santa Cruz, CA, USA).

2.7. Cell proliferation and viability assays

Cell proliferation was assessed with the Cell Counting Kit-8 (CCK8, Dojin Laboratories, Kumamoto, Japan) assay. Briefly, 4×10^4 cells were seeded into the wells of 96-well plates. 2, 4 or 7 d later, 10 μl of the kit reagent was added to each well. 2 h later, the plates were scanned at 450 nm using a microplate reader. CCK8 was also used to determine cell viability after drug treatments, including for daunorubicin and dexamethasone treatment (Solarbio, Beijing, China). Cells were seeded and, after 72 h,10 μl of the kit reagent was added to each well. 2 h later, the plates were scanned at 450 nm using a microplate reader. Cell viability was assessed based on the value of the fluorescent

signal of live cells with no drug treatment. The experiments were performed 3 times independently in triplicate.

2.8. Colony formation assays

Cells were suspended in 1 mL of complete MethoCult™ medium and plated in 6-well plates at a concentration of 4×10^3 cells/well. Colonies were maintained at 37 °C in 5% CO_2 and 95% humidity for 7 d and then counted and scored on day 7 after staining with 1% crystal violet (Sigma, St. Louis, MO, USA). Only colonies with ≥50 cells were scored. The experiments were performed 3 times independently in triplicate.

2.9. Cell-cycle analyses

Cells were seeded into 6-well plates and starved by adding serum-free medium for G1 synchronization. After 24 h, medium containing 10% fetal bovine serum was added for an additional 48 h. The cells were fixed in 75% ethanol, stained with propidium iodide (BD Pharmingen, San Jose, CA, USA) and analyzed by flow cytometry. The results were analyzed with ModFit LT2.0 software (Coulter Electronics, Hialeah, FL, USA).

2.10. Cell apoptosis analyses

Cell apoptosis was measured using AnnexinV-fluorescein isothiocyanate (FITC) and propidium iodide (PI) staining according to the manufacturer's instructions (Annexin V-FITC apoptosis detection kit I, BD Biosciences). The cell death data were acquired using a FACSCalibur flow cytometer (BD, USA).

2.11. Endpoints and statistical analyses

Relapse-free survival (RFS) was calculated from the date of first complete remission to the date of first relapse. Survival was calculated from the date of diagnosis to the date of death from any cause. Patients were queried at the date of last follow-up to determine whether they were still alive, or were censored on the date they were last known to be alive. The differences across groups were compared using the Pearson Chi-square analysis or Fisher exact test for categorical data and the Mann-Whitney U test or Student *t*-test for continuous variables. A receiver operating characteristic (ROC) curve was used to identify the optimal cutoff levels that best discriminated patients with different outcomes (death). Survival functions were estimated via the Kaplan-Meier method and were compared using the log-rank test. A Cox proportional hazard regression model was used to identify associations between the RASD1 transcript levels and OS and RFS. Variables associated with $P < 0.20$ in the univariate analysis were entered into multivariable analysis performed via the Cox models. The level for statistical significance between differences was set at $P < 0.05$ for all of the univariate tests. The analyses were performed in Graphpad Prism™ 5.01 (San Diego, California, USA) and SPSS software version 18.0 (Chicago, IL, USA).

3. Results

3.1. Patient characteristics

The baseline characteristics of the 53 patients are summarized in Table 2. About half of the patients were male (55%). The median age was 35 years old. The median WBC level at diagnosis was 10.0 (range 1.3–586.0) $\times 10^9$ cells/L. Forty patients were diagnosed with com-B-ALL, 10 patients with pro-B-ALL and only 3 patients with pre-B-ALL. Regarding the cytogenetic features, 14 patients had a normal karyotype, 19 had BCR-ABL fusions (Ph+), 5 had MLL-translocations, 1 had a complex karyotype, and 14 had other abnormalities. Twenty-six patients had IKZF1-deletions and twenty-five patients were classified into a poor-risk group. The MRD-test based on FCM was positive (≥0.1%) in 23 patients at the end of the induction therapy. Twenty-nine patients received allo-HSCTs, and the other patients received only chemotherapy.

Table 2
Association of RASD1 transcript levels with the clinical features of adult B-ALL.

	Total N = 53	L-RASD1 N = 20	H-RASD1 N = 33	*P*
Male/female	29/24	11/9	18/15	0.974
Age (years): median (range)	35 (14–62)	33 (15–52)	41 (14–62)	0.418
WBC($\times 10^9$/L) : median (range)	10.0 (1.3–586)	7.4 (1.3–586)	11.3 (1.9–342)	0.656
PLT ($\times 10^9$/L) : median (range)	54.5 (4.0–310)	48.0 (4.0–95)	59.0 (4.0–310)	0.195
Immune phenotype				
Common-B-ALL	40	17	23	0.325
Pre-B-ALL	3	0	3	0.282
Pro-B-ALL	10	3	7	0.725
Karyotype				
Normal	14	4	10	0.410
BCR-ABL	19	10	9	0.094
MLL-translocation	5	2	3	1.000
Complex karyotype	1	0	1	1.000
Other karyotypes	14	4	10	0.410
IKZF1-deletion	26	9	17	0.646
Risk group				0.145
Poor risk	25	12	13	
Standard risk	28	8	20	
MRD ≥ 0.1%	23	11	12	0.185
Allo-HSCT	29	11	18	0.974

L-RASD1: RASD1/ABL% < 0.665%; H-RASD1: RASD1/ABL% ≥ 0.665%.

3.2. Abnormal RASD1 expression at diagnosis in B-ALL patients

In our previous work, we identified 20 genes that were abnormally highly expressed in B-ALL patients, and we verified the high RASD1 expression levels in all of the B-ALL patients by using RQ-PCR to verify differences in the RASD1 mRNA levels in bone marrow cells from 26 adults with newly-diagnosed B-ALL compared with cells from 23 healthy controls [5]. Furthermore, by using the public in silico transcriptomics (IST) database (http://ist.medisapiens.com/), we also observed elevated RASD1 expression levels in B-ALL malignancies (Fig. 1). In fact, the RASD1 expression levels were higher in B-ALL malignancies compared with those in most of the other malignancies included in the IST.

3.3. High RASD1 expression levels predicted poor outcomes in adult B-ALL patients

We used ROC curves to identify the optimal cutoff value to differentiate patients based on death. A value of 0.665% was identified as the optimal cutoff value based on its maximal Youden index (0.334) among all of the values. Therefore, RASD1 ≥ 0.665% and < 0.665% were defined as high expression and low expression, respectively. In the entire cohort, 33 (62.3%) patients had high RASD1 expression (≥0.665%). As shown in Table 1, there were no significant associations between RASD1 expression level and age, sex, WBC, platelet level, immune phenotype, BCR-ABL fusion status, MLL-translocation status, IKZF1-deletion status, risk group, or MRD-test result at the end of induction therapy and/or post-remission therapy.

The median follow-up was 18 months (range 1–90 months). The complete remission rates after one cycle of induction therapy in subjects with high and low RASD1 expression levels were similar (82% vs. 100%; P = 0.072). Patients with high RASD1 expression had significantly lower 3-year OS (H-RASD1 44% (20%–61%) vs. L-RASD1 79% (60%–97%)); P = 0.037; Fig. 2A) than patients with low RASD1

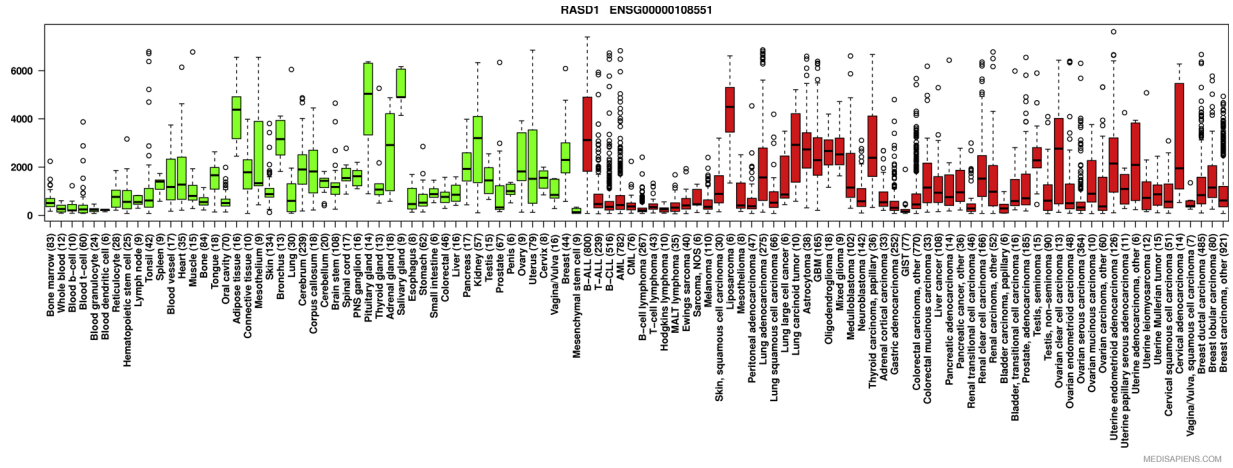

Fig. 1. RASD1 is overexpressed in B-ALL. Box plot depicting the expression profiles of RASD1 mRNA in human normal and cancer tissues based on microarray samples from the in silico transcriptomics (IST) database (Kilpinen et al., 2008). Each box represents the quartile distribution (25–75%) range with the median indicated with a black horizontal line. The 95% range including individual outlier samples is also displayed. The y-axis indicates the relative gene expression level.

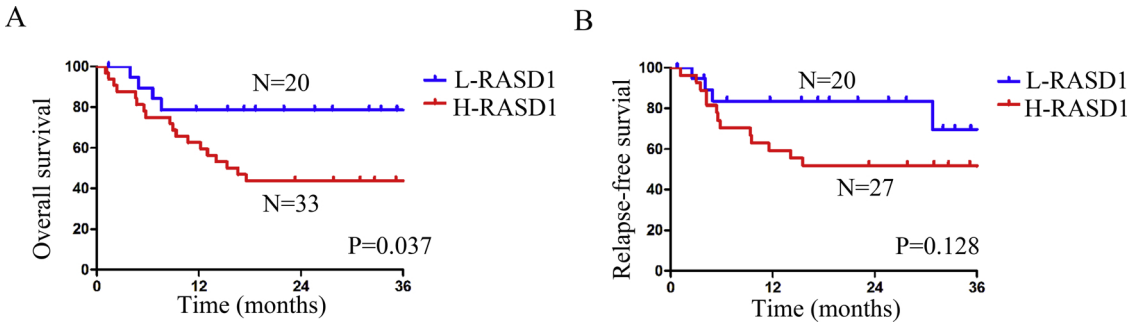

Fig. 2. Associations between RASD1 expression and OS and RFS in adult B-ALL patients. (A) OS and (B) RFS were compared between subjects with high or low RASD1 transcript levels.

expression. Patients with high RASD1 expression had similar 3-year RFS compared to patients with low RASD1 expression (H-RASD1 52% (33%–71%) vs. L-RASD1 70% (41%–98%); P = 0.128; Fig. 2B).

3.4. High RASD1 expression independently predicts poor outcomes in adult B-ALL patients

To further assess the effect of RASD1 expression on the prognoses of adult B-ALL patients, we set up a Cox regression model including age (≥ vs. < 35 years), BCR-ABL fusion status (N/Y), MLL-translocation status (no/yes), treatment (chemotherapy only vs. chemotherapy/allotransplant), MRD-test result at the end of induction therapy (negative/positive) and RASD1 transcript levels (low/high). A univariate analysis was performed and the results are shown in Table 3. High RASD1 expression, an MRD-positive result at the end of induction and no allo-HSCT were significantly related to poorer OS; an MRD-positive result at the end of induction and no allo-HSCT were significantly related to poorer RFS. However, age, BCR-ABL fusion status and MLL-translocation status had no effects on relapse or survival. The effects of the variables associated with P < 0.20 in the univariate analysis were analyzed by multivariable analysis. As shown in Table 3, both high RASD1 expression, an MRD-positive result at the end of induction and no allo-HSCT were independent adverse prognostic factors for OS. Furthermore, an MRD-positive result at the end of induction and no allo-HSCT were independent adverse prognostic factors for RFS.

3.5. RASD1 promotes cell proliferation

To elucidate the potential role of RASD1 in B-ALL, we performed in vitro experiments using the human B-ALL cell line, BALL-1. We developed a RASD1-overexpressing BALL-1 cell line (RASD1-OE; Fig. 3A–B). RASD1 overexpression in the RASD1-OE cells markedly increased their proliferation compared with that of cells transfected with control lentiviral particles (Fig. 3C). We also tested whether RASD1 overexpression promoted colony formation. RASD1 overexpression of significantly increased the number of colony-forming units compared with that of the controls (Fig. 3D).

Bioinformatic analyses also showed that high RASD1 expression in cells correlated with positive regulation of cell proliferation as well as

Table 3
Predictors of OS and RFS: univariate analysis and multivariate analysis.

	Univariate analysis		Multivariate analysis	
	HR(95%CI)	P	HR(95%CI)	P
OS				
Age	1.8 (0.8–4.2)	0.188	1.0 (0.4–2.5)	0.998
BCR-ABL	0.9 (0.4–2.2)	0.844	NA	NA
MLL-translocation	1.4 (0.3–6.2)	0.623	NA	NA
MRD	2.1 (0.9–4.9)	0.080	4.6 (1.9–11.1)	0.001
Allo-HSCT	0.2 (0.1–0.4)	0.000	0.1 (0.0–0.3)	0.000
RASD1 level	3.0 (1.0–8.9)	0.047	4.9 (1.5–15.9)	0.008
RFS				
Age	1.7 (0.7–4.3)	0.268	NA	NA
BCR-ABL	0.9 (0.3–2.5)	0.886	NA	NA
MLL-translocation	0.9 (0.1–6.5)	0.888	NA	NA
MRD	1.4 (0.5–3.6)	0.508	NA	NA
Allo-HSCT	0.2 (0.1–0.5)	0.001	0.2 (0.1–0.5)	0.001
RASD1 level	2.3 (0.8–7.2)	0.140	2.8 (0.9–8.5)	0.077

Abbreviations: NA not available; HR hazard ratio; CI confidence interval.

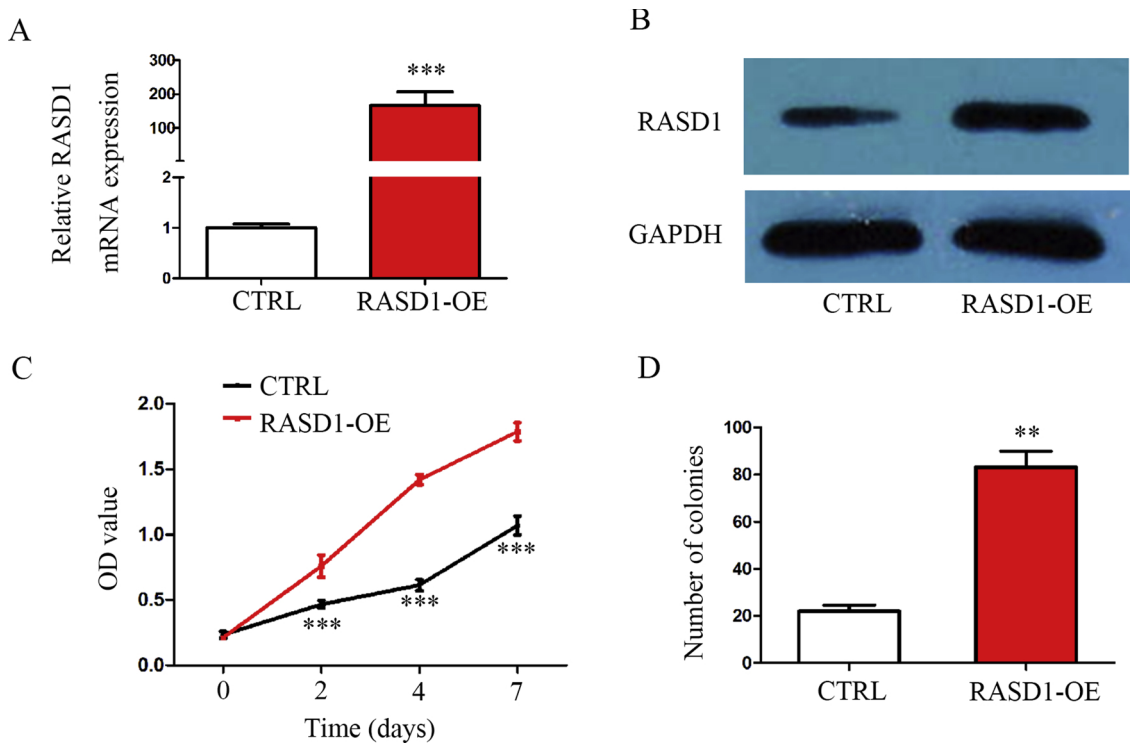

Fig. 3. RASD1 promotes cell proliferation in vitro. (A–B) Ectopic RASD1 expression in BALL-1 cells was demonstrated by RQ-PCR (A) and western blot (B); (C) Ectopic RASD1 expression significantly enhanced BALL-1 cell viability; (D) Ectopic RASD1 expression increased the number of colonies in BALL-1 cells; The values are the mean ± standard deviation (SD). **, $P < 0.01$; ***, $P < 0.001$, compared with the control group.

nucleosome assembly and telomere organization (Table 4). RASD1 was moderately expressed in normal human B-cells (ARS = 40.56). In our previous study, we reported that moderately expressed genes had more plastic or variable expression under diverse experimental conditions [22]. The gene plasticity (GPL) score of RASD1 was 11 in normal B-cells (400 GSMs), making it suitable for virtual sorting, an immune informatics method useful for evaluating immune cell subpopulations and their functions based on highly plastic genes.

3.6. RASD1 promotes cell-cycle progression and inhibits cell apoptosis

Data from flow cytometry analyses showed that increased RASD1 expression promoted cell-cycle progression: the number of RASD1-OE cells in S and G2/M phases increased, while the number of RASD1-OE cells in G0 and G1 phases decreased substantially compared with the numbers observed for the control cells (Fig. 4A–B). Annexin V and PI double-staining assays showed that the numbers of RASD1-OE cells undergoing early and total apoptosis were significantly lower than the numbers observed for the control cells (Fig. 4C–D), indicating that increased RASD1 expression inhibits cell apoptosis.

3.7. RASD1 promotes drug resistance

Drug resistance is the primary reason for treatment-failure and relapse in B-ALL. We studied the relationship between RASD1 transcript levels and B-ALL sensitivity to dexamethasone and daunorubicin. RASD1-OE BALL-1 cells showed significantly increased dexamethasone and daunorubicin resistance compared with that of the control cells (Fig. 5).

4. Discussion

To identify novel B-ALL biomarkers, we previously studied differentially-expressed genes in normal B cells and B-ALL using data from the ImmuSort database [23] (http://immusort.bjmu.edu.cn; Table 1), which contains data from 400 B-cell samples from normal subjects and 690 samples from B-ALL patients [5]. We focused on the top 20 differentially-expressed genes in the B-ALL samples and used RQ-PCR to verify the differential mRNA levels of these genes in bone marrow cells from 26 adults with B-ALL compared with cells from 23 normal subjects [5]. Finally, we focused on RASD1, whose abnormal expression correlates with many different kinds of tumors.

Table 4
Functional annotation of RASD1 high expression B-cells.

Term	Name	P-value	Fold-enrichment	Adjusted P-value (Bonferroni)
GO:0006334	nucleosome assembly	1.90E-17	11.93	2.76E-14
GO:0006335	DNA replication-dependent nucleosome assembly	4.15E-14	25.08	3.02E-11
GO:0032200	telomere organization	1.64E-13	27.44	5.95E-11
GO:0008284	positive regulation of cell proliferation	2.36E-05	2.91	0.002859
GO:0016233	telomere capping	2.80E-05	16.10	0.003122
GO:0034080	CENP-A containing nucleosome assembly	6.17E-05	10.05	0.006387

The annotations are from biological processes of Gene Ontology (GO) via the DAVID website (https://david.ncifcrf.gov).

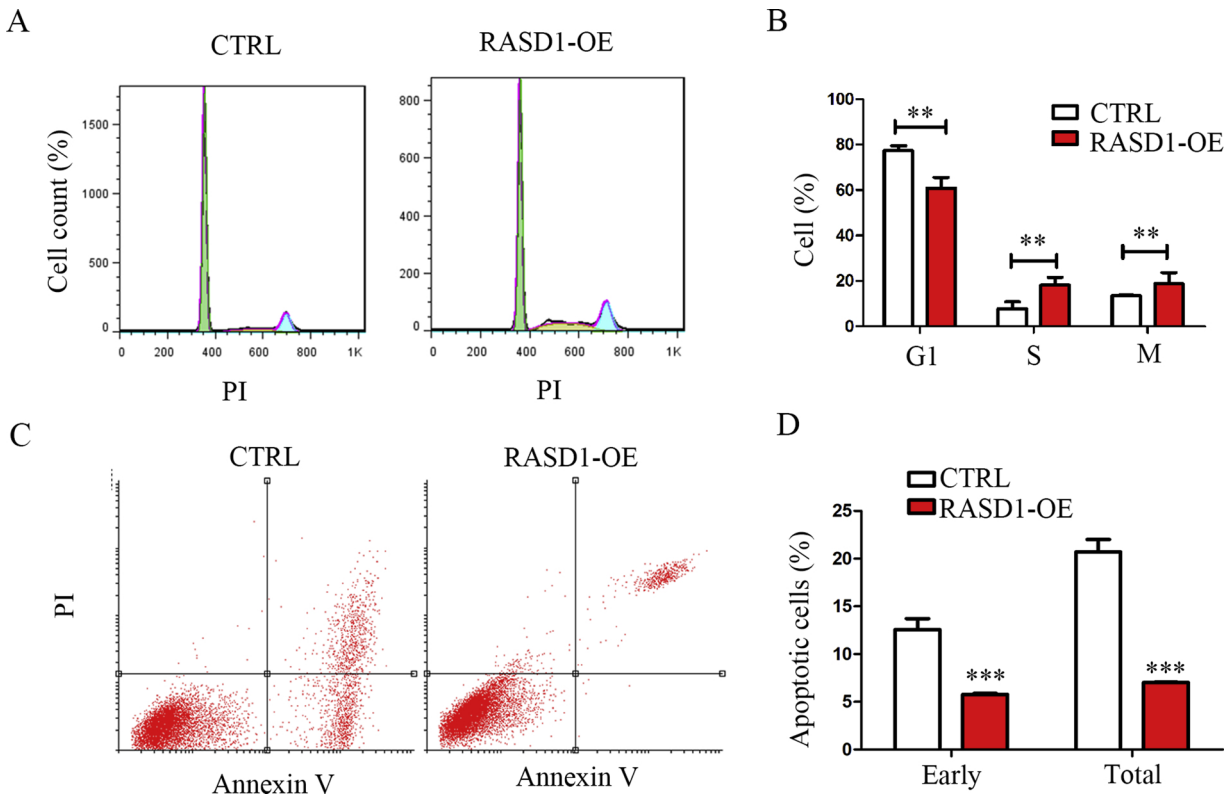

Fig. 4. RASD1 promotes cell-cycle progression and inhibits cell apoptosis. (A) Cell cycle distributions determined by flow cytometry; (B) Ectopic RASD1 expression significantly inhibited BALL-1 cell apoptosis. The values are the mean ± SD. **, $P < 0.01$; ***, $P < 0.001$, compared with the control group.

RASD1, a member of the RAS family, plays important roles in tumor growth and expansion. RASD1 is located on the human 17p11.2 chromosome, and mutations in this region are correlated with cancer development. In a variety of tumor studies, RASD1 plays a role in inhibiting tumor cell growth and migration and in promoting tumor cell apoptosis [8–11]. RASD1 overexpression inhibited the proliferation of breast cancer, renal cell carcinoma and lung adenocarcinoma cell lines [8,9]; furthermore, RASD1 inhibits glioma cell migration by inhibiting the AKT/mTOR signaling pathway [10], and RASD1 methylation in multiple myeloma leads to gene inactivation and dexamethasone resistance [11]. However, it has also been reported that RASD1 plays a role in promoting cancer progression. RASD1 expression is elevated in osteosarcoma and prostate cancer, and RASD1 overexpression promotes the proliferation of osteosarcoma cells [12–15]. These findings suggest that RASD1, unlike other RAS family members, may play different roles in different cancer cell types. It has been reported that RASD1 can regulate the proliferation and activity of normal B cells [24]; however, no reports on RASD1 and B-ALL have been reported so far. Our findings suggest that RASD1 plays a role as an oncogene in B-ALL. RASD1 was abnormally overexpressed in B-ALL, and high RASD1 transcript levels predicted poor prognosis in adult B-ALL patients. Functional analyses indicated that RASD1 overexpression promoted cell proliferation and cell colony formation, findings that are consistent with our bioinformatic analyses. Moreover, we also found that RASD1 overexpression promoted cell cycle progression, drug resistance and inhibited cell apoptosis.

Although high RASD1 transcript levels were associated with OS, this association was not significant in RFS, possibly because all of the patients who did not achieve CR had high RASD1 expression levels and were only included in the RFS analysis. In our multivariate regression analyses, high RASD1 transcript levels were independently-associated with poorer OS, regardless of post-remission therapy. Furthermore, we found a positive MRD-test result at the end of induction therapy and chemotherapy-only were independently associated with worse prognosis whereas other variables including age, BCR-ABL status and MLL-translocation status were not. Yao et al. have previously discussed the

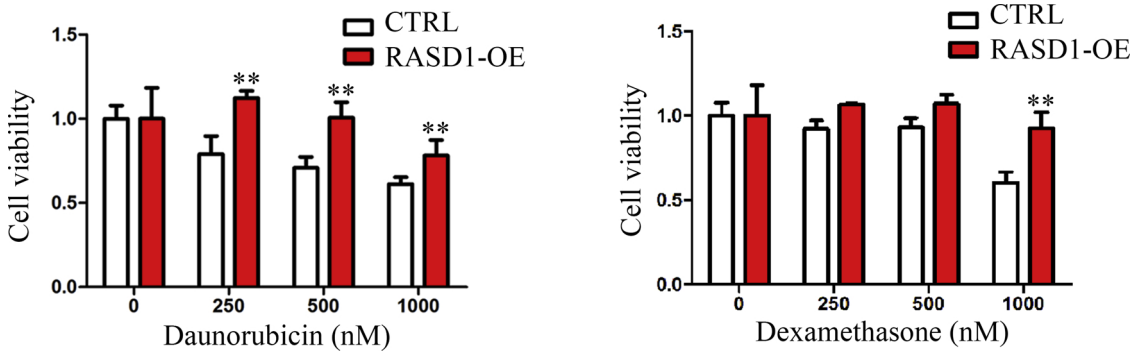

Fig. 5. Ectopic RASD1 expression in BALL-1 cells significantly increased their daunorubicin and dexamethasone resistance. The values are the mean ± SD. **, $P < 0.01$ compared with the control group.

possible reasons for the lack of a significant association between BCR-ABL status and prognosis [17]. There were only 5 patients with MLL-translocations in this group, which may be the reason for the lack of association between MLL-translocation status and prognosis. Compared to post-therapy markers like MRD and treatment, a method for determining whether the initial leukemia could be categorized as “high-risk” before beginning therapy would be more useful for stratifying patients toward risk-directed therapy.

There are several limitations to our study. First, it was retrospective and susceptible to selection bias. Second, the small sample sizes resulted in relatively low statistical power. Third, the prognostic value of RASD1 had better be further validated in a validation cohort. Fourth, there is the potential for an interaction between RASD1 transcript levels and the type of post-remission therapy. Because of these limitations, our conclusions must be validated in a larger, independent prospective cohort. If validated, determination of the RASD1 transcript levels in adult B-ALL patients might inform therapeutic decisions. Moreover, consideration could be given to interventional down-regulation of RASD1 expression as a therapy target.

Declarations of conflicts of interest

None.

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