Fms-like Tyrosine Kinase (FLT) 3 and FLT3 Internal Tandem Duplication in Different Types of Adult Leukemia: Analysis of 147 Patients

Hong-Ling Peng[1], Guang-Sen Zhang[1], Fan-Jie Gong[1], Jian-Kai Shen[1], Yang Zhang[2], Yun-Xiao Xu[1], Wen-Li Zheng[1], Chong-Wen Dai[1], Min-Fei Pei[1], Jun-Jie Yang[1].

[1]Division of Hematology/Institute of Molecular Hematology, Second Xiang-Ya Hospital, Central South University, Changsha, Hunan, People's Republic of China
[2]Division of Oncology, Second Xiang-Ya Hospital, Central South University, Changsha, Hunan, People's Republic of China

Aim To assess the expression level of fms-like tyrosine kinase 3 *(FLT3),* the incidence of FLT3/internal tandem duplications *(ITD)* mutation, and prognostic value of *FLT3* changes in different types of adult leukemia.

Methods Bone marrow mononuclear cells were isolated from 147 adult patients with leukemia. Reverse transcriptase polymerase chain reaction (PCR) was used to screen *FLT3/ITD* mutation and quantitative PCR was performed to evaluate the expression of the *FLT3* transcript. Flow cytometry was used for detection of *FLT3* receptor protein expression on bone marrow mononuclear cells. Pearson correlation analysis was performed to estimate the significance of *FLT3*.

Results FLT3 expression was higher in acute myeloid leukemia and B-acute lymphoid leukemia than in T-acute lymphoid leukemia ($P = 0.006$, $P = 0.001$) and chronic myelogenous leukemia ($P < 0.001$). In chronic myelogenous leukemia, *FLT3* expression in blast transformation phase was higher than in acceleration phase ($P = 0.023$). Surface expression of *FLT3* protein was correlated with high percentage of bone marrow blasts and with *FLT3* mRNA expression ($r = 0.366$, $P < 0.001$) in acute leukemia. *FLT3/ITDs* in the juxtamembrane domain were found in 25% of patients with acute myeloid leukemia and 7% of patients with acute lymphoid leukemia. *FLT3/ITD* positive sequences had 36, 42, and 57 nucleotides. *FLT3/ITD* mutation was associated with a higher white blood cell count, higher marrow blast percentage, and elevated serum lactate dehydrogenase ($P = 0.045$, $P = 0.014$, $P < 0.001$, respectively) and not associated with a higher *FLT3* mRNA and FLT3 protein expression, and lower complete remission ($P = 0.091$, $P = 0.060$, $P = 0.270$, respectively).

Conclusion *FLT3* expression levels differed in different types of adult leukemia. Overexpression of *FLT3* and presence of a positive *FLT3/ITD* mutation in acute leukemia were associated with unfavorable clinical characteristics and poor prognosis.

> **Correspondence to:**
Guang-Sen Zhang
Division of Hematology, Institute of Molecular Hematology
Second Xiang-Ya Hospital, Central South University
Changsha, Hunan, People's Republic of China
zgsllzy@public.cs.hn.cn

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The fms-like tyrosine kinase 3 *(FLT3)* gene belongs to the class III receptor tyrosine kinases and is predominantly expressed on hematopoietic progenitor cells in the bone marrow, thymus, and lymph nodes (1). An abnormality in the *FLT3* gene is implicated in the pathogenesis of acute myeloid leukemia (2-4). Approximately 25% of patients with adult acute myeloid leukemia harbor internal tandem duplications (ITD) within the juxtamembrane domain of the *FLT3* gene (5,6). *FLT3/ITD*s cause structural changes in the juxtamembrane and this disrupts the autoinhibitory conformation of the receptor (7) by promoting constitutive activation of both receptor (8-10) and downstream effectors, which all leads to a bad prognosis (11,12). In the last decade, *FLT3/ITD* mutations have been reported in 13%-32% of adult patients with acute myeloid leukemia and in a small number of patients with acute lymphoid leukemia (13-16). Patients with this abnormality have increased incidence of leukocytosis and decreased overall survival in comparison with patients without this abnormality. These findings indicate that *FLT3/ITD*s not only play an important role in the pathogenesis mechanism of leukemia but also have a prognostic value.

A previous study has demonstrated that high levels of *FLT3* were expressed in leukemia and lymphoma cell lines including pre-B, myeloid, and monocytic cell lines (17). Also, several studies have shown that high levels of *FLT3* were expressed in 70%-100% of patients with acute myeloid leukemia and B-acute lymphoid leukemia and in about 30% of patients with T-acute lymphoid leukemia (18,19). Likewise, a small number of chronic myelogenous leukemia blast crisis and chronic lymphocytic leukemia cells has been shown to express *FLT3* (18,20). These data indicate that FLT3 expression may play a role in the survival or proliferation of leukemic blasts. Using Western blotting, Carow et al (18) found no FLT3 expression in the normal bone marrow, but identified FLT3 protein in 14 of 14 B-cell acute lymphoid leukemia cases, 36 of 41 acute myeloid leukemia cases, and 1 of 4 T-cell acute lymphoid leukemia cases. Though *FLT3* expression in leukemia and its clinical significance have been widely investigated, little is known about *FLT3* expression level and its clinical significance in Chinese patients with adult leukemia. Most of the studies have used Western blot assay as *FLT3* protein assay, whereas flow cytometry on intact leukemic cell surface has been rarely used. We used flow cytometry on cell surface to investigate the expression of *FLT3* receptor and quantitative polymerase chain reaction (PCR) to investigate *FLT3* mRNA expression, as well as performed identification of *FLT3/ITD*s in different types of adult leukemia.

Patients and methods

The study included 120 patients with newly diagnosed acute leukemia – 60 with acute myeloid leukemia, 30 with B-acute lymphoid leukemia, 30 with T-acute lymphoid leukemia; 27 with chronic myeloid leukemia; and 30 controls (Table 1). Diagnosis was based on May-Grunwald-Giemsa-stained bone marrow smears and cytochemistry performed according to the French-American-British (FAB) group criteria (21). Leukocyte differentiation antigens were analyzed by immunofluorescent method for some cases. Complete remission was defined as normocellular bone marrow containing less than 5% blasts and showing evidence of normal maturation of other bone marrow elements. Patients and controls provided informed consent to use their samples for this study.

mRNA expression analysis

Patients and controls' bone marrow mononuclear cells were separated on a Ficoll-Hypaque

Table 1. Clinical and demographic characteristics of 147 Chinese patients and controls with different types of adult leukemia*

Type of adult leukemia	No. of patients (male/female)	Age (years; median, range)
Controls	30 (17/13)	48 (18-76)
Idiopathic thrombocytopenic purpura	5 (0/5)	40 (18-60)
Iron deficiency anemia	15 (9/6)	50 (25-76)
Normal	10 (8/2)	49 (20-72)
Chronic myelogenous leukemia:	27 (15/12)	52 (23-77)
chronic phase	10 (6/4)	49 (23-73)
acceleration phase	7 (4/3)	51 (30-71)
lymphoid blast	3 (2/1)	56 (35-77)
blast crisis	7 (3/4)	53 (32-76)
Acute myeloid leukemia (FAB type):	60 (36/24)	47 (15-75)
M2	20 (12/8)	45 (21-69)
M3	15 (8/7)	45 (15-60)
M4	7 (4/3)	53 (25-71)
M5	15 (10/5)	48 (19-75)
M6	3 (2/1)	52 (35-69)
Acute lymphoid leukemia	60 (30/30)	45 (17-77)
T acute lymphoid leukemia	30 (16/14)	46 (19-77)
B-acute lymphoid leukemia	30 (14/16)	44 (17-70)

*Abbreviations: M2 – acute myeloblastic leukemia with maturation; M3 – acute promyelocytic leukemia; M4 – acute myelomonocytic leukemia; M5 – acute monocytic leukemia; M6 – acute erythroid leukemia; FAB – French-American-British classification of acute myeloid leukemia (21).

density gradient. Total RNA was extracted from bone marrow mononuclear cells by using Trizol Kit (Invitrogen, Carlsbad, CA, USA). cDNA was synthesized from each RNA by a random primer and Moloney murine leukemia virus reverse transcriptase (Promage, Madison, WI, USA) according to the manufacturer's recommendations. FLT3 gene expression was assessed by reverse transcriptase PCR (RT-PCR) and quantitative PCR. The primer pairs of *FLT3* for RT-PCR were 5′-TGTCGAGCAGTACTCTAAACA-3′ and 5′-ATCCTAGTACCTTCCCAAACTC-3′ (22). The *β-actin* gene, with primers 5′-TGACGGGGTCACCCACAC-3′ and 5′-CTAGAAGCATTTGCGGTGGA-3′, served as an internal control. Reagents consisted of Taq polymerase, dNTP, buffer, and $MgCl_2$. Reaction was performed in three steps as follows: denaturation at 94°C, annealing at 50°C, and elongation at 72°C. After 35 cycles, PCR products were fractionated through 2.5% agarose gels and viewed under UV illumination after ethidium bromide staining.

Expression of FLT3 mRNA was analyzed with quantitative PCR in 20 controls and 50 patients with acute myeloid leukemia (16 acute myeloblastic leukemia with maturation; 12 acute promyelocytic leukemia; 5 acute myelomonocytic leukemia; 15 acute monocytic leukemia; 2 acute erythroid leukemia), 20 patients with B-acute lymphoid leukemia, 20 patients with T-acute lymphoid leukemia, and 27 patients with chronic myelogenous leukemia. Quantitative PCR was done by Quantitect SYBR green PCR reagent (Qiagen, Miami, FL, USA) following the manufacturer's recommendations and using a quantitative PCR instrument RotorGene 6000 (Corbett Research, Mortlake, Australia). The primer sequences of *FLT3* were forward – 5′-TCAAGTGCTGTGCATACAATTCCC-3′ and reverse – 5′-CACCTGTACCATCTGTAGCTGGCT-3′; *glyceraldehyde 3-phosphate dehydrogenase (GAPDH)* served as a reference with the following sequences: forward – 5′-CCAAAATCAAGTGGGGCGATG-3′ and reverse – 5′-AAAGGTGGAGGAGTGGGTGTCG-3′ (23). Thermal cycling for *FLT3* was performed as follows: step 1 – at 95°C for 15 minutes; step 2 – at 95°C for 15 seconds; step 3 – at 50°C for 30 seconds, step 4 – at 72°C for 1 minute. Steps 2-4 were repeated for 35 cycles. The copy number of each sample was calculated by relative quantification. A dilution series of 5 different concentrations was used to generate two standard curves, one for FLT3 and another for *GAPDH. FLT3 and GAPDH* were simultaneously amplified and the threshold cycle (Ct) of each gene was obtained. Ct values of the sample and the corresponding standard curve were used to calculate the amounts of *GAPDH* and *FLT3* mRNA. *FLT3* mRNA expression was then presented as ratio of *FLT3/GAPDH* mRNA in the sample, as the expression of *GAPDH* was constant across samples.

Screening for mutation of FLT3/ITD

Amplified products were resolved on 2.5% agarose gels. *FLT3/ITD* mutations were

identified as bands migrating above the expected 366 bp size of the wild-type *FLT3* fragment. Amplified cDNA fragments were purified and sequence analysis performed by ABI BigDye (Applied Biosystems, Foster City, CA, USA).

Flow cytometry assay of FLT3 receptor protein expression

Bone marrow mononuclear cells were washed twice with phosphate buffered saline (PBS) and incubated at 37°C for 1 hour with concentration of 100 μg/mL goat antihuman *FLT3* antibody (R&D System, Inc, Minneapolis, MN, USA). The cells were washed with PBS three times and incubated with fluorescence isothiocyanate-conjugated rabbit anti-goat immunoglobulin antibody for 1 hour. Flow cytometry analysis was performed by gating all cells acquired after Ficoll separation. The percentages of positive cells were determined using isotype controls. The correlation between the amounts of *FLT3*-positive cells and corresponding percentage of blasts (according to morphology classification in the bone marrow) was analyzed in acute leukemia.

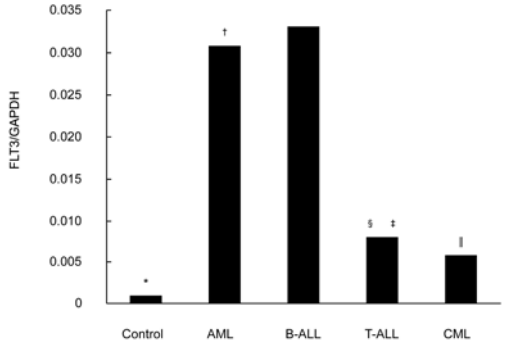

Figure 1. Analysis of fms-like tyrosine kinase 3 *(FLT3)* mRNA expression levels in controls and patients with acute myeloid leukemia (AML), B-acute lymphoid leukemia (B-ALL), T-acute lymphoid leukemia (T-ALL), and chronic myelogenous leukemia (CML). The data were calculated from the ratio of *FLT3* to glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*, internal control) by quantitative polymerase chain reaction. Ratios for controls, AML, B-ALL, T-ALL, CML were 0.001, 0.031, 0.033, 0.008, and 0.006.respectively. Asterisk indicates $P < 0.001$, control vs leukemia; dagger indicates $P = 0.091$, AML vs B-ALL; double dagger indicates $P = 0.006$, AML vs T-ALL; section mark indicates $P = 0.001$, B-ALL vs T-ALL; parallel mark indicates $P < 0.001$, AML vs CML (Student Newman-Keuls test).

Statistical analysis

Difference in median values of age, peripheral white blood cell counts, copy number, and lactate dehydrogenase between two groups were analyzed with the Mann-Whiney U test and among three or more groups with student Newman-Keuls test for distribution. The analysis of the distribution between two variables was performed by Pearson correlation test. Analysis of the frequencies was performed by Fisher exact test for 2×2 tables. Statistical analyses were performed with Statistical Package for the Social Sciences (SPSS Inc., Chicago, IL, USA). All P values were two-tailed and considered statistically significant if $P < 0.05$.

Results

Expression of FLT3 transcript in leukemia

RT-PCR combined with gel-image analysis system was used to determine the expression of *FLT3* transcript in 147 patients with leukemia and 30 controls. Of 147 patients, there were 120 patients with acute leukemia and 27 patients with chronic myelogenous leukemia. Of these, 138 (93.9%) showed FLT3 mRNA expression (117 patients with acute leukemia and 21 with chronic myelogenous leukemia). Quantitative PCR showed that *FLT3* mRNA was expressed in 90 patients with acute leukemia, 27 patients with chronic myelogenous leukemia, and 20 controls (Figure 1). There was a significant difference between patients with acute myeloid leukemia and patients with chronic myelogenous leukemia in *FLT3*expression (Figure 1). In patients with acute myeloid leukemia, B-acute lymphoid leukemia, and T-acute lymphoid leukemia, mean percentage of blasts was 61.0%, 56.5%, 55.8%, respectively, and the expression level in acute myeloid leukemia and B-acute lymphoid leukemia was significantly higher than in T-acute lymphoid leukemia. In chronic myelog-

Table 2. Expression level of the fms-like tyrosine kinase 3 *(FLT3)* transcript and clinical characteristics in chronic myelogenous leukemia (CML) used by quantitative polymerase chain reaction*

Characteristics	CML chronic phase (n = 10)	CML acceleration phase (n = 7)	CML blast crisis (n = 10)	*P*†
PBWBC count (× 10^9/L, mean±SD)	41.0 ± 7.48	80.6 ± 8.13	113.2 ± 46.4	0.001‡
				0.089§
Percentage of blasts in the bone marrow	0.030 ± 0.009	0.15.6 ± 0.025	0.642 ± 0.182	0.001‡
				0.001§
Expression level of *FLT3* mRNA (*FLT3/GAPDH*)	0.002	0.004	0.017	0.307‡
				0.023§
Surface expression of *FLT3* receptor protein	0.067 ± 0.019	0.101 ± 0.01.9	0.158 ± 0.030	0.002‡
				0.001§

*Abbreviations: PBWBC – peripheral blood white blood cell; GAPDH – glyceraldehyde 3-phosphate dehydrogenase.
†Student Newman-Keuls test.
‡CML chronic phase in comparison with CML acceleration phase.
§CML acceleration phase in comparison with CML blast crisis.

Table 3. Distribution of fms-like tyrosine kinase 3/internal tandem duplications *(FLT3/ITD)* mutations in acute myeloid leukemia

FAB type*	No. of *FLT3/ITD*+ patients
M2 (n = 20)	3
M3 (n = 15)	3
M4 (n = 7)	2
M5 (n = 15)	6
M6 (n = 3)	1

*Abbreviations: M2 – acute myeloblastic leukemia with maturation; M3 – acute promyelocytic leukemia; M4 – acute myelomonocytic leukemia; M5 – acute monocytic leukemia; M – acute erythroid leukemia; FAB – French-American-British Classification of acute myeloid leukemia (21).

enous leukemia, the expression of FLT3 transcript was associated with disease progression of transformation (Table 2).

Incidence of FLT3/ITD mutation in acute leukemia

Of 60 patients with acute myeloid leukemia, 15 had *FLT3/ITD* mutation. Patients with acute monocytic leukemia had higher *FLT3/ITD* mutation rate than patients with other FAB types (Table 3). Of 60 patients with acute lymphoid leukemia, 4 had *FLT3/ITD* mutations. No *FLT3/ITD* mutation was found in chronic myelogenous leukemia. Sequence analysis performed in 3 *FLT3/ITD* mutations showed that mutations involved exon 14, the sizes of the inserting nucleotides were 36, 42, and 57 pairs, and all had tandem duplication. The location of each mutation also varied, with the *ITD* in two patients starting at codon 597 and in another patient at codon 603. The deduced amino acid sequences for *FLT3/ITD* mutation are shown in Figure 2.

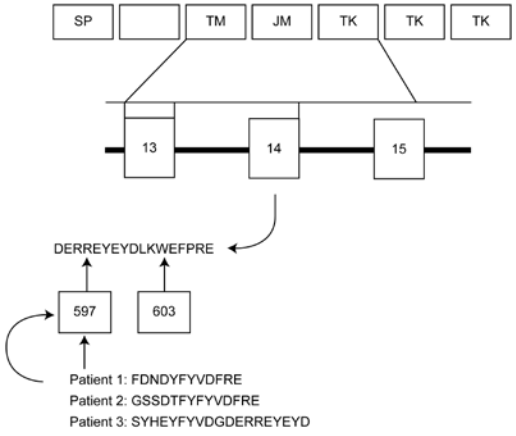

Figure 2. Sequence analysis results of fms-like tyrosine kinase 3/internal tandem duplications *(FLT3/ITD)* mutations in three patients with acute leukemia and deduced amino acid sequences for mutation region. Abbreviations: SP – signal peptide; TM – trans-membrane domain; JM – juxtamembrane domain;TK – tyrosine kinase domain.

Characteristics of patients with FLT3/ITD mutation

Nineteen of 120 patients with acute leukemia had *FLT3/ITD* mutation (Table 4). The presence of the mutation was not related to sex or age. However, it was associated with a higher peripheral white blood cell count and a higher percentage of blasts in the bone marrow at diagnosis. Patients with acute leukemia with *FLT3/ITD* had significantly higher serum LDH concentration than patients with acute leukemia without *FLT3/ITD*. As to the expression level, *FLT3* transcript in 12 patients with tandem duplication was upregulated, but the difference was not significant. The expressive signal of *FLT3* protein in patients with acute leukemia with *FLT3/*

Table 4. Expression levels and clinical characteristics of patients with positive and negative fms-like tyrosine kinase 3/internal tandem duplications (*FLT3/ITD*+ and *FLT3/ITD*-) acute leukemia analyzed by reverse transcriptase-polymerase chain reaction and flow cytometry

Characteristic	No. of patients		*P*
	FLT3/ITD+ AL	FLT3/ITD- AL	
Age (years; median, range)*	44 (21-70)	47 (15-77)	0.813‡
Age >60 y	6	29	
Sex:*			0.821‡
male	10	56	
female	9	45	
Expression level of *FLT3* mRNA†	0.062	0.034	0.091§
Surface expression level of *FLT3* protein (%)*	22.0	16.0	0.060§
Peripheral blood white blood cell count (× 10^9/L)*	41.6	29.5	0.045§
Blasts in the bone marrow (%)*	79	56	0.014§
Serum lactate dehydrogenase concentration (μ/L)*	996.8	213.6	<0.001§
Outcome:*			0.270‡
complete remission	14	85	
failure	5	16	
complete remission rate	73.7%	84.2%	

*19 *FLT3/ITD*- and 101 *FLT3/ITD*+ cases.
†12 *FLT3/ITD*- and 78 *FLT3/ITD*+ cases.
‡Fisher exact test.
§Mann-Whiney U test.

ITD mutation was stronger than in patients with acute leukemia without *FLT3/ITD* mutation.

The complete remission rate for 120 patients with acute leukemia in this study was 82.5%. Patients with *FLT3/ITD* mutation had lower complete remission rate than patients without *FLT3/ITD* mutation (73.68% vs 84.16%), although the relationship was not significant.

FLT3 receptor expression and its correlation with bone marrow blasts

Mean expression level (percentage of *FLT3* positive cells) of *FLT3* protein in controls was 2.20 ± 1.06%, which was significantly lower than in patients with leukemia (17.38 ± 9.45%, $P < 0.001$) (Table 2). The expression of FLT3 was significantly higher in patients with acute leukemia (18.82 ± 9.66%) than in patients with chronic myelogenous leukemia in chronic (6.74 ± 1.85%, $P < 0.001$) and acceleration phase (10.11% ± 1.92, $P = 0.019$), but no significant difference was observed in comparison with patients in the blast transformation

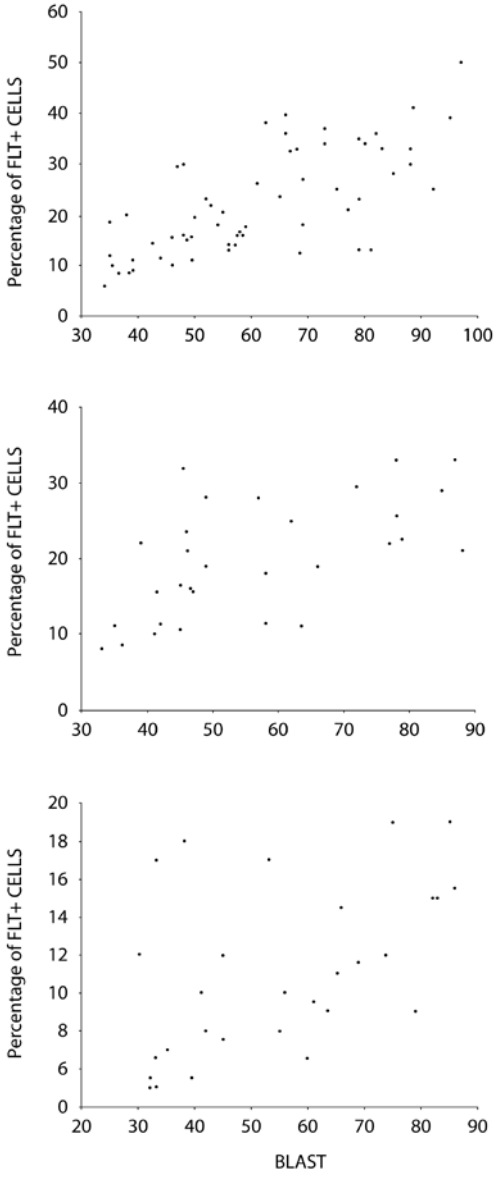

Figure 3. Surface expression level of fms-like tyrosine kinase 3 *(FLT3)* analyzed by flow cytometry according to the percentage of bone marrow blasts, *FLT3* expression levels increase with the number of bone marrow blasts in acute leukemia. (**A**) Correlation of *FLT3* expression with blasts in bone marrow in acute myeloid leukemia (r = 0.716; $P < 0.001$); (**B**) Correlation of *FLT3* expression with blasts in bone marrow in B-acute lymphoid leukemia (r = 0.607; $P < 0.001$); (**C**) Correlation of *FLT3* expression with blasts in bone marrow in T-acute lymphoid leukemia (r = 0.524; $P = 0.003$).

phase (15.81% ± 3.02, $P = 0.307$). In the acute leukemia group, Pearson correlation analysis showed that the percentage of FLT3-positive cells was significantly related to blast count (Figure 3). The expression of FLT3 transcript

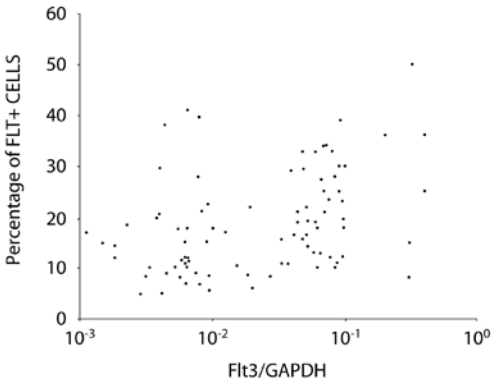

Figure 4. Correlation between surface expression of fms-like tyrosine kinase 3 *(FLT3)* protein and mRNA expression levels. Correlation of surface expression of *FLT3* with *FLT3*/glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) in 90 patients with acute leukemia analyzed by quantitative polymerase chain reaction. Pearson correlation shows a significant correlation between receptor expression and mRNA expression ($r = 0.366$, $P < 0.001$).

was correlated with that of FLT3 receptor protein (Figure 4).

Discussion

Our study showed that FLT3 mRNA was expressed in all patients with B- acute lymphoid leukemia and acute myeloid leukemia and 90% of patients with T-acute lymphoid leukemia. Compared with controls, the mean expression level of the *FLT3* transcript in acute lymphoid leukemia and acute myeloid leukemia was evidently increased. This is in close agreement with previously published results (18,20). Due to a limited number of acute myeloid leukemia cells available for this analysis, we did not investigate the correlations with FAB type and cytogenetics, which had previously been demonstrated as important factors for outcome of acute myeloid leukemia (5,6,11,15). Larger scale investigations on the associations between FLT3 expression level and cytogenetics should be performed in future research.

We found *FLT3* mRNA and protein expression in chronic myelogenous leukemia, which is consistent with the results by Carow et al (18). Compared with the chronic phase of chronic myelogenous leukemia, 7 patients in the acceleration phase had significantly increased white blood cell count and number of blasts in the bone marrow, but not the expression level of the *FLT3* transcript. Also, patients in the acceleration phase had significantly higher surface expression of *FLT3* receptor protein than patients in the chronic phase. In chronic myelogenous leukemia, FLT3 protein expression increased more than mRNA expression. This was different than in acute leukemia, probably because FLT3 mRNA level is associated with cell types and stage of blast maturation and perhaps has an unknown biological cause. Although larger scale analysis is required to clarify the clinical significance of increased *FLT3* expression, we believe that *FLT3* expression may contribute to chronic myelogenous leukemia disease progression and that its estimation may be helpful for identification of chronic myelogenous leukemia blast transformation. This is in accordance with the study by Lin et al (24), who detected *FLT3* gene mutations in chronic myeloproliferative diseases and found *FLT3* mutations correlated with an increased number of blasts. *FLT3* may participate in blast transformation in chronic myeloproliferative diseases, including chronic myelogenous leukemia.

No data have been available on the expression of *FLT3* protein in acute leukemia. We found that the surface expression of *FLT3* receptor protein in bone marrow mononuclear cells was related to *FLT3* mRNA. This is in line with the findings of Kuchenbauer et al (25), who reported that CD135 receptor expression was correlated with *FLT3* mRNA. On the other hand, Ozeki et al (26) found that surface expression level of *FLT3* was not related to the expression of the *FLT3* transcript. This might be due to the fact that they analyzed a limited number of acute myeloid leukemia samples. Kuchenbauer et al (27) reported higher Spearman rank correlation than

we did, probably because they used direct conjugated antibody. As to the mechanism of *FLT3* protein overexpression, they suggested an autocrine stimulatory mechanism of *FLT3* receptor-ligand (*FL*). *FLT3-FL* loop, which is expressed in all cell lines, plays an important role in the pathobiology of leukemia. Combined with various cytokines, *FL* has synergistic or additive mitogenic effect, which leads to significant anti-apoptotic effects on primary acute myeloid leukemia cells (27). *FLT3* proteins on the cell surface were internalized when exogenous *FL* stimulation was administered (3). Our results showed that an increased *FLT3* protein levels in bone marrow mononuclear cells were related to the number of blast cells in the bone marrow, suggesting that *FLT3* protein assay may be a useful biomarker for making leukemia prognosis.

In our cohort, the frequency of *FLT3/ITD* mutation was 25% in acute myeloid leukemia and 7% in acute lymphoid leukemia, which is consistent with findings of a Japanese study (23%) and Kottaridis et al (27%) (14,15). Our initial findings showed that Chinese patients with leukemia shared the same gene alternation as patients from different genetic backgrounds, suggesting a common mechanism for the pathogenesis of acute leukemia. We found a significant association between the presence of the *FLT3/ITD* mutation, a higher white blood cell count, a higher marrow blast percentage, and elevated serum LDH. This suggests that *FLT3/ITD* is an independent prognostic factor in acute leukemia. Other studies have also reported that high *FLT3* expression levels were associated with an unfavorable prognosis (26,28).

In our study, the expression of *FLT3* transcript in patients with tandem duplication was increased, but the increase was not significant. Ozeki et al (26) also reported the association between *FLT3/ITD* mutation and expression of FLT. The explanation for such an association is that expression levels of *FLT3/ITD* depend on the co-expression of the wild type and the mutant alleles. It has been detected that *FLT3* expression levels were related to the relative proportions of wild type and mutant *FLT3* (25). Future analysis of *FLT3* expression levels in patients with *FLT3/ITD* mutation should examine the relative fragments of wild type and mutant-*FLT3*.

To further assess the prognostic significance of *FLT3/ITD* mutation, we analyzed whether *FLT3/ITD* mutation influenced the complete remission rate of leukemia. The results showed that patients with *FLT3/ITD* had a little lower complete remission rate than patients without *FLT3/ITD* mutation, but the difference was not significant, which is in accordance with other studies (14,15). Lack of effect of *FLT3/ITD* mutation on the complete remission rate might be explained by a lack of effect of *FLT3/ITD* mutation on chemosensitivity of leukemic cells at diagnosis. Though it did not affect complete remission rate, *FLT3* mutation has been found to predict relapse rate and overall survival (14,29). In fact, the presence of *FLT3/ITD* mutation has been suggested to have a major impact on long-term outcome (15).

Recently, Stirewalt et al (30) has reported that the size of *FLT3/ITD* has prognostic significance in patients with acute myeloid leukemia. They showed that increased *ITD* size was associated with decreased overall survival and relapse free survival. We sequenced three PCR products of patients with acute leukemia with *FLT3/ITD* mutation, detecting the insertion sizes of 36 bp, 42 bp, and 57 bp. Insertion nucleotides >40 bp were regarded as large; two patients in our group had large size *ITD* mutation. The patient with 42 bp insertion died three months after two courses of chemotherapy. The patient with 57 bp insertion died 5 months after diagnosis. The third patient, with a secondary acute monocytic type of leuke-

mia, experienced myelodysplastic syndrome with spontaneous splenic rupture and died 10 months later. It seems that larger *ITD* size is related to poorer clinical outcomes. However, more studies and patients are needed to confirm the effect of *FLT3/ITD* mutation size on prognosis.

In summary, our results suggest that *FLT3* expression levels exhibit significant difference in various types of adult leukemia and that the presence of *FLT3/ITD* mutation is an independent prognostic factor for acute leukemia. Such results may provide a new target for leukemia therapy.

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