DNMT3A low-Expression is Correlated to Poor Prognosis in Childhood B-ALL and Confers Resistance to Daunorubicin on Leukemic Cells

Weijing Li (lwj1985@126.com)
Beijing Children's Hospital https://orcid.org/0000-0002-5860-0696
Shuguang Liu
Beijing Children's Hospital
Chanjuan Wang
Beijing Children's Hospital
Lei Cui
Beijing Children's Hospital
Xiaoxi Zhao
Beijing Children's Hospital
Wei Liu
Zhengzhou Children's Hospital
Ruidong Zhang
Beijing Children's Hospital
Zhigang Li
Beijing Children's Hospital https://orcid.org/0000-0001-5212-8215

Research Article

Keywords: Childhood B-ALL, DNMT3A expression, Genome editing of DNMT3A, DNR drug resistance

Posted Date: March 9th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-193259/v2

Version of Record: A version of this preprint was published at BMC Cancer on March 18th, 2023. See the published version at https://doi.org/10.1186/s12885-023-10724-6.

Abstract

Background

Little is known about *DNMT3A* expression and its prognostic significance in childhood B cell acute lymphoblastic leukemia (B-ALL).

Methods

We determined *DNMT3A* mRNA expression in 102 children with B-ALL. Correlations with relapse-free survival (RFS) and common clinical characteristics were analyzed. *DNMT3A* was stably knocked out by CRISPR/Cas9 gene editing technology in Reh and 697 B-ALL cell lines. Cell proliferation activity after treated with daunorubicin (DNR) was determined by CCK8 assay in *DNMT3A* KO Reh and 697 cell lines.

Results

DNMT3A expression in B-ALL patients who were in continuous complete remission (CCR) was higher than in those who got relapse (P=0.0111). Receiver operating characteristic curve showed prognostic significance of *DNMT3A* expression (P=0.003). Low expression of *DNMT3A* (<0.197) was significantly correlated with poor RFS (P<0.001) in children with B-ALL. Knock-out of *DNMT3A* in Reh and 697 cell lines significantly increased IC50 of DNR (P=0.0201 and 0.0022 respectively), indicating elevated resistance to DNR.

Conclusion

Low expression of *DNMT3A* associates with poor prognosis in children with B-ALL. Knock-out of *DNMT3A* confers resistance to DNR on leukemic cells.

Background

B cell acute lymphoblastic leukemia (B-ALL) is the most common childhood malignancy. Although cure rate of childhood B-ALL has been greatly improved with risk-adjusted therapy [1, 2], relapsed leukemia is still a leading cause of death for children mainly due to therapy resistance [2–4]. Thus, it is of great significance to clarify the mechanisms of therapy resistance and relapse of B-ALL.

DNA methyltransferase 3A (DNMT3A) catalyzes de novo DNA methylation and plays important roles in the pathogenesis of malignancies including leukemia. Furthermore, *DNMT3A* mutations in acute myeloid leukemia (AML) and T cell ALL are associated with poor prognosis of the patients [5–11]. Our previous studies have shown that *DNMT3A* mutations can be found in a few of children with B-ALL, and are correlated with poor prognosis [12]. However, the expression level of *DNMT3A* and its prognostic significance in B-ALL remains unclear.

In this study, we assessed the relationship between expression level of *DNMT3A* and prognosis in Chinese childhood B-ALL. Moreover, CRISPR/Cas9 has been used to knock out *DNMT3A* gene in leukemic Reh and 697 cell lines in order to explore the role of *DNMT3A* expression playing in resistance to chemotherapeutic drugs. We showed that low expression of *DNMT3A* was correlated with poor treatment outcome, knock-out of this gene resulted in obvious resistance to DNR, a common chemotherapeutic drug in treatment of ALL.

Methods

Patients

From July 2010 to May 2014,a total of 226 consecutive childhood patients with newly diagnosed B-ALL were admitted to Beijing Children's Hospital. The criterion for the patient's inclusion was ≥70% leukemic cells in BM samples.

102 B-ALL patients with available diagnostic bone marrow (BM) samples were enrolled in this study. One hundred twenty-four patients not fulfilling the inclusion criterion were excluded from this study. No difference was found between patients included and excluded in terms of patients' characteristics and survival to suggest selection bias (Supplemental Table S1). BM samples from 11 patients at continuous CR were collected and used as control. ALL patients were diagnosed and treated in accordance with the Chinese Children's Leukemia Group ALL 2008 Protocol (CCLG-ALL 2008) at Beijing Children's Hospital [4].

Among the 102 patients, there were 64 boys and 38 girls, aged from 1 to 13 years with a median age of 4. Chromosome karyotype analysis was performed in 66 patients and the karyotype results were interpreted according to the International System for Human Gytogenomic Nomenclature guide-lines[13]. Of the 66 patients, normal and abnormal karyotype was seen in 22 and 32 patients respectively and no metaphase schizophrenia was found in the 12 patients. Fusion gene was detected in all 102 patients by a nested multiplex reverse transcription polymerase chain reaction (RT-PCR) system, as described by Gao C. et al. [14]. Thirty-four patients carried 4 types of fusion genes including *ETV6-RUNX1*, *TCF3-PBX1*, *BCR-ABL1*, and *FUS-ERG.* The details of stratification and treatment according to CCLG-ALL 2008 were described previously [4]. Ninety-four patients were in continuous complete remission (CR), 8 patients relapsed 2 to 62 months after diagnosis. The follow-up time ranged from 1.67 to 92 months (median, 59 months). MRD at d33 (the end of induction of remission) and d78 (before consolidation therapy) were detected using RQ-PCR targeted at Ig/TCR (*immunoglobulin* and *T cell receptor* gene rearrangements) according to European MRD (Minimal residual disease) laboratory guidelines [15–18]. Informed consents were obtained from all the children's parents or legal guardians.

Cell lines

Human B-ALL cell lines Reh and 697, as a kind gift from Dr. Suning Chen at the first affiliated Hospital of Soochow University (Suzhou, China), was cultured in RPMI 1640 (GIBCO, USA) supplemented with 10% fetal bovine serum (FBS, AusGeneX, Brisbane) and 1% penicillin/streptomycin. HEK293T cell, kindly

provided by Dr. Fen Chang at Peking University Health Science Center (Beijing, China), was cultured in DMEM supplemented with 10% FBS and 1% penicillin/streptomycin. All cells were maintained at 37°C in a humidified atmosphere containing 5% CO2.

Nucleic acid extraction

Mononucleated cells were separated from 1 ml of patients' BM aspirate by centrifugation with Ficoll 400 (MD Pacific Technology CO., Ltd.) and stored at -70˚C until use. Total RNA of samples was extracted and reverse transcribed using Trizol Reagent (Invitrogen, USA) and MMLV reverse transcriptase (Promega, USA) according to the manufacturers' instructions respectively. Genomic DNA of Reh and 697 cell lines were extracted using a Blood & Cell Culture DNA Midi Kit (TIANGEN, China) according to the manufacturer's protocol.

Quantitative analysis of DNMT3A expression

Real-time quantitative polymerase chain reaction (RQ-PCR) was performed using Power SYBR™ Green PCR Master Mix (Applied Biosystems 4367659) by an ABI Prism 7500 Sequence Detection System (Applied Biosystems, Foster City, CA, USA). *GUS* (β-Glucuronidase) expression was used as an internal control. The cycling condition included pre-denaturation at 95°C for 30 seconds, followed by 40 cycles of 5 s at 95°C, 30s at 55°C and 30s at 72°C. Primers were shown in Table 1. We used the cDNA samples obtained from 697 cell line as a calibrator. The relative expression of *DNMT3A* was calculated by the method of $2^{-\Delta\Delta Ct}$. The levels of *DNMT3A* and *GUS* were tested in triplicates.

Table 1
Oligo sequences

Oligo name	Sequence	Description
DNMT3A ex7 sg F	CACCGGGGGCCCGGGGAGTCTCAGA	sgRNA primer
DNMT3A ex7 sg R	AAACTCTGAGACTCCCCGGGCCCCC	
DNMT3A ex7 F	TTTCACGGCAAGGCAGCTGGTTG	PCR primer (445bp) for T7e1 assay
DNMT3A ex7 R	AGAGGAGAGCAGGACGGGAGGAG	
DNMT3A ex23 F	GCCACCTCTTCGCTCCGCTG	RQ-PCR primer(239bp)for clinical samples
DNMT3A ex23 R	GATGATGTCCAACCCTTTTCGCAA	
GUS F	GAAAATATGTGGTTGGAGAGCTCATT	RQ-PCR primer(101bp)as internal control for clinical samples
GUS R	CCGAGTGAAGATCCCCTTTTTA	
The capital letters underlined indicate BsmbI sticky end.		

Lenti DNMT3A-sgRNA-Cas9 constructs

The cDNA sequence encoding sgRNA which targets a conserved sequence in exon 7 of human *DNMT3A* gene was synthesized and subcloned into LentiCRISPR-v2 plasmid (Addgene 52961, kindly provided by Dr. Jian Huang at Temple University, Philadelphia, PA) to make the lentiDNMT3A-sgRNA-Cas9 construct. Briefly, the forward and reverse primers including 20 bp target *DNMT3A* sequence and BsmbI sticky ends were annealed and inserted into the lentiCRISPR-v2 plasmid digested with FastDigest Esp3I (Thermo Fisher Scientific, #FD0454) (Fig. 1b). sgRNA primer sequences have been reported by Gundry MC et al. previously and were shown in Table 1 [19].

Lentivirus production and infection

To produce lentivirus, 6μg of transfer plasmid lentiDNMT3A-sgRNA-Cas9 or control plasmid lenti-CRISPR-v2 were co-transfected into HEK 293T cells with 4.5μg of packaging plasmids psPAX2 (AddGene 12260) and 3μg of VSV-G (AddGene 8454) using FuGENE® 6 Transfection Reagent (Promega E2692) according to the manufacturer's instructions. After incubation for 48h, the culture supernatants containing lentivirus were harvested and filtered with 0.45μm filter and stored at −80°C. The Reh and 697 cell lines (5×10^5) was infected with the lentivirus at an M.O.I. of 40 separately, using spin-transduction (centrifuging the plate coated with 8μg/ml polybrene (SANTA CRUZ) at 1200g for 2 hours at 25°C), then were cultured for 24 hours in the incubator. On the next day, the medium was changed with fresh RPMI 1640 complete medium and the cells were cultured for another 24 hours.

T7EN1 assays for quantifying frequencies of indel mutations

Lentivirus-infected cells were selected by 1μg/ml puromycin for 2 days. Genomic DNA was extracted and used to amplify the genomic region flanking the *DNMT3A* sgRNA target site with KAPA2G Robust HotStart ReadyMix (KAPA BIOSYSTEMS KK5702) and PCR primers listed in Table 1. Then T7EN1 assay was performed using T7 Endonuclease I (NEB #M0302L) according to the Instruction Manual. The digested DNA was analyzed on electrophoresis system using a 2% agarose gel.

Western blotting

A fraction of lentivirus-infected cells was lysed in NE-PER® Nuclear and Cytoplasmic Extraction Reagents (Thermo Fisher Scientific, USA). The lysates were denatured in 5× SDS loading buffer by boiling at 95°C for 10minutes and were subjected on a NuPAGE™ 4-12% Bis-Tris Protein Gels (Invitrogen). After transferred to Biotrace NT nitrocellulose Transfer Membrane (PALL, 66485), the expression of proteins was detected using following antibodies: 1:300 DNMT3A (D23G1) Rabbit mAb (CST 3598) alone was incubated firstly, then 1:300 DNMT3A (D2H4B) Rabbit mAb (CST 32578) and 1:2000 Lamin B1 Mouse mAb (proteintech 66095-1-Ig) were mixed and incubated together on the next day in the same blot after finishing the secondary antibody incubation and band scanning for DNMT3A(D23G1) mAb. Secondary antibody included Goat anti-Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 680 (Invitrogen A21058) and Goat anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor Plus 800 (Invitrogen A32735). The bands were scanned by LICOR Odyssey CLX.

Cell viability

The lentivirus-infected Reh and 697 cell lines were plated into 96-well plate separately, 10^4/well. After treatment with 100μl DNR (0.005, 0.01, 0.05, 0.1, 0.5, 1μg/ml), the cells were cultured for 24h at 37°C in a humidified atmosphere containing 5% CO_2. Twenty microliters of Cell Counting Kit-8 (CCK-8, Yeasen 40203ES60*, Shanghai, China) solution were added to each well and mixed gently. After incubation for 1 h, optical density (OD) at 450 nm was determined using a Spectra MAX 190 microplate reader. After calibrated with non-cellular background, cell viability was calculated using a non-treatment control regarded as 100% of cell viability.

Statistical analysis

Receiver Operating Characteristic (ROC) curve was used to decide the cut-off value of low- and high-expression of *DNMT3A* (*DNMT3A*^{low} and *DNMT3A*^{high}) in leukemic cells of children with B-ALL. Fisher's exact test was used to test the differences in clinical characteristics and relapse rates between *DNMT3A*^{low} and *DNMT3A*^{high} patients. Relapse free survival (RFS) was defined as the date of leukemia diagnosis to the date of recurrence. Survival estimates were calculated using the Kaplan-Meier method, and the groups were compared using the log-rank test. The independent prognostic significance of *DNMT3A* expression and the common clinical features was analyzed by Cox proportional hazards model

(Method: Enter). All data were analyzed with the SPSS 16.0 software package and a *P* value <0.05 was considered statistically significant. The fitting curves of inhibitory effects of DNR on cell proliferation were plotted by GraphPad Prism 8, and half maximal inhibitory concentration (IC50) was also calculated by the software.

Results

DNMT3A expression in childhood B-ALL

Firstly, we determined *DNMT3A* expression in 102 newly diagnosed (ND) B-ALL patients and 11 patients with non-malignant hematological disease (control) by relative quantitative PCR. As a result, *DNMT3A* expression in ND patients with B-ALL, ranged from 0.0006594 to 1.713 with a median of 0.4363, was significantly higher than that in control patients (range: 0.08055 to 0.1865, median: 0.1147; *P* =0.0004, Fig. 2a). Interestingly, *DNMT3A* expression in ND B-ALL patients who got relapse was significantly decreased compared with that in patients who were in CCR at the last follow-up (*P*=0.0111, Fig. 2b).

Low expression of DNMT3A indicated poor prognosis in ND B-ALL patients

ROC curve analysis was performed to evaluate the prognostic value of *DNMT3A* expression. When regarded as a continuous value, DNMT3A expression had a good predictive significance for relapse of B-ALL in children, with an area under curve (AUC) of 0.819 (95% CI: 0.686-0.952; *P*=0.003), Fig. 3A), which indicated that *DNMT3A* expression could be a potential prognostic biomarker for ND B-ALL patients.

According to the ROC curve, 0.197 was determined as the optimal cutoff value for DNMT3A expression levels, with a sensitivity and specificity of 75% and 87.2% respectively. Using this cut-off, we divided 102 patients into two groups, 22 cases with low *DNMT3A* expression (≤0.197, $DNMT3A^{low}$) and 80 cases with high *DNMT3A* expression (>0.197, $DNMT3A^{high}$). There was a significantly higher relapse rate in $DNMT3A^{low}$ group (6 out of 22 vs 2 out of 88, Fisher's exact test, *P*=0.001). Moreover, poor RFS was observed in the patients of $DNMT3A^{low}$ group (*P*<0.001) (Fig. 3b).

In multivariate analysis for prognosis (Table 2), DNMT3A expression, Age, WBC at diagnosis, Fusion gene (*ETV6-RUNX1, TCF3-PBX1, BCR-ABL1*), MRD at day 33 and day 78 were used as covariates in a cohort of 96 cases due to no MRD result about 6 patients. Only DNMT3A expression reached *P* value<0.1 in the respective univariate analysis for RFS. Adjusted for all these factors, DNMT3A expression remained as only independent prognostic factors for RFS of patients with B-ALL in the multivariate models (HR=19.195, 95% CI: 3.159~116.651, *P*=0.001, Table 2). These findings indicated that low expression of *DNMT3A* in leukemic cells at diagnosis could be a useful indicator for disease relapse in childhood B-ALL.

Table 2 Multivariate analysis of prognostic factors for relapse-free survival in children with B-cell acute lymphoblastic leukemia.

Variables	Relapse-free survival				
	Univariate*	Multivariate#			
	P	Hazard ratio (HR)	*P*	95% CI for HR	
				Lower	Upper
DNMT3A expression: Low (n=21) &. High (n=75)	<0.001	19.195	0.001	3.159	116.651
Age years <1 or ≥10 (n=7) & 1~10 (n=89)	0.255	0.000	0.988	0.000	
WBC counts at diagnosis (*10^9/L): <50(n=77) &≥50 (n=19)	0.169	1.353	0.738	0.231	7.929
ETV6-RUNX1: Positive(n=28) & negative (n=68)	0.59	0.252	0.093	0.51	1.258
TCF3-PBX1 Positive(n=2)& negative (n=94)	0.566	1.025	1.000	0.000	
BCR-ABL1 Positive(n=2)& negative (n=94)	0.514	1.446	1.000	0.000	
MRD at day33: <10^{-3} (n=85) & ≥10^{-3} (n=11)	0.237	3.517	0.212	0.487	25.380
MRD at day78: <10^{-4}(n=93) &≥10^{-4} (n=3)	0.463	0.000	0.996	0.000	

HR indicates Hazard ratio; WBC, white blood count; MRD, minimal residual disease.

***Univariate analysis was performed by Kaplan-Meier Log-rank test.**

#All factors in the univariate analysis were selected in Cox regression of the multivaritate analysis.

Comparison of clinical features between DNMT3A^{low} **and** DNMT3A^{high} **patients**

In the next step, we analyzed the correlation of *DNMT3A* expression with common clinical characteristics such as age, gender, white blood cell (WBC) count at diagnosis and fusion genes. However, no correlation was found between *DNMT3A* expression and above clinical characteristics (Table 3).

Table 3
Correlation of *DNMT3A* expression with clinical characteristics in BCP-ALL

	DNMT3A^{low}, n (%)	*DNMT3A*^{high}, n (%)	*P*
Age			
<1 or ≥10	1(4.5)	6 (7.5)	1.000
1~10	21(95.5)	74 (92.5)	
Gender			
Male	16 (72.7)	48 (60)	0.327
Female	6 (27.3)	32 (40)	
WBC(*10^9/L)			
<50	15 (68.2)	68 (85)	0.118
≥50	7 (31.8)	12 (15)	
Fusion gene			
Negative	19 (86.4)	49 (61.25)	0.264
ETV6-RUNX1	3 (13.6)	25 (31.25)	
TCF3-PBX1	0 (0)	3 (3.75)	
BCR-ABL1	0 (0)	2 (2.5)	
FUS-ERG	0 (0)	1 (1.25)	
MRD at d33			
$<10^{-3}$	18(85.7)	67 (89.3)	0.701
$\geq10^{-3}$	3(14.3)	8(10.7)	
MRD at d78			
$<10^{-4}$	21 (100)	72 (96)	1.000
$\geq10^{-4}$	0(0)	3(4)	

We further analyzed the association of *DNMT3A* expression with MRD at d33 and MRD at d78 respectively, but no significant correlation between them was found (Fisher's exact test, $P>0.05$, Table 3).

Knock-out of DNMT3A **enhanced resistance of Reh and 697 cell lines to DNR**

To confirm the correlation of low expression of *DNMT3A* with poor prognosis of children with B-ALL, firstly, we disrupted *DNMT3A* in Reh and 697 cell lines separately. T7 endonuclease I (T7EN1) assay

showed high efficiency of the sgRNA to direct Cas9-mediated ablation of *DNMT3A* (Fig. 4a and 4b). Furthermore, as expected, Western blotting indicated that *DNMT3A* expression was remarkably reduced after infection with *DNMT3A*-sgRNA lentivirus (Fig. 4c and 4d).

DNR is one of the main chemotherapeutic drugs in induction therapy of B-ALL. We next tested whether knock-out of *DNMT3A* gene could cause Reh and 697 cell lines to be tolerant to DNR by CCK8 assay. These cells were treated by different concentrations of DNR for 24h. It was shown that IC50 was significantly increased in the *DNMT3A*-knockout cells, indicating decreased cell viability (Fig. 5a, Control vs. *DNMT3A* KO, 0.01218 vs. 0.01631 μg/ml, P=0.0201; Fig. 5b, Control vs. *DNMT3A* KO, 0.06449 vs. 0.1052 μg/ml, P=0.0022). These results demonstrated that sgRNA mediated Cas9 knock-out of *DNMT3A* can causes Reh and 697 cell lines to be resistant to DNR, implying that *DNMT3A* expression plays an important role in the sensitivity of B-ALL leukemic cells to chemotherapeutic drugs such as DNR.

Discussion

In recent two decades, *DNMT3A* mutations have been found in approximately 20% of adult AML patients, 9% of adult T-ALL and 0~1.4% of childhood AML, and the hotspots of mutations are mainly located in exon 23 which encodes the catalytic methyltransferase domain [5–11]. *DNMT3A* mutations are associated with poor prognosis and used for risk stratification in AML [5–10], and is associated with increased age and adverse outcome in adult T-ALL [11]. However, few studies focused on the role of *DNMT3A* in B-ALL. Our previous study has shown that *DNMT3A* mutations can be found in exon 23 and its adjacent intron regions in a few of children with B-ALL (5/182, 2.7%), and may have adverse impact on prognosis [12].

As there are only a few B-ALL patients with *DNMT3A* mutations, we sought to determine the prognostic significance of *DNMT3A* expression in B-ALL. As expected, low expression leading to decreased methyltransferase activity was associated with relapse in 102 patients with B-ALL. Furthermore, knock-out of *DNMT3A* increased IC50 of DNR in Reh and 697 cell lines, indicating the relationship of low expression of *DNMT3A* and chemoresistance.

It was reported that Dnmt3a loss in HSCs leads to hypomethylation of genes with a causal role in cancer, such as *Runx1* and *Gata3*. Runx1 promotes murine erythroid progenitor proliferation and inhibits differentiation by preventing Pu.1 downregulation [20]. Gata3 targets Runx1 in the embryonic hematopoietic stem cell niche [21]. Thus, previous studies and ours' suggest that deletion or low-expression of *Dnmt3a* result in differentiation inhibition of HSCs and allow HSCs to be propagated indefinitely in vivo [22–25], which may play an important role in leukemogenesis and resistance to chemotherapy. This may provide us with an alternative target of therapy for childhood B-ALL.

It has been reported that *DNMT3A* expression is directly transactivated by transcription factor WT1 in Wilms' tumor cells [26] and is negatively regulated by p53 at the transcriptional level in lung cancer[27]. In fact, overexpression of the WT1 transcript was demonstrated in children with B-ALL at diagnosis [28], which may contribute to increased expression of *DNMT3A* in leukemic cells. However, increased

expression of *TP53* by 2 to 20-fold higher in pediatric primary B-ALL than in healthy controls [29] would inhibit *DNMT3A* expression. Thus, the regulation mechanism of *DNMT3A* expression is quite complicated in different types of cancer cells and needs to be clarified especially in childhood B-ALL.

Conclusions

In summary, we associated low expression of *DNMT3A* with poor prognosis in Chinese pediatric patients with B-ALL and resistance to DNR. Furthermore, successful disruption of *DNMT3A* in Reh and 697 cell lines may facilitate the studies on mechanism of relapse and chemotherapeutic resistance for childhood B-ALL. Future prospective studies with large sample size, long-term follow-up, more leukemia cell lines and more mechanism research were recommended to confirm the DNMT3A role in childhood with B-ALL.

Abbreviations

B-ALL: B cell acute lymphoblastic leukemia; RFS: relapse-free survival; DNR: daunorubicin; CCR: continuous complete remission; DNMT3A: DNA methyltransferase 3A; AML: acute myeloid leukemia; BM: bone marrow; CCLG-ALL 2008: Chinese Children's Leukemia Group ALL 2008 Protocol; CR: continuous complete remission; Ig/TCR: *immunoglobulin* and *T cell receptor* gene rearrangements; MRD: Minimal residual disease; RQ-PCR: Real-time quantitative polymerase chain reaction; ROC: Receiver Operating Characteristic; RFS: Relapse free survival; WBC: white blood cell; T7EN1: T7 endonuclease I.

Declarations

Ethics approval and consent to participate

This study was submitted to and approved by our institutional ethics committee of Beijing Children's Hospital Affiliated to Capital Medical University.

Informed consents were obtained from all the children's parents or legal guardians.

Consent for publication

Not applicable.

Competing interests

The authors declare no conflict of interest.

Acknowledgments

The authors would like to thank all staff members in the hematology oncology center and in the laboratory of hematologic diseases who have taken care of the patients and given the help for the studies.

Authors' contributions

ZGL designed this research and revised the manuscript. WJL performed experiments, interpreted/analyzed data, prepared figures and wrote the manuscript. CJW also performed part of the experiments and LC helped in analyzing data and preparing figures. As co-corresponding authors, WL and RDZ contributed equally to study design and organized informed consent. SGL and XXZ contributed patient material and provided clinical data. All authors have read and approved the manuscript.

Funding

This study was supported by the grants from WJL's Beijing Natural Science Foundation of China [No. 7194263]; ZGL's National Natural Science Foundation of China [No. 81870114] and Scientific Research Common Program of Beijing Municipal Commission of Education [No. KM201810025025]; LC's National Natural Science Foundation of China [No. 81970135] and Beijing Natural Science Foundation of China [No.7202044] and RDZ's Beijing Municipal Science & Technology Commission [No. Z181100001718100]. The roles these funders had were showed in part of "Authors' contributions" in detail.

Availability of data and materials

All data generated and analyzed during this study are included in this manuscript and original data as supplemental part.

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Figures

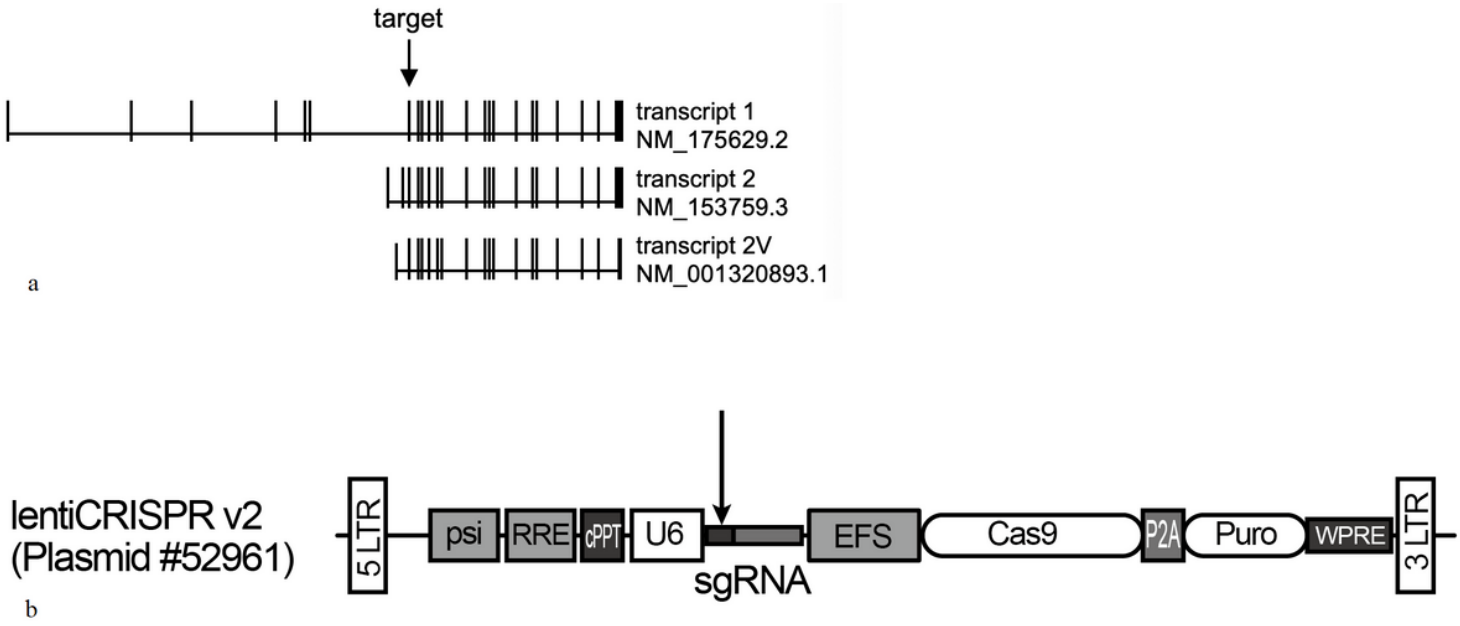

Figure 1

Schematic diagram of sgRNA targeting *DNMT3A*. a. The structure of *DNMT3A* gene and the three common transcripts. Black vertical lines: exons. Horizontal lines: introns. Arrow: the location of sgRNA targeting exon 7. b. The structure of lentiCRISPR v2 plasmid. The arrows indicate the sgRNA sequence.

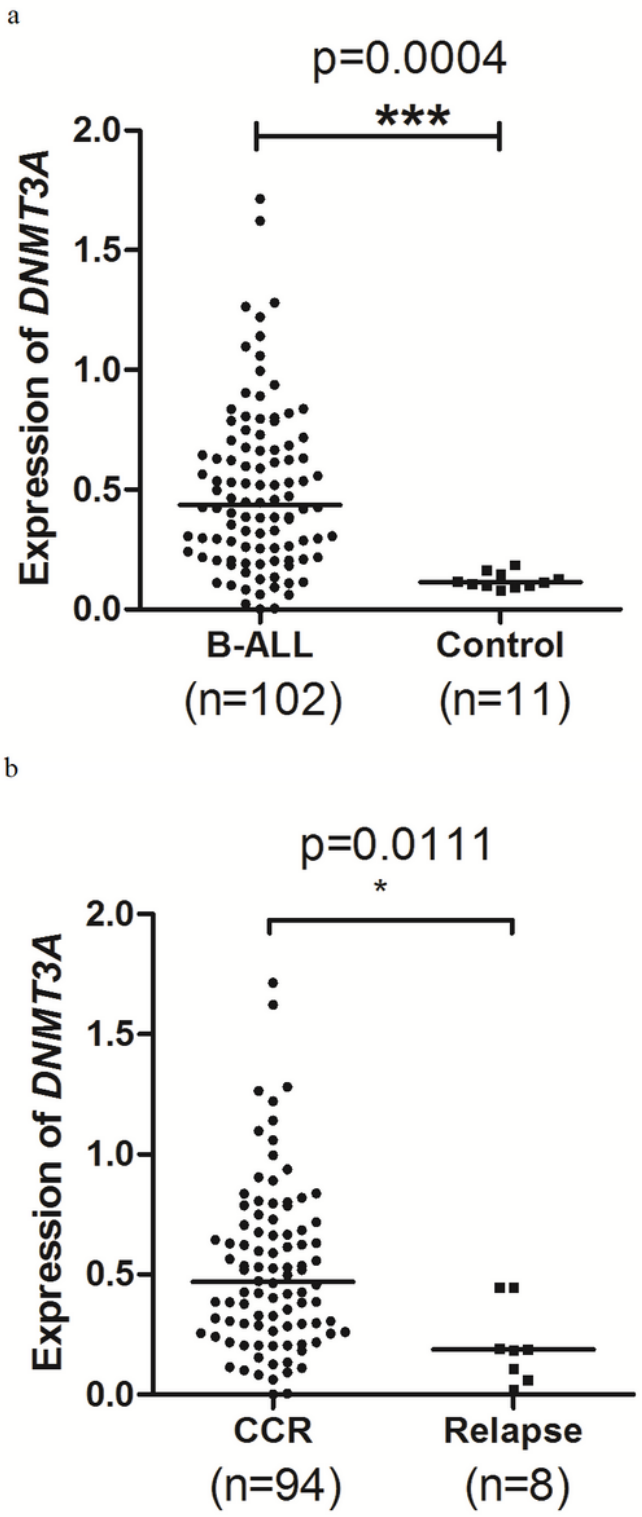

Figure 2

***DNMT3A* expression in ND B-ALL patients and controls.** a. *DNMT3A* expression was significantly increased in ND B-ALL patients compared with that of controls. b. *DNMT3A* expression was significantly decreased in ND B-ALL patients who relapsed compared with that in ND patients in CCR.

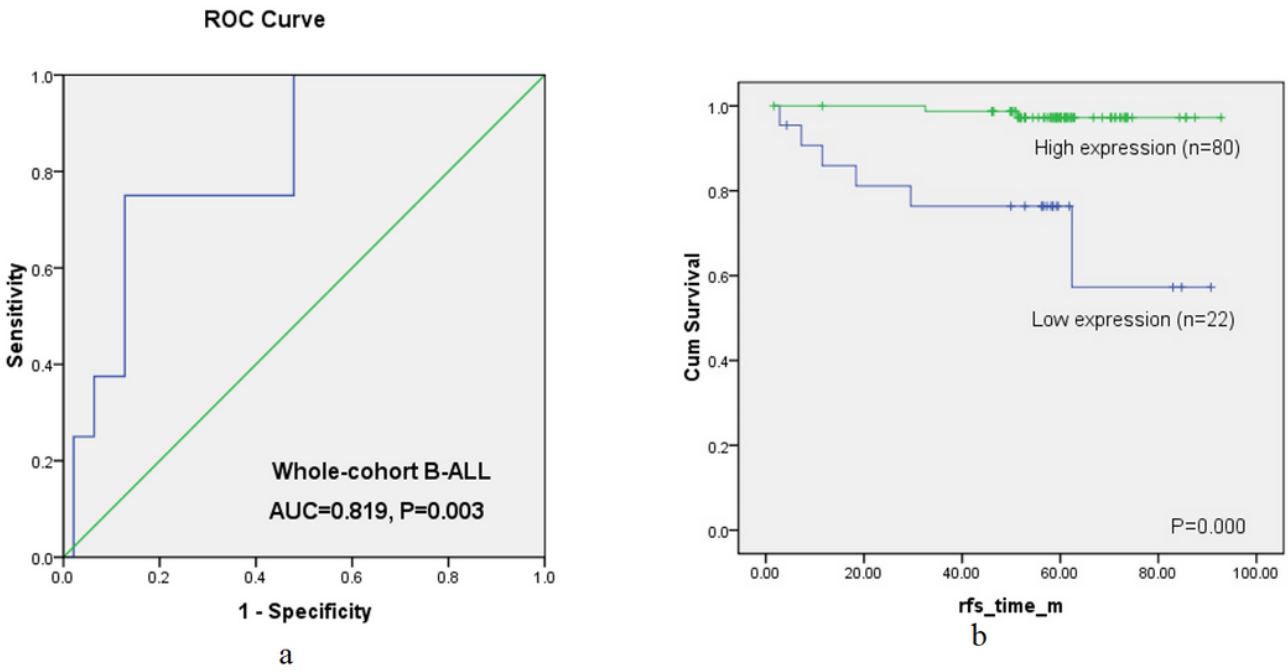

Figure 3

Prognostic significance of *DNMT3A* expression in 102 children with B-ALL. a. ROC curve analysis of *DNMT3A* expression with relapse as an event. b. The patients in *DNMT3A*^{low} group had poorer RFS than those in *DNMT3A*^{high} group (P<0.001).

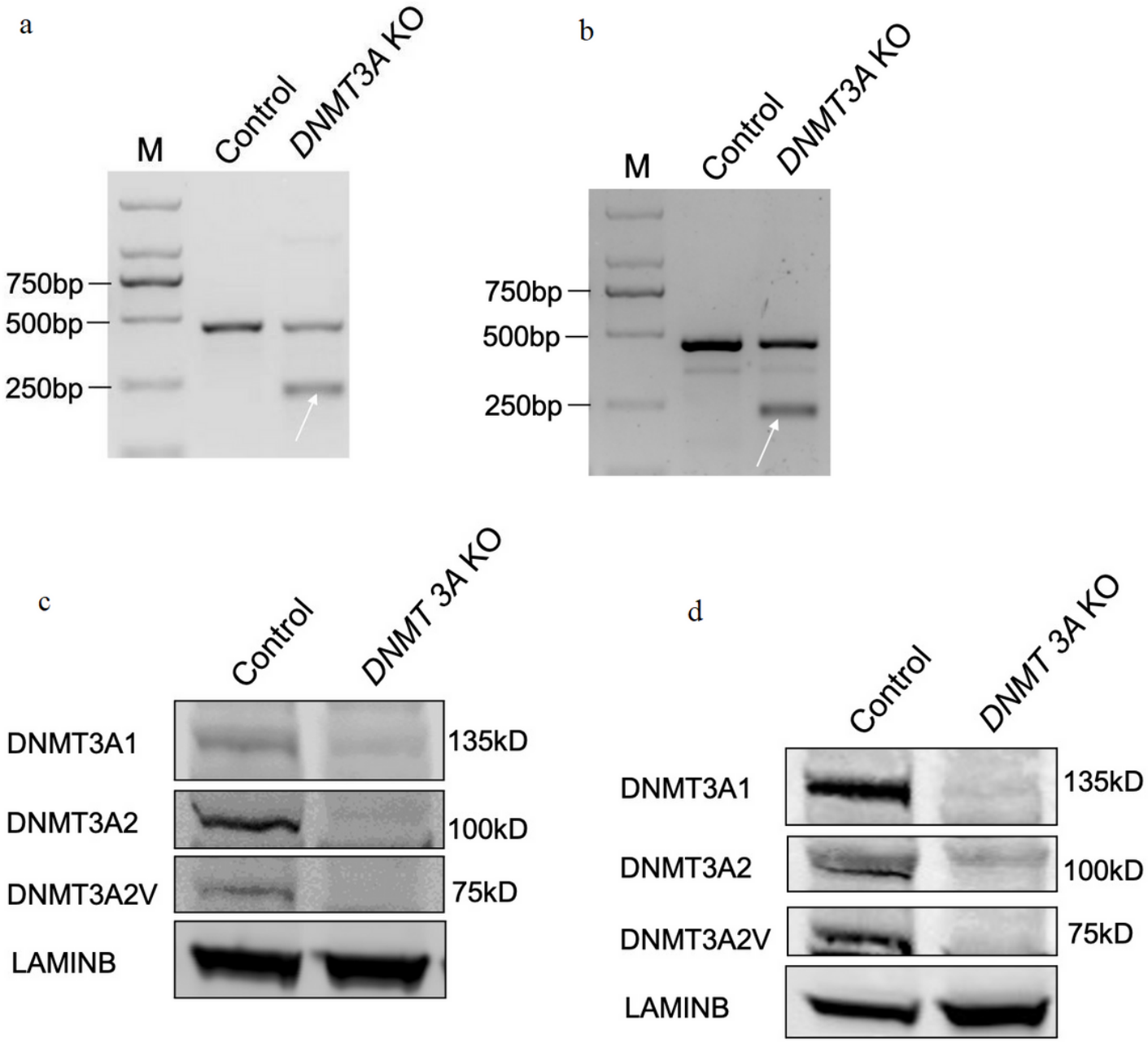

Figure 4

LentiCRISPR/Cas9 mediated editing of *DNMT3A* gene in Reh and 697 cell lines. a and b. T7e1 assay analysis of specific sgRNA-mediated indels at DNMT3A locus in Reh and 697 cell lines separately. The lower migrating bands marking by a white arrow represent the disrupted gene alleles. c and d. Expression of three DNMT3A protein variants was significantly reduced in Reh and 697 cell lines infected by *DNMT3A*-sgRNA lentivirus separately.

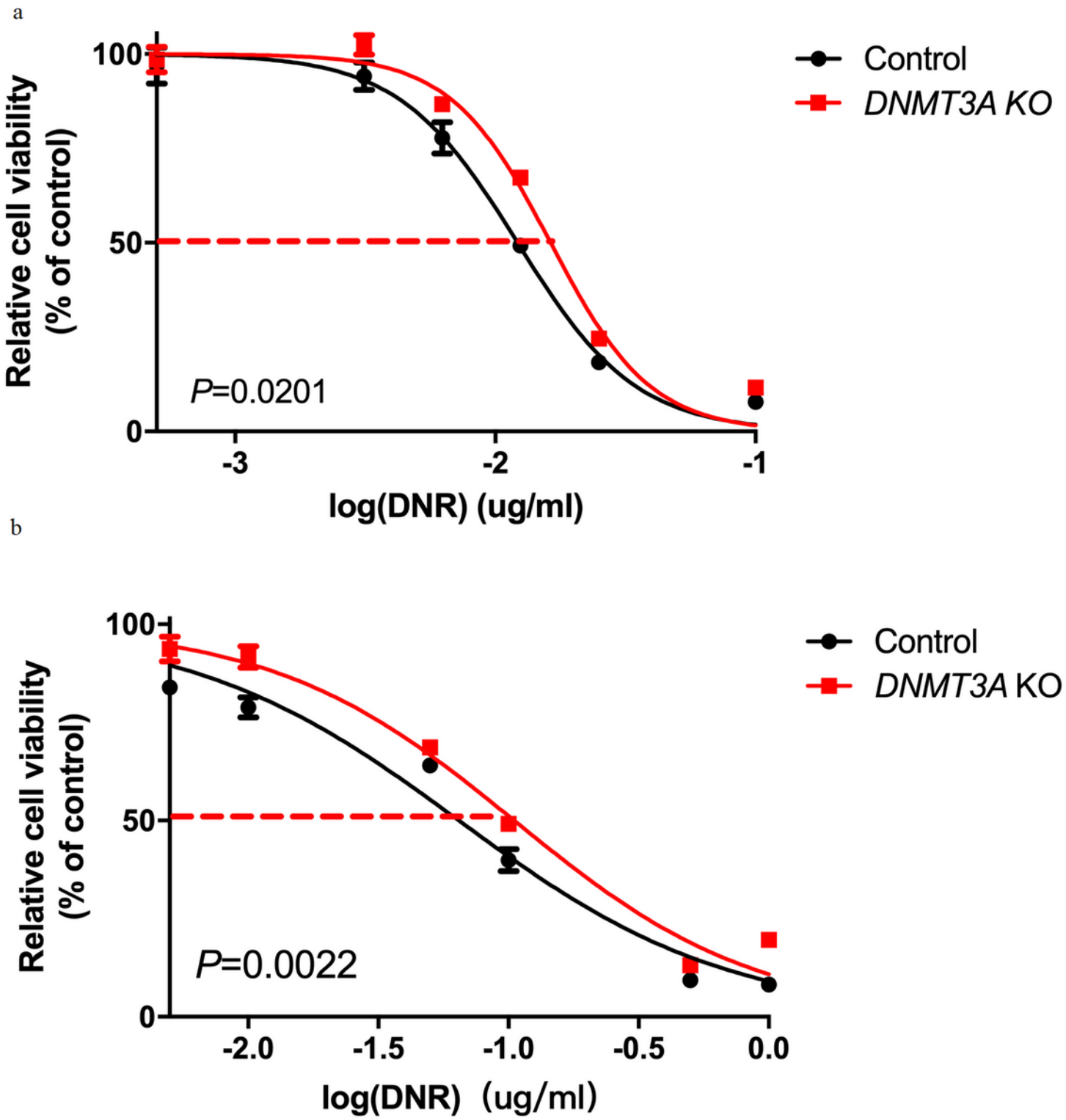

Figure 5

Knock-out of *DNMT3A* gene increased resistance of Reh and 697 cell lines to DNR. IC50 of DNR significantly increased in *DNMT3A*-knockout cells (Independent-samples T test, *P* = 0.0201 in Reh cell line (Fig.5a) and *P*=0.0022 in 697 cell line (Fig.5b)). The standard errors of the means are shown (n = 3 experiments for each drug concentration).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- TableS1.doc