Front. Med. 2021, 15(4): 608–620
https://doi.org/10.1007/s11684-021-0836-7

RESEARCH ARTICLE

Clinical significance of $CD34^+CD117^{dim}$/$CD34^+CD117^{bri}$ myeloblast-associated gene expression in t(8;21) acute myeloid leukemia

Xueping Li[1,*], Yuting Dai[1,2,*], Bing Chen[1], Jinyan Huang[1], Saijuan Chen[1], Lu Jiang (✉)[1]

[1]*Shanghai Institute of Hematology, State Key Laboratory of Medical Genomics, National Research Center for Translational Medicine at Shanghai, Ruijin Hospital Affiliated to Shanghai Jiao Tong University School of Medicine, Shanghai 200025, China;* [2]*School of Life Sciences and Biotechnology, Shanghai Jiao Tong University, Shanghai 200240, China*

Abstract **t(8;21)(q22;q22) acute myeloid leukemia (AML) is a highly heterogeneous hematological malignancy with a high relapse rate in China. Two leukemic myeloblast populations ($CD34^+CD117^{dim}$ and $CD34^+CD117^{bri}$) were previously identified in t(8;21) AML, and $CD34^+CD117^{dim}$ cell proportion was determined as an independent factor for this disease outcome. Here, we examined the impact of $CD34^+CD117^{dim}$/$CD34^+CD117^{bri}$ myeloblast-associated gene expression on t(8;21) AML clinical prognosis. In this study, 85 patients with t(8;21) AML were enrolled. The mRNA expression levels of $CD34^+CD117^{dim}$-associated genes (*LGALS1*, *EMP3*, and *CRIP1*) and $CD34^+CD117^{bri}$-associated genes (*TRH*, *PLAC8*, and *IGLL1*) were measured using quantitative reverse transcription PCR. Associations between gene expression and clinical outcomes were determined using Cox regression models. Results showed that patients with high *LGALS1*, *EMP3*, or *CRIP1* expression had significantly inferior overall survival (OS), whereas those with high *TRH* or *PLAC8* expression showed relatively favorable prognosis. Univariate analysis revealed that CD19, $CD34^+CD117^{dim}$ proportion, *KIT* mutation, minimal residual disease (MRD), and expression levels of *LGALS1*, *EMP3*, *CRIP1*, *TRH* and *PLAC8* were associated with OS. Multivariate analysis indicated that *KIT* mutation, MRD and *CRIP1* and *TRH* expression levels were independent prognostic variables for OS. Identifying the clinical relevance of $CD34^+CD117^{dim}$/$CD34^+CD117^{bri}$ myeloblast-associated gene expression may provide new clinically prognostic markers for t(8;21) AML.**

Keywords t(8; 21)(q22; q22) AML; $CD34^+CD117^{dim}$/ $CD34^+CD117^{bri}$ cell population; gene expression; prognosis

Introduction

Acute myeloid leukemia (AML) with t(8;21)(q22;q22) is a malignant hematological proliferative disease characterized by the accumulation of clonal proliferative myeloid cells that are arrested at different hematological stages [1]. The fusion protein *RUNX1-RUNX1T1*, which is produced by the (8;21) translocation, could block myeloid cell differentiation. However, this protein is not sufficient to induce leukemia, and additional genetic alterations are required to address t(8;21) AML pathogenesis [2]. t(8;21) is one of the most frequent chromosomal translocations in AML and accounts for 10%–15% of adult *de novo* AML cases [3–6]. Most of the t(8;21) AML cases belong to the AML-M2b subtype in the FAB nomenclature. Although t(8;21) AML is a favorable subtype [7], it has a high relapse rate in China and some other countries, leading to poor prognosis. Thus, its pathogenesis needs investigation.

In our previous study, $CD34^+$ myeloblasts are classified into two heterogeneous cell populations, namely, $CD34^+CD117^{dim}$ and $CD34^+CD117^{bright\ (bri)}$, by using the antibody combination of CD34 and CD117 in t(8;21) AML, according to CD117 expression level. These myeloblasts are blocked at different myeloid stages and have distinct characteristics that manifest through several approaches, including single-cell RNA sequencing (scRNA-seq), RNA sequencing (RNA-seq), and morphological and immunophenotypic analyses [8]. The $CD34^+CD117^{dim}$ cell population is located at the earliest stage of myeloid differentiation, exhibits high expression of granulocyte-monocyte progenitor markers, and presents a leukemia stem cell gene

Received September 23, 2020; accepted November 9, 2020
Correspondence: Lu Jiang, jianglu125@126.com

*These authors contributed equally to this work.

expression signature. Bulk RNA-seq data in 62 patients with t(8;21) AML revealed that several genes are aberrantly upregulated in patients with different proportions of cell populations. Normal hematopoiesis and cellular differentiation are believed to strictly depend on transcriptional regulation systems. Aberrantly high gene expression in heterogeneous cell populations may lead to the abnormal phenotypes of these cells. Hence, the role of the overexpressed genes in the $CD34^{+}CD117^{dim}$/$CD34^{+}CD117^{bri}$ population in t(8;21) AML deserves further investigation. In addition, our previous study revealed that the proportion of the $CD34^{+}CD117^{dim}$ population is associated with the disease clinical outcome. Combined with *KIT* mutation, which is a well-established prognostic factor in t(8;21) AML, t(8;21) AML could be further stratified into one low-risk subgroup, two intermediate-risk subgroups, and one high-risk subgroup. Whether the highly expressed genes in $CD34^{+}CD117^{dim}$/$CD34^{+}CD117^{bri}$ cells are associated with t(8;21) AML prognosis need to be addressed. Therefore, the present work aimed to investigate the gene expression and prognostic value of the overexpressed genes in $CD34^{+}CD117^{dim}$ and $CD34^{+}CD117^{bri}$ cells and the correlations between gene expression and gene mutations in t(8;21) AML.

Materials and methods

Patient characteristics

This study enrolled 85 patients with *de novo* t(8;21) AML and 21 healthy donors from Ruijin Hospital Affiliated to Shanghai Jiao Tong University School of Medicine, Shanghai, China. Informed consent was obtained from all the patients and healthy donors in accordance with the *Helsinki Declaration* II.

The patients were treated with standard first-line "3 + 7" induction regimens consisting of idarubicin (12 mg/m^2 for days 1–3) and Ara-C (100 mg/m^2 for days 1–7), followed by 2–4 courses of high-dose cytarabine-based therapy (2 g/m^2 every 12 h for days 1–3, a total of six doses) or allogeneic hematopoietic stem cell transplantation (allo-HSCT) as consolidation therapy. Among the 85 patients, 16 (18.82%) received allo-HSCT.

Gene expression analysis via qRT-PCR

Quantitative reverse transcription PCR (qRT-PCR) was conducted to detect the gene expression levels of *LGALS1, ANXA2, EMP3, TRH, PLAC8*, and *IGLL1* by using TB Green™ Premix Ex *Taq*II (Tli RNaseH Plus) (TAKARA, Japan) on an ABI ViiA 7 detection system (Life technologies, USA). Positive and negative controls were included in all assays. All values were normalized to *GAPDH* mRNA levels and presented as ΔCT values. The primer sequences are listed in Table S1.

Gene expression analysis via 10x Genomics scRNA-seq

Gene expression data and cell annotations of 10x Genomics scRNA-seq were obtained from our previously study [8]. Gene set enrichment analysis (GSEA) was performed using the clusterProfiler R package [9]. Gene sets were downloaded from the Molecular Signatures Database (MSigDB, v7.1) of the Broad Institute. Hallmark gene sets (H) and Kyoto Encyclopedia of Genes and Genomes (KEGG) gene sets (C2) were used to perform GSEA in 1000-gene set. R package enrichplot was employed to visualize the GSEA results. Uniform manifold approximation and projection (UMAP) were utilized to reduce dimensionality by using the first 20 principal components for visualization. Clusters were identified using differentially expressed genes with an adjusted *P* value (p_val_adj) $\leqslant 0.05$ and an average log fold change (avg_logFC) $\geqslant 0.2$. The *P* values of the highly expressed genes in $CD34^{+}CD117^{dim}$ and $CD34^{+}CD117^{bri}$ populations were calculated using two-sided Wilcoxon test. Heatmap and ggplot2 R packages were employed for the visualization of the gene expression data.

TCGA cohort analysis

Data from the public database The Cancer Genome Atlas (TCGA-LAML) were used to analyze the gene expression levels of patients with different AML subtypes [10]. The raw expression matrix of fragments per kilobase per million (FPKM) and clinical data were downloaded using TCGAbiolinks [11]. $\log_2$(FPKM+1) was applied to evaluate the expression level of each gene.

Statistical analysis

The clinical characteristics of the two groups were analyzed using χ^2-test or Fisher's exact test for categorical parameters and Mann–Whitney U test for continuous variables. Overall survival (OS) was measured from the date of disease diagnosis to the date of death (failure) or the last follow-up time (censored). A Cox regression model was employed for the univariate and multivariate analyses of OS. Kaplan–Meier method was applied to estimate the probabilities of OS, and the log-rank test was utilized to compare the *P* values. Major molecular remission (MMR) was based on the *RUNX1-RUNX1T1* transcript level as previously described [12–14]. Statistical analyses were performed with SPSS 25.0 (IBM) and GraphPad Prism 6.0.

Results

Gene expression in t(8;21) AML

The previous scRNA-seq data of nine patients with t(8;21) AML [8] were analyzed. GSEA results revealed that

$CD34^{+}CD117^{dim}$ cells had highly expressed genes enriched in the epithelia-mesenchymal transition (EMT), apical junction, IL6-JAK-STAT3 signaling, and TNFA signaling via the NF-κB pathways. The genes associated with DNA repair and G2M checkpoint were activated in the $CD34^{+}CD117^{bri}$ population (Fig. 1A). Given that the genes related to cell adhesion and EMT pathways were highly expressed in the $CD34^{+}CD117^{dim}$ population, the genes participating in these pathways were further evaluated. The results showed that *CRIP1*, *LGALS1*, and *EMP3* associated with cell adhesion and EMT were overexpressed in the $CD34^{+}CD117^{dim}$ population, with significant *P* values ($P < 0.0001$, Fig. 1B). Thus, these genes were selected as candidate genes in the following study. The highly expressed genes in $CD34^{+}CD117^{bri}$ cells with significant *P* values were also analyzed. *PLAC8*, *TRH*, and *IGLL1* (Fig. 1B) were selected for subsequent study because they could be specifically quantified by the qRT-PCR primers. The RNA-seq data obtained from the bone marrow mononuclear cell (BMMC) samples of 62 patients with t(8;21) AML also confirmed that *CRIP1*, *LGALS1*, and *EMP3* were overexpressed in patients with a high proportion of $CD34^{+}CD117^{dim}$ cells, and *PLAC8, TRH*, and *IGLL1* were highly expressed in those with a high proportion of $CD34^{+}CD117^{bri}$ cells (Fig. 1C). In our previous study, $CD34^{+}CD117^{dim}$ ratio was found to be associated with clinical outcome, and patients with a high proportion of $CD34^{+}CD117^{dim}$ or $CD34^{+}CD117^{bri}$ populations present poor or favorable clinical outcomes, respectively. Hence, whether the highly expressed genes in the $CD34^{+}CD117^{dim}$ (*LGALS1, CRIP1*, and *EMP3*) or $CD34^{+}CD117^{bri}$ (*TRH, PLAC8*, and *IGLL1*) population may be related to inferior or favorable prognoses, respectively, in t(8;21) AML was determined in the present work.

Gene expression in AML

The expression levels of *LGALS1*, *CRIP1*, *EMP3*, *TRH*, *PLAC8*, and *IGLL1* in BMMC samples from different AML subtypes, including M0–M7 in the FAB nomenclature, were compared using the bulk RNA-seq data from the TCGA AML project. *TRH* and *IGLL1* levels in t(8;21) AML were significantly higher than their mean expression in other AML subtypes, whereas *LGALS1* expression in patients with t(8;21) AML was lower than its mean value in other AML subtypes. However, all AML subtypes presented high expression levels for all the six genes, with most of their $\log_2$(FPKM+1) value exceeding 10 (Fig. 2).

The expression levels of *LGALS1*, *EMP3*, *PLAC8*, *TRH, IGLL1*, and *CRIP1* in BMMC samples from 85 patients with t(8;21) AML and 21 healthy donors from our cohort were compared using qRT-PCR. The clinical characteristics of these 85 patients are summarized in Table 1. The expression levels of *LGALS1*, *CRIP1*, *TRH*, and *IGLL1* in patients with t(8;21) AML were significantly higher than those in the healthy donors (Fig. 3A). Although *EMP3* and *PLAC8* levels were higher in patients with t(8;21) AML than in normal controls, the differences did not reach statistical significance (Fig. 3A).

Clinical features and outcomes related to gene expression in AML

The clinical relevance of the expression levels of *LGALS1*, *CRIP1*, *EMP3*, *PLAC8*, *TRH,* and *IGLL1* were further analyzed. For the 85 patients with t(8;21) AML in our cohort, the median relative expression levels of *LGALS1*, *CRIP1*, *EMP3*, *PLAC8*, *TRH*, and *IGLL1* to *GAPDH* were 0.1358, 0.0434, 0.082, 0.1039, 0.0691, and 0.06, respectively. The patients were further classified into high and low groups according to the median values of these genes. A nearly bimodal distribution was achieved (Fig. 3B); thus, the median values were defined as the cutoff values. The clinical features of the high and low groups for each gene were compared (Table 2). Patients with high *CRIP1* expression harbored more marrow blasts ($P = 0.004$) than those with low expression. They also had more cytogenetic abnormalities other than t(8;21) ($P = 0.016$) and tended to lose the sex chromosome ($P = 0.001$) compared with those with low *CRIP1* expression. The expression levels of *CRIP1* in patients with other abnormal karyotypes were higher than those in patients with t(8;21), except for those with t(15;17) in TCGA database (Fig. S1). This finding confirmed our result from another aspect. The high *EMP3* expression group had high blood platelet counts at diagnosis ($P = 0.047$). *FLT3*-ITD mutation and *PML-RARA* fusion transcript are correlated with low blood platelet counts [15–17], leading to poor prognosis in acute promyelocytic leukemia. However, whether blood platelet count is associated with clinical outcome in t(8;21) AML remains unknown [12,18–20]. Patients with high *IGLL1* expression tended to have a high median age ($P = 0.001$) and more myeloblasts ($P = 0.005$), whereas those with low *PLAC8* expression tended to have *KIT* gene mutations ($P = 0.017$). No significant difference in gender, median white blood count, median hemoglobin, AM cell proportion, induction cycles, and relapse rate was observed among the different expression groups (Table 2).

The clinical outcomes of 85 patients with t(8;21) AML in the cohort were further analyzed. Patients with high expression levels of *LGALS1*, *EMP3*, and *CRIP1* presented significantly worse OS than those with low gene expression levels ($P = 0.0146$, $P = 0.0007$, and $P = 0.0354$, respectively). The high *TRH* and *PLAC8* expression groups showed more favorable prognosis than the low expression groups ($P = 0.0126$ and $P = 0.0059$, respectively, Fig. 4). The gene expression combination could further stratify these patients into two groups with significant differences (Fig. S2). Poor relapse-free survival

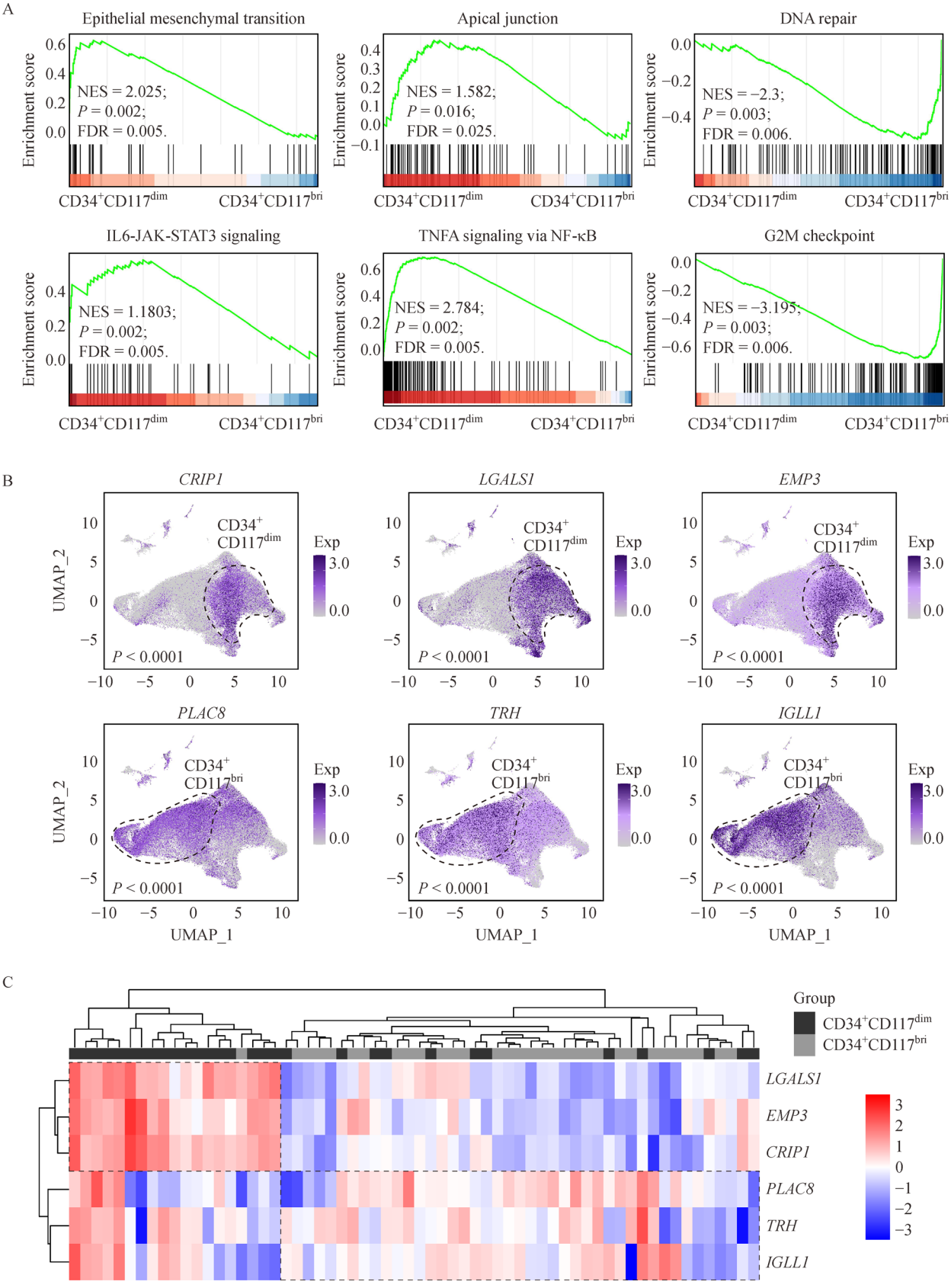

Fig. 1 GSEA and expression of highly expressed genes in the $CD34^{+}CD117^{dim}$ and $CD34^{+}CD117^{bri}$ populations. (A) Representative GSEA plots showing the activated pathways in the $CD34^{+}CD117^{dim}$ and $CD34^{+}CD117^{bri}$ populations. Normalized enrichment score (NES), nominal *P* value, and false discovery rate (FDR) value are given. (B) Uniform manifold approximation and projection (UMAP) plots displaying the expression patterns of the highly expressed genes in the $CD34^{+}CD117^{dim}$ and $CD34^{+}CD117^{bri}$ populations from nine patients with primary t(8;21) AML after batch effects were removed. Each dot represents a cell, and the purple color indicates cells expressing the marker genes. Statistical significance was determined using a two-sided Wilcoxon test. (C) Hierarchical cluster analysis using six differentially expressed genes in the $CD34^{+}CD117^{dim}$ and $CD34^{+}CD117^{bri}$ populations in 62 patients with t(8;21) AML. The relative expression levels of genes (rows) among patients (columns) are shown.

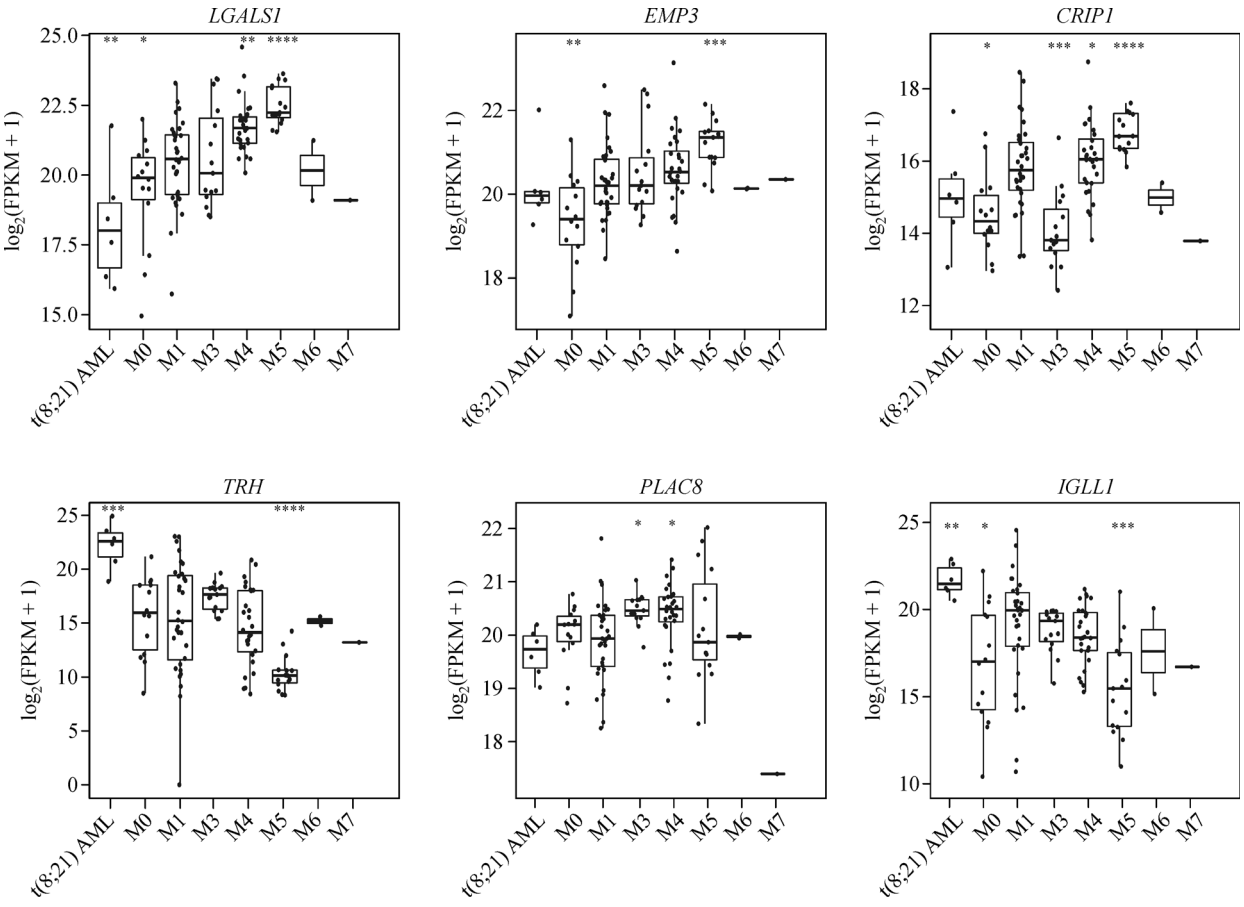

Fig. 2 Gene expression levels of *LGALS1*, *EMP3*, *PLAC8*, *TRH*, *IGLL1*, and *CRIP1* in BMMC samples from patients with each AML subtype, including M0–M7 in the FAB nomenclature. t(8;21) AML represents the M2b subtype because most of the t(8;21) AML cases belong to this subtype. The gene expression of each AML subtype was compared with the mean value of the gene expression in other AML subtypes. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$. Statistical significance was determined using two-sided Wilcoxon test.

Table 1 Clinical characteristics of 85 patients with t(8;21) AML

Characteristics	Number (%) or median (range)
Age, median (range) (year)	40 (15–74)
Gender, male/female	46/39
WBC, median (range) ($\times 10^9$/L)	9.56 (0.80–94.6)
HGB, median (range) (g/L)	71.0 (41.0–116.0)
PLT, median (range) ($\times 10^9$/L)	32.0 (4.0–132.0)
Marrow blasts, median (range) (%)	45.5 (9.0–92.0)
AM, median (range) (%)	12.0 (0.0–45.0)
Karyotype[a]	
t(8;21) alone/all patients	26/78
Loss of X or Y chromosome/all patients	40/78
Molecular mutations	
KIT mutation/all patients	28/85
Induction cycle to attain CR	
1 cycle/> 1 cycle	75/10
HSCT/Chemotherapy	16/85

[a]78 patients were available for cytogenetic analysis.

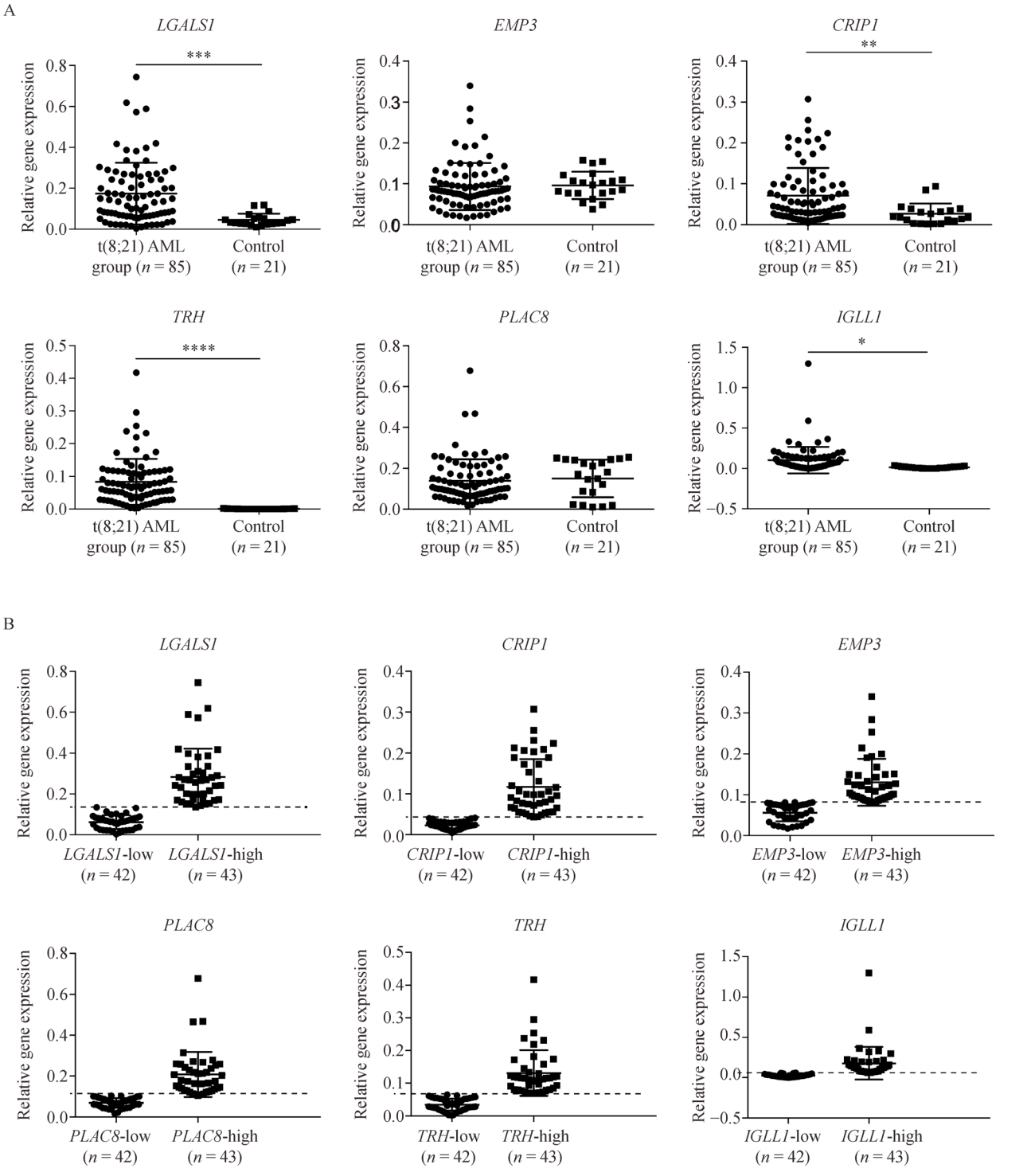

Fig. 3 Relative quantification of *LGALS1*, *EMP3*, *PLAC8*, *TRH*, *IGLL1*, and *CRIP1* expression. (A) Relative expression levels of *LGALS1*, *EMP3*, *PLAC8*, *TRH*, *IGLL1*, and *CRIP1* in BMMC samples from patients with t(8;21) AML and healthy donors. (B) Relative expression levels of *LGALS1*, *EMP3*, *PLAC8*, *TRH*, *IGLL1*, and *CRIP1* in BMMC samples from 85 patients with t(8;21) AML. The cutoff value of each gene is shown as a dashed line. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$. Statistical significance was determined using two-sided Student's *t* test.

was observed for patients with high *LGALS1* expression and those with low *TRH* and *PLAC8* expression ($P = 0.0323$, $P = 0.0296$, and $P = 0.006$, respectively, Fig. S3). Data from TCGA AML project confirmed these results and showed that patients with high expression levels of *LGALS1*, *EMP3*, and *CRIP1* had poor clinical outcome

Table 2 Gene expression and patient clinical characteristics

Gene expression	Age (year, median (range))	Male/female (*n*/*n*)	WBC count ($\times 10^9$/L, median (range))	HGB (g/L, median (range))	PLT ($\times 10^9$/L, median (range))	Marrow blasts (%, median (range))	AM (%, median (range))	Karyotype		*KIT* mutation /all patients	Induction cycle to attain CR (1 cycle/>1 cycle)	Relapse[b] /CR
								t(8;21) alone/all	Loss of X or Y chromosome /all			
CRIP1												
Low ($n = 42$)	40.0 (17–67)	19/23	9.02 (1.4–66.0)	72.50 (45.0–116.0)	26.50 (4.0–94.0)	35.35 (9.0–85.5)	12.75 (1.5–39.0)	17/36	11/36	14/42	38/4	18/39
High ($n = 43$)	41.0 (15–74)	27/16	10.8 (0.80–94.6)	70.0 (41.0–111.0)	33.0 (9.0–132.0)	51.5 (21.5–92.0)	10.0 (0.0–45.0)	9/42	29/42	14/43	37/6	22/41
P	0.981	0.104	0.191	0.644	0.689	0.004	0.341	0.016	0.001	0.939	0.766[a]	0.502
LGALS1												
Low ($n = 42$)	40 (17–65)	24/18	10.17 (0.80–66.0)	77.5 (43.0–116.0)	32.50 (4.0–132.0)	43.0 (9.0–92.0)	14.25 (0.0–45.0)	13/40	22/40	16/42	38/4	16/39
High ($n = 43$)	41 (15–74)	22/21	9.56 (1.4–94.6)	67.0 (41.0–110.0)	27.0 (8.0–94.0)	49.0 (22.0–82.0)	10.0 (1.0–39.0)	13/38	18/38	12/43	37/6	24/41
P	0.623	0.580	0.559	0.093	0.712	0.124	0.135	0.873	0.500	0.318	0.766[a]	0.117
EMP3												
Low ($n = 42$)	43.50 (17–67)	22/20	10.18 (2.4–66.0)	72.50 (41.0–116.0)	23.50 (4.0–73.0)	41.75 (9.0–92.0)	13.25 (0.0–45.0)	16/38	16/38	11/42	36/6	18/39
High ($n = 43$)	40.0 (15–74)	24/19	9.54 (0.80–94.6)	70.0 (49.0–111.0)	35.0 (6.0–132.0)	49.0 (12.0–82.0)	10.0 (1.0–38.0)	10/40	24/40	17/43	39/4	22/41
P	0.524	0.751	0.970	0.998	0.047	0.380	0.141	0.109	0.114	0.191	0.707[a]	0.502
TRH												
Low ($n = 42$)	43.50 (17–67)	22/20	12.55 (0.8–94.6)	64.50 (41.0–116.0)	23.5 (4.0–132.0)	38.75 (9.0–92.0)	13.0 (0.0–38.0)	16/41	19/41	15/42	37/5	23/40
High ($n = 43$)	40.0 (15–74)	24/19	8.9 (1.4–82.0)	75.0 (43.0–116.0)	34.0 (8.0–55.0)	51.5 (13.0–86.5)	12.0 (1.5–45.0)	10/37	21/37	13/43	38/5	17/40
P	0.195	0.751	0.058	0.284	0.616	0.123	0.754	0.262	0.358	0.591	1.000[a]	0.180
PLAC8												
Low ($n = 42$)	41.0 (17–65)	22/20	12.43 (1.4–94.6)	68.0 (41.0–113.0)	26.5 (6.0–132.0)	43.75 (9.0–86.5)	10.00 (1.0–38.0)	14/37	17/37	19/42	39/3	24/40
High ($n = 43$)	40.0 (15–74)	24/19	9.17 (0.8–52.8)	76.0 (42.0–116.0)	33.0 (4.0–73.0)	47.0 (13.0–92.0)	13.0 (0.0–45.0)	12/41	23/41	9/43	36/7	16/40
P	0.735	0.751	0.086	0.350	0.839	0.542	0.667	0.423	0.370	0.017	0.332[a]	0.074
IGLL1												
Low ($n = 42$)	36 (17–65)	22/20	12.67 (0.8–82.0)	69.5 (42.0–116.0)	22.0 (4.0–132.0)	40.25 (9.0–78.0)	14.0 (1.0–45.0)	14/39	22/39	13/42	40/2	18/41
High ($n = 43$)	47 (15–74)	24/19	8.9 (1.4–94.6)	73.0 (41.0–116.0)	33.0 (6.0–94.0)	55.5 (21.5–92.0)	10.0 (0.0–39.0)	12/39	18/39	15/43	35/8	22/39
P	0.001	0.751	0.115	0.967	0.347	0.005	0.076	0.631	0.365	0.700	0.100[a]	0.263

[a]Induction cycle was compared and tested using continuity correction. The remaining binary variables were tested by Pearson's Chi-square.
[b]Among the 85 patients, 5 failed to achieve CR.

(P = 0.00026, P = 0.033, and P = 0.08, respectively), whereas those with high expression levels of *TRH* and *PLAC8* presented favorable outcomes (P = 0.00013 and P = 0.0014, respectively, Fig. S4). Given the significantly higher *LGALS1* expression in patients with other AML subtypes than in patients with t(8;21) AML, the relationship of *LGALS1* expression and prognosis in patients with other AML subtypes was also examined by using the data from TCGA database. The result showed that patients with high *LGALS1* expression presented significantly poor OS in other AML subtypes (P = 0.00017, Fig. S5).

Univariate and multivariate analyses were performed to assess whether *LGALS1*, *CRIP1*, *EMP3*, *PLAC8*, *TRH,* and *IGLL1* expression levels are independent prognostic factors associated with OS (Table 3). The results of univariate analysis showed the association of OS with CD19 positive rate (HR, 0.400; 95% CI, 0.191 – 0.836; P = 0.015), $CD34^{+}CD117^{dim}$ proportion (HR, 2.444; 95% CI, 1.059 – 5.639; P = 0.036), *KIT* mutation (HR, 3.340; 95% CI, 1.602 – 6.964; P = 0.001), MRD status (HR, 0.356; 95% CI, 0.147 – 0.861; P = 0.022), *LGALS1* expression (HR, 2.579; 95% CI, 1.165 – 5.707; P = 0.019), *CRIP1* expression (HR, 2.188; 95% CI, 1.027 – 4.659; P = 0.042), *EMP3* expression (HR, 3.710; 95% CI, 1.637 – 8.412; P = 0.002), *PLAC8* expression (HR, 0.341; 95% CI, 0.153 – 0.756; P = 0.008), and *TRH* expression (HR, 0.379; 95% CI, 0.173 – 0.830; P = 0.015). The results of multivariate analysis revealed that *KIT* mutation (HR, 3.926; 95% CI, 1.635 – 9.427; P = 0.002), MRD status (HR, 0.286; 95% CI, 0.108 – 0.758; P = 0.012), *CRIP1* expression (HR, 2.651; 95% CI, 1.017 – 6.907; P = 0.046), and *TRH* expression (HR, 0.237; 95% CI, 0.093 – 0.602; P = 0.002) remained independent prognostic factors for OS, thus suggesting their important roles. However, no statistically significant difference was found in the $CD34^{+}CD117^{dim}$ proportion and expression of *CRIP1* and *PLAC8*. This finding could be attributed to the small sample size. Further studies are thus required to confirm these results.

Correlation between gene expression and mutation in gene families

The relationship between gene expression and mutation in gene families obtained from the previous 62 RNA-seq data was analyzed. Patients with high *PLAC8* expression harbored fewer gene mutations in the RTK/Ras family (*KIT*, *NRAS*, *FLT3*, *JAK2*, and *KRAS*) than those with low expression (40.6% vs. 66.7%, P = 0.046). Patients with high *IGLL1* expression had fewer gene mutations in epigenetic modifiers (*ASXL2*, *TET2*, *ARID2*, *ASXL1*, *KDM6A*, *KMT2D*, *EZH2*, *IDH2*, and *JMJD1C*) than those with low expression levels (26.7% vs. 55.2%, P = 0.026). However, no significant difference in the RTK/Ras family, epigenetic modifiers, transcriptional factors, cohesion complex or signaling pathways for *LGALS1*, *EMP3*, *CRIP1*, and *TRH* was observed between the high and low expression groups (Table 4).

Discussion

In this study, scRNA-seq and bulk RNA-seq data from our previous work [8] were used and qRT-PCR was performed

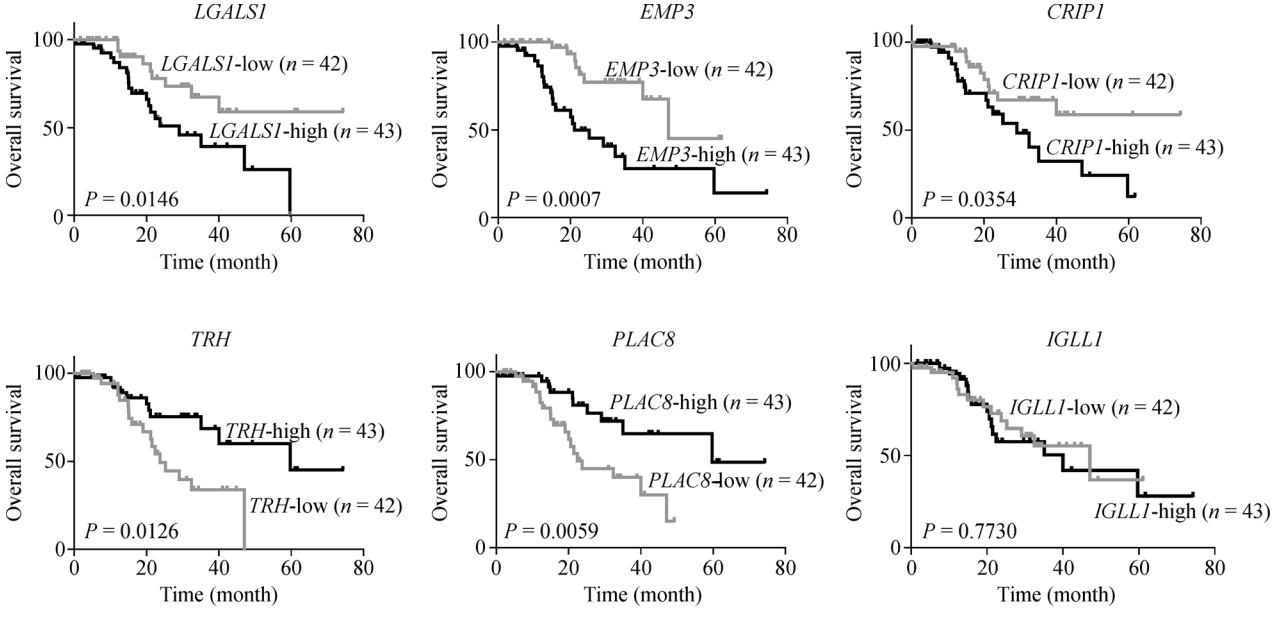

Fig. 4 Comparison of overall survival between patients with high and low gene expression levels. Survival curves were estimated using the Kaplan–Meier method and compared using the log-rank test.

Table 3 Univariate and multivariate analyses for the clinical characteristics of OS in 85 patients with t(8;21) AML

Factor	OS			
	Univariate		Multivariate	
	HR (95% CI)	*P* value	HR (95% CI)	*P* value
Age (year)	1.019 (0.994–1.044)	0.138		
Gender (male vs. female)	0.730 (0.351–1.521)	0.401		
Marrow blasts (%)	1.011 (0.992–1.031)	0.249		
AM (%)	0.986 (0.954–1.020)	0.421		
WBC ($\times 10^9$/L)	1.015 (0.996–1.035)	0.115		
PLT ($\times 10^9$/L)	1.001 (0.985–1.016)	0.944		
CD56 (positive/negative)	1.797 (0.729–4.428)	0.203		
CD19 (positive/negative)	0.400 (0.191–0.836)	0.015		NS
t(8;21) (alone vs. additional change)	1.267 (0.577–2.783)	0.555		
Loss of sex chromosomes	0.694 (0.328–1.471)	0.341		
$CD34^+CD117^{dim}$% (High vs. low)	2.444 (1.059–5.639)	0.036		NS
KIT mutation	3.340 (1.602–6.964)	0.001	3.926 (1.635–9.427)	0.002
LGALS1 expression	2.579 (1.165–5.707)	0.019		NS
EMP3 expression	3.710 (1.637–8.412)	0.002	2.222 (0.866–5.702)	0.097
CRIP1 expression	2.188 (1.027–4.659)	0.042	2.651 (1.017–6.907)	0.046
TRH expression	0.379 (0.173–0.830)	0.015	0.237 (0.093-0.602)	0.002
PLAC8 expression	0.341 (0.153–0.756)	0.008		NS
IGLL1 expression	1.123 (0.539–2.338)	0.757		
Minimal residual disease (achieving MMR vs. not achieving MMR)	0.356 (0.147–0.861)	0.022	0.286 (0.108-0.758)	0.012
Transplant status	0.413 (0.140–1.216)	0.109		

Factors with $P < 0.10$ in the univariate analyses were subjected to multivariate analysis. Major molecular remission (MMR) was based on the *RUNX1-RUNX1T1* transcript level.

to investigate the gene expression levels of the highly expressed genes in $CD34^+CD117^{dim}$ and $CD34^+CD117^{bri}$ cell populations in t(8;21) AML. The clinical characteristics of these genes and the related clinical outcomes were also analyzed.

scRNA-seq data revealed that *EMP3*, *CRIP1*, and *LGALS1* were highly expressed in $CD34^+CD117^{dim}$ cells. *EMP3* belongs to the peripheral myelin protein 22-kDa (PMP22) gene family, is overexpressed in breast cancer, and is related to high *HER-2* expression. *HER-2* and *EMP3* co-expression is the most important indicator of clinical outcome for patients with urothelial carcinoma. Besides, high *EMP3* expression is related to poor prognosis in several tumors [21]. In this work, patients with high *EMP3* expression tended to have relatively high blood platelet counts at diagnosis. This finding was reminiscent of a recent report, which showed that early platelet recovery time was an independent prognostic factor in AML [22], and thus suggested the importance of monitoring the recovery of platelet throughout the therapy. Thus, the relationship between gene expression and platelet recovery time deserves further study. *CRIP1* functions as an intracellular zinc transport protein and acts as an oncogene in regulating migration and invasion through excessive zinc-induced EMT in colorectal cancer [23]. This protein is an independent prognostic marker with remarkable predictive power in several solid tumors [24–26]. *LGALS1* (galectin 1) is a glycan that binds β-galactoside and a wide array of complex carbohydrates. This protein is upregulated in several types of cancer cells, and its abnormal expression is linked to the development, progression, and metastasis of cancers [27]. Human Cell Landscape database showed that *LGALS1* and *EMP3* are expressed in stromal cell populations (Fig. S6). All the above genes are involved in EMT that is associated with several pathways, such as TGF-β, NF-κB, and PI3K-AKT signaling pathways [28–30]. During this process, epithelial cells acquire migratory and stem-like traits that lead them to be tumorigenic and malignant [31]. The clinical importance of EMT in solid tumors is well acknowledged, and EMT signatures could be used as indicators of poor clinical outcomes in various solid tumors [28,30–35]. However, the role of EMT in hematological malignancies is poorly understood. A study using an inducible transgenic mouse model of MLL-AF9-driven leukemia reported that EMT-associated genes are linked to poor prognosis in AML [36]. The present work confirmed this observation and showed that high *EMP3*, *CRIP1,* and *LGALS1* expression was significantly associated with poor OS in t(8;21) AML. The external cohort of the TCGA

Table 4 Correlation between gene expression levels and mutation in gene families[a]

Group	RTK/Ras mutation /all	Epigenetic mutation /all	TF mutation/all	Cohesin complex mutation/all	Signaling mutation /all
LGALS1	31/59	24/59	11/59	10/59	7/59
Low (n = 42)	16/30	15/30	6/30	3/30	5/30
High (n = 43)	15/29	9/29	5/29	7/29	2/29
P	0.902	0.138	0.786	0.271[b]	0.424[c]
EMP3	31/59	24/59	11/59	10/59	7/59
Low (n = 42)	15/30	15/30	4/30	5/30	5/30
High (n = 43)	16/29	9/29	7/29	5/29	2/29
P	0.691	0.138	0.287	1.000[b]	0.424[c]
CRIP1	31/59	24/59	11/59	10/59	7/59
Low (n = 42)	13/28	15/28	4/28	5/28	3/28
High (n = 43)	18/31	9/31	7/31	5/31	4/31
P	0.371	0.055	0.414	1.000[b]	1.000[c]
TRH	31/59	24/59	11/59	10/59	7/59
Low (n = 42)	17/29	15/29	5/29	7/29	6/29
High (n = 43)	14/30	9/30	6/30	3/30	1/30
P	0.358	0.089	0.786	0.271[b]	0.052[c]
PLAC8	31/59	24/59	11/59	10/59	7/59
Low (n = 42)	18/27	13/27	4/27	5/27	1/27
High (n = 43)	13/32	11/32	7/32	5/32	6/32
P	0.046	0.283	0.488	1.000[b]	0.112[c]
IGLL1	31/59	24/59	11/59	10/59	7/59
Low (n = 42)	18/29	16/29	6/29	5/29	6/29
High (n = 43)	13/30	8/30	5/30	5/30	1/30
P	0.150	0.026	0.692	1.000[b]	0.052[c]

[a]85 patients with t(8;21) AML were grouped into high and low groups according to the expression levels of each gene as measured by qRT-PCR. Gene mutation was analyzed in 62 patients with t(8;21) AML and previous RNA-seq data. Among the 85 patients, 59 had previous RNA-seq data.
[b]Cohesin complex mutation was compared and tested using continuity correction.
[c]Signaling mutation was compared and tested using Fisher's Exact Test.
The remaining binary variables were tested by Pearson's Chi-square.

AML project also confirmed our findings. However, these results should be validated in a large sample size. Further investigations are also needed to confirm the function of these genes in t(8;21) AML.

TRH and *IGLL1* were highly expressed in the $CD34^{+}CD117^{bri}$ population, and their expression levels in t(8;21) AML were significantly higher than those in other AML subtypes. This finding was consistent with previous studies [37,38] showing that *TRH* expression is significantly higher in t(8;21) AML than in inv(16) AML. However, the functions of these two genes in AML remain poorly understood. One recent scRNA-seq study on primary glioblastoma showed that high *TRH* expression is inversely correlated with tumor invasion and may be related to cell proliferation [39]. This result was consistent with our finding that cell cycle pathways were activated in the $CD34^{+}CD117^{bri}$ population. Another work on epigenetic and genetic heterogeneity in AML also revealed that high *IGLL1* expression is correlated with cell cycle and DNA repair [40]. Given that patients with a high proportion of $CD34^{+}CD117^{bri}$ cells presented favorable clinical outcomes, those with high expression levels of these two genes may have a good prognosis. t(8;21) AML is considered a favorable AML subtype. Whether high *TRH* and *IGLL1* expression could be used as a candidate prognostic marker in AML deserves further study.

Correlations between gene expression and genetic alterations were also analyzed. Although the result was preliminary, patients with low *PLAC8* expression tended to have more gene mutations in the RTK/Ras family. *PLAC8* is a conserved cysteine-rich protein that plays an important role in normal cellular processes and various diseases, and this action is highly reliant on cellular and physiological contexts [41,42]. Although *PLAC8* overexpression is associated with poor prognosis in several solid tumors [41–43], it was found to be related to favorable clinical outcomes in t(8;21) AML in this study. Whether the prognostic effect of high *PLAC8* expression is affected by gene mutations in the RTK/Ras family or whether these genes could regulate *PLAC8* expression needs further investigation.

Summary

This study is the continuation of our previous work. A correlation was found between the clinical outcome and the highly expressed genes identified in different cell populations. The results suggested that cell adhesion and EMT might play important roles in t(8;21) AML pathogenesis. Identifying new biomarkers may lead to the development of new tools for future tailored therapy in t(8;21) AML.

Acknowledgements

This study was supported by the National Natural Science Foundation of China (No. 81800147), Shanghai Sailing Program (No. 18YF1413700), the National Key Research and Development Plan of China (No. 2018YFA0107800), the National Natural Science Foundation of China (No. 81890994), the National Key Research and Development Plan of China (No. 2017YFA0506200), and the Innovative Research Team of High-level Local Universities in Shanghai.

Compliance with ethics guidelines

Xueping Li, Yuting Dai, Bing Chen, Jinyan Huang, Saijuan Chen, and Lu Jiang declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article. This study was approved by the Ruijin Hospital Review Board and was in accordance with the principle of the *Helsinki Declaration* II.

Electronic Supplementary Material Supplementary material is available in the online version of this article at https://doi.org/10.1007/s11684-021-0836-7 and is accessible for authorized users.

References

1. Döhner H, Weisdorf DJ, Bloomfield CD. Acute myeloid leukemia. N Engl J Med 2015; 373(12): 1136–1152
2. Wang YY, Zhou GB, Yin T, Chen B, Shi JY, Liang WX, Jin XL, You JH, Yang G, Shen ZX, Chen J, Xiong SM, Chen GQ, Xu F, Liu YW, Chen Z, Chen SJ. AML1-ETO and C-KIT mutation/overexpression in t(8;21) leukemia: implication in stepwise leukemogenesis and response to Gleevec. Proc Natl Acad Sci USA 2005; 102(4): 1104–1109
3. Döhner H, Estey E, Grimwade D, Amadori S, Appelbaum FR, Büchner T, Dombret H, Ebert BL, Fenaux P, Larson RA, Levine RL, Lo-Coco F, Naoe T, Niederwieser D, Ossenkoppele GJ, Sanz M, Sierra J, Tallman MS, Tien HF, Wei AH, Löwenberg B, Bloomfield CD. Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel. Blood 2017; 129(4): 424–447
4. Papaemmanuil E, Gerstung M, Bullinger L, Gaidzik VI, Paschka P, Roberts ND, Potter NE, Heuser M, Thol F, Bolli N, Gundem G, Van Loo P, Martincorena I, Ganly P, Mudie L, McLaren S, O'Meara S, Raine K, Jones DR, Teague JW, Butler AP, Greaves MF, Ganser A, Döhner K, Schlenk RF, Döhner H, Campbell PJ. Genomic classification and prognosis in acute myeloid leukemia. N Engl J Med 2016; 374(23): 2209–2221
5. Faber ZJ, Chen X, Gedman AL, Boggs K, Cheng J, Ma J, Radtke I, Chao JR, Walsh MP, Song G, Andersson AK, Dang J, Dong L, Liu Y, Huether R, Cai Z, Mulder H, Wu G, Edmonson M, Rusch M, Qu C, Li Y, Vadodaria B, Wang J, Hedlund E, Cao X, Yergeau D, Nakitandwe J, Pounds SB, Shurtleff S, Fulton RS, Fulton LL, Easton J, Parganas E, Pui CH, Rubnitz JE, Ding L, Mardis ER, Wilson RK, Gruber TA, Mullighan CG, Schlenk RF, Paschka P, Döhner K, Döhner H, Bullinger L, Zhang J, Klco JM, Downing JR. The genomic landscape of core-binding factor acute myeloid leukemias. Nat Genet 2016; 48(12): 1551–1556
6. Yushu H, Shougeng B, Zhijian X, Yingchang M, Chao HZ. Acute myeloid leukemia M2b. Haematologica 1999; 84(3): 193–194
7. Grimwade D, Hills RK, Moorman AV, Walker H, Chatters S, Goldstone AH, Wheatley K, Harrison CJ, Burnett AK; the National Cancer Research Institute Adult Leukaemia Working Group. Refinement of cytogenetic classification in acute myeloid leukemia: determination of prognostic significance of rare recurring chromosomal abnormalities among 5876 younger adult patients treated in the United Kingdom Medical Research Council trials. Blood 2010; 116(3): 354–365
8. Jiang L, Li XP, Dai YT, Chen B, Weng XQ, Xiong SM, Zhang M, Huang JY, Chen Z, Chen SJ. Multidimensional study of the heterogeneity of leukemia cells in t(8;21) acute myelogenous leukemia identifies the subtype with poor outcome. Proc Natl Acad Sci USA 2020; 117(33): 20117–20126
9. Yu G, Wang LG, Han Y, He QY. clusterProfiler: an R package for comparing biological themes among gene clusters. OMICS 2012; 16 (5): 284–287
10. The Cancer Genome Atlas Research Network. Genomic and epigenomic landscapes of adult *de novo* acute myeloid leukemia. N Engl J Med 2013; 368(22): 2059–2074
11. Colaprico A, Silva TC, Olsen C, Garofano L, Cava C, Garolini D, Sabedot TS, Malta TM, Pagnotta SM, Castiglioni I, Ceccarelli M, Bontempi G, Noushmehr H. TCGAbiolinks: an R/Bioconductor package for integrative analysis of TCGA data. Nucleic Acids Res 2016; 44(8): e71
12. Zhu HH, Zhang XH, Qin YZ, Liu DH, Jiang H, Chen H, Jiang Q, Xu LP, Lu J, Han W, Bao L, Wang Y, Chen YH, Wang JZ, Wang FR, Lai YY, Chai JY, Wang LR, Liu YR, Liu KY, Jiang B, Huang XJ. MRD-directed risk stratification treatment may improve outcomes of t(8;21) AML in the first complete remission: results from the AML05 multicenter trial. Blood 2013; 121(20): 4056–4062
13. Schnittger S, Weisser M, Schoch C, Hiddemann W, Haferlach T, Kern W. New score predicting for prognosis in PML-RARA^{+}, AML1-ETO^{+}, or CBFBMYH11^{+} acute myeloid leukemia based on quantification of fusion transcripts. Blood 2003; 102(8): 2746–2755
14. Leroy H, de Botton S, Grardel-Duflos N, Darre S, Leleu X, Roumier C, Morschhauser F, Lai JL, Bauters F, Fenaux P, Preudhomme C. Prognostic value of real-time quantitative PCR (RQ-PCR) in AML with t(8;21). Leukemia 2005; 19(3): 367–372
15. Gale RE, Hills R, Pizzey AR, Kottaridis PD, Swirsky D, Gilkes AF, Nugent E, Mills KI, Wheatley K, Solomon E, Burnett AK, Linch DC, Grimwade D; the NCRI Adult Leukaemia Working Party.

Relationship between FLT3 mutation status, biologic characteristics, and response to targeted therapy in acute promyelocytic leukemia. Blood 2005; 106(12): 3768–3776

16. Noguera NI, Breccia M, Divona M, Diverio D, Costa V, De Santis S, Avvisati G, Pinazzi MB, Petti MC, Mandelli F, Lo Coco F. Alterations of the FLT3 gene in acute promyelocytic leukemia: association with diagnostic characteristics and analysis of clinical outcome in patients treated with the Italian AIDA protocol. Leukemia 2002; 16(11): 2185–2189
17. Kuchenbauer F, Schoch C, Kern W, Hiddemann W, Haferlach T, Schnittger S. Impact of FLT3 mutations and promyelocytic leukaemia-breakpoint on clinical characteristics and prognosis in acute promyelocytic leukaemia. Br J Haematol 2005; 130(2): 196–202
18. Jiao B, Wu CF, Liang Y, Chen HM, Xiong SM, Chen B, Shi JY, Wang YY, Wang JH, Chen Y, Li JM, Gu LJ, Tang JY, Shen ZX, Gu BW, Zhao WL, Chen Z, Chen SJ. AML1-ETO9a is correlated with C-KIT overexpression/mutations and indicates poor disease outcome in t(8;21) acute myeloid leukemia-M2. Leukemia 2009; 23(9): 1598–1604
19. Willekens C, Blanchet O, Renneville A, Cornillet-Lefebvre P, Pautas C, Guieze R, Ifrah N, Dombret H, Jourdan E, Preudhomme C, Boissel N. Prospective long-term minimal residual disease monitoring using RQ-PCR in RUNX1-RUNX1T1-positive acute myeloid leukemia: results of the French CBF-2006 trial. Haematologica 2016; 101(3): 328–335
20. Christen F, Hoyer K, Yoshida K, Hou HA, Waldhueter N, Heuser M, Hills RK, Chan W, Hablesreiter R, Blau O, Ochi Y, Klement P, Chou WC, Blau IW, Tang JL, Zemojtel T, Shiraishi Y, Shiozawa Y, Thol F, Ganser A, Löwenberg B, Linch DC, Bullinger L, Valk PJM, Tien HF, Gale RE, Ogawa S, Damm F. Genomic landscape and clonal evolution of acute myeloid leukemia with t(8;21): an international study on 331 patients. Blood 2019; 133(10): 1140–1151
21. Wang YW, Cheng HL, Ding YR, Chou LH, Chow NH. EMP1, EMP 2, and EMP3 as novel therapeutic targets in human cancer. Biochim Biophys Acta Rev Cancer 2017; 1868(1): 199–211
22. Çiftçiler R, Haznedaroğlu IC, Sayınalp N, Özcebe O, Aksu S, Demiroğlu H, Göker H, Malkan UY, Büyükaşık Y. The impact of early versus late platelet and neutrophil recovery after induction chemotherapy on survival outcomes of patients with acute myeloid leukemia. Turk J Haematol 2020; 37(2): 116–120
23. He G, Zhu H, Yao Y, Chai H, Wang Y, Zhao W, Fu S, Wang Y. Cysteine-rich intestinal protein 1 silencing alleviates the migration and invasive capability enhancement induced by excessive zinc supplementation in colorectal cancer cells. Am J Transl Res 2019; 11(6): 3578–3588
24. Li HG, Zhao LH, Zhang ZH, Liu JZ, Ren K, Li SY, Su ZJ. The impact of cysteine-rich intestinal protein 1 (CRIP1) on thyroid carcinoma. Cell Physiol Biochem 2017; 43(5): 2037–2046
25. Zhang L, Zhou R, Zhang W, Yao X, Li W, Xu L, Sun X, Zhao L. Cysteine-rich intestinal protein 1 suppresses apoptosis and chemosensitivity to 5-fluorouracil in colorectal cancer through ubiquitin-mediated Fas degradation. J Exp Clin Cancer Res 2019; 38(1): 120
26. Ye C, Ma S, Xia B, Zheng C. Weighted gene coexpression network analysis identifies cysteine-rich intestinal protein 1 (CRIP1) as a prognostic gene associated with relapse in patients with acute myeloid leukemia. Med Sci Monit 2019; 25: 7396–7406
27. Chou FC, Chen HY, Kuo CC, Sytwu HK. Role of galectins in tumors and in clinical immunotherapy. Int J Mol Sci 2018; 19(2): E430
28. Feng Y, Ji D, Huang Y, Ji B, Zhang Y, Li J, Peng W, Zhang C, Zhang D, Sun Y, Xu Z. TGM3 functions as a tumor suppressor by repressing epithelialtomesenchymal transition and the PI3K/AKT signaling pathway in colorectal cancer. Oncol Rep 2020; 43(3): 864–876
29. Wang YS, Li H, Li Y, Zhang S, Jin YH. (20S)G-Rh2 inhibits NF-κB regulated epithelial-mesenchymal transition by targeting annexin A2. Biomolecules 2020; 10(4): E528
30. Schliekelman MJ, Taguchi A, Zhu J, Dai X, Rodriguez J, Celiktas M, Zhang Q, Chin A, Wong CH, Wang H, McFerrin L, Selamat SA, Yang C, Kroh EM, Garg KS, Behrens C, Gazdar AF, Laird-Offringa IA, Tewari M, Wistuba II, Thiery JP, Hanash SM. Molecular portraits of epithelial, mesenchymal, and hybrid states in lung adenocarcinoma and their relevance to survival. Cancer Res 2015; 75(9): 1789–1800
31. Mani SA, Guo W, Liao MJ, Eaton EN, Ayyanan A, Zhou AY, Brooks M, Reinhard F, Zhang CC, Shipitsin M, Campbell LL, Polyak K, Brisken C, Yang J, Weinberg RA. The epithelial-mesenchymal transition generates cells with properties of stem cells. Cell 2008; 133(4): 704–715
32. Rahimi M, Sharifi-Zarchi A, Firouzi J, Azimi M, Zarghami N, Alizadeh E, Ebrahimi M. An integrated analysis to predict microRNAs targeting both stemness and metastasis in breast cancer stem cells. J Cell Mol Med 2019; 23(4): 2442–2456
33. Byers LA, Diao L, Wang J, Saintigny P, Girard L, Peyton M, Shen L, Fan Y, Giri U, Tumula PK, Nilsson MB, Gudikote J, Tran H, Cardnell RJ, Bearss DJ, Warner SL, Foulks JM, Kanner SB, Gandhi V, Krett N, Rosen ST, Kim ES, Herbst RS, Blumenschein GR, Lee JJ, Lippman SM, Ang KK, Mills GB, Hong WK, Weinstein JN, Wistuba II, Coombes KR, Minna JD, Heymach JV. An epithelial-mesenchymal transition gene signature predicts resistance to EGFR and PI3K inhibitors and identifies Axl as a therapeutic target for overcoming EGFR inhibitor resistance. Clin Cancer Res 2013; 19 (1): 279–290
34. Tan TZ, Miow QH, Miki Y, Noda T, Mori S, Huang RY, Thiery JP. Epithelial-mesenchymal transition spectrum quantification and its efficacy in deciphering survival and drug responses of cancer patients. EMBO Mol Med 2014; 6(10): 1279–1293
35. Lamouille S, Xu J, Derynck R. Molecular mechanisms of epithelial-mesenchymal transition. Nat Rev Mol Cell Biol 2014; 15(3): 178–196
36. Stavropoulou V, Kaspar S, Brault L, Sanders MA, Juge S, Morettini S, Tzankov A, Iacovino M, Lau IJ, Milne TA, Royo H, Kyba M, Valk PJM, Peters AHFM, Schwaller J. MLL-AF9 expression in hematopoietic stem cells drives a highly invasive AML expressing EMT-related genes linked to poor outcome. Cancer Cell 2016; 30 (1): 43–58
37. Bullinger L, Rücker FG, Kurz S, Du J, Scholl C, Sander S, Corbacioglu A, Lottaz C, Krauter J, Fröhling S, Ganser A, Schlenk RF, Döhner K, Pollack JR, Döhner H. Gene-expression profiling identifies distinct subclasses of core binding factor acute myeloid leukemia. Blood 2007; 110(4): 1291–1300
38. Hsu CH, Nguyen C, Yan C, Ries RE, Chen QR, Hu Y, Ostronoff F,

Stirewalt DL, Komatsoulis G, Levy S, Meerzaman D, Meshinchi S. Transcriptome profiling of pediatric core binding factor AML. PLoS One 2015; 10(9): e0138782

39. Patel AP, Tirosh I, Trombetta JJ, Shalek AK, Gillespie SM, Wakimoto H, Cahill DP, Nahed BV, Curry WT, Martuza RL, Louis DN, Rozenblatt-Rosen O, Suvà ML, Regev A, Bernstein BE. Single-cell RNA-seq highlights intratumoral heterogeneity in primary glioblastoma. Science 2014; 344(6190): 1396–1401
40. Li S, Garrett-Bakelman FE, Chung SS, Sanders MA, Hricik T, Rapaport F, Patel J, Dillon R, Vijay P, Brown AL, Perl AE, Cannon J, Bullinger L, Luger S, Becker M, Lewis ID, To LB, Delwel R, Löwenberg B, Döhner H, Döhner K, Guzman ML, Hassane DC, Roboz GJ, Grimwade D, Valk PJ, D'Andrea RJ, Carroll M, Park CY, Neuberg D, Levine R, Melnick AM, Mason CE. Distinct evolution and dynamics of epigenetic and genetic heterogeneity in acute myeloid leukemia. Nat Med 2016; 22(7): 792–799
41. Jia Y, Ying X, Zhou J, Chen Y, Luo X, Xie S, Wang QC, Hu W, Wang L. The novel KLF4/PLAC8 signaling pathway regulates lung cancer growth. Cell Death Dis 2018; 9(6): 603
42. Zou L, Chai J, Gao Y, Guan J, Liu Q, Du JJ. Down-regulated PLAC8 promotes hepatocellular carcinoma cell proliferation by enhancing PI3K/Akt/GSK3β/Wnt/β-catenin signaling. Biomed Pharmacother 2016; 84: 139–146
43. Mao M, Chen Y, Jia Y, Yang J, Wei Q, Li Z, Chen L, Chen C, Wang L. PLCA8 suppresses breast cancer apoptosis by activating the PI3k/AKT/NF-κB pathway. J Cell Mol Med 2019; 23(10): 6930–6941