AML1/RUNX1 mutations in 470 adult patients with de novo acute myeloid leukemia: prognostic implication and interaction with other gene alterations

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Somatic mutation of the *AML1/RUNX1(RUNX1)* gene is seen in acute myeloid leukemia (AML) M0 subtype and in AML transformed from myelodysplastic syndrome, but the impact of this gene mutation on survival in AML patients remains unclear. In this study, we sought to determine the clinical implications of *RUNX1* mutations in 470 adult patients with de novo non-M3 AML. Sixty-three distinct *RUNX1* mutations were identified in 62 persons (13.2%); 32 were in N-terminal and 31, C-terminal. The *RUNX1* mutation was closely associated with male sex, older age, lower lactic dehydrogenase value, French-American-British M0/M1 subtypes, and expression of HLA-DR and CD34, but inversely correlated with CD33, CD15, CD19, and CD56 expression. Furthermore, the mutation was positively associated with *MLL/PTD* but negatively associated with *CEBPA* and *NPM1* mutations. AML patients with *RUNX1* mutations had a significantly lower complete remission rate and shorter disease-free and overall survival than those without the mutation. Multivariate analysis demonstrated that *RUNX1* mutation was an independent poor prognostic factor for overall survival. Sequential analysis in 133 patients revealed that none acquired novel *RUNX1* mutations during clinical courses. Our findings provide evidence that *RUNX1* mutations are associated with distinct biologic and clinical characteristics and poor prognosis in patients with de novo AML. (Blood. 2009; 114:5352-5361)

Introduction

Acute myeloid leukemia (AML) is a heterogeneous group of neoplastic disorders with great variability in the pathogenesis, clinical course, and response to therapy.[1] In recent years, frequent events of acquired gene mutations, as well as deregulation of gene expression, were identified in AML.[2-4] A 2-hit model proposes that cooperation between 2 classes of gene mutations contributes to the leukemogenesis.[5,6] Class I mutations activate genes in the kinase signaling pathways, such as *RAS*, *FLT3*, *KIT*, and *PTPN11*, leading to cell survival and proliferation. Class II mutations, such as *PML/RARA*, *CBFB/MYH11*, *RUNX1/RUNX1T1*, *MLL/PTD*, *CEBPA*, and *SPI1* mutations, inactivate transcriptional factors or cofactors resulting in impaired hematopoietic differentiation. Among these, *FLT3/ITD* and *MLL/PTD* are associated with inferior overall and relapse-free survival, whereas mutations in *CEBPA* and *NPM1* (without concomitant *FLT3/ITD*) are linked to a favorable outcome.[7-9]

The *AML1/RUNX1* (hereafter referred to as *RUNX1*) gene,[10] consisting of 10 exons (exons 1-6, 7A, 7B, 7C, and 8), is one of the most frequently deregulated genes in leukemia through chromosomal translocations and point mutations.[11,12] RUNX1 protein consists of 3 main structure domains: runt homology domain (RHD, residues 50-177), transcription activation domain (TAD,

residues 291-371), and repression domain (residues 446-453). The RHD, a highly conserved 128–amino acid protein motif, is responsible for both DNA binding and heterodimerization with the β-subunit of CBF. The TAD is responsible for the interaction with MOZ,[13] a transcription coactivator of RUNX1 currently named MYST histone acetyltransferase 3.[14] RUNX1 is required for definitive hematopoiesis, and its functional dysregulation leads to leukemia.[15,16] Monoallelic germline mutation of the *RUNX1* gene occurs in rare cases of familial platelet disorder with predisposition to AML.[17] Acquired *RUNX1* mutation was mostly reported in therapy-related myelodysplastic syndrome (MDS) or MDS/AML but was also found in some de novo AMLs, especially French-American-British (FAB) subtype M0.[11,18-24] This mutation has been shown to be associated with poor outcome in MDS,[20,25] but its prognostic implication in de novo AML remains unclear.

In this study, we assessed the clinical implication of *RUNX1* mutation in 470 unselected adults with de novo AML and its interactions with other gene alterations. Sequential analysis of *RUNX1* mutation during the clinical course was also performed on 133 patients to investigate the stability and pathogenic role of this mutation in AML. To the best of our knowledge, this is the first study to address the prognostic implication of *RUNX1* mutation in a

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The online version of this article contains a data supplement.

large cohort of patients with de novo AML. We found that *RUNX1* mutation is an independent poor-risk factor for overall survival (OS) in these patients.

Methods

Subjects

This study was approved by the institutional review board of the National Taiwan University Hospital, and written informed consent was obtained from all participants in accordance with the Declaration of Helsinki. From November 1995 to March 2007, a total of 470 adult patients who were newly diagnosed as having de novo non-M3 AML at National Taiwan University Hospital were enrolled consecutively. Patients with antecedent hematologic diseases or therapy-related AML were excluded. Diagnosis and classification of AML were made according to the FAB Cooperative Group Criteria. Patients with AML M3 subtype were excluded because their treatment and survival differ significantly from those of other AML patients. Among them, 330 patients (70.2%) received induction chemo-therapy (idarubicin 12 mg/m^2 per day on days 1-3 and cytarabine 100 mg/m^2/d on days 1-7) and then consolidation chemotherapy with 2 to 4 courses of high-dose cytarabine (2000 mg/m^2 every 12 hours on days 1-4, total 8 doses), with or without an anthracycline (idarubicin or novantrone) after achieving complete remission (CR). The remaining 140 patients received palliative therapy with supportive care or low-dose chemotherapy resulting from underlying comorbidity or based on the decision of the patients. Ninety-six patients underwent allogeneic hematopoietic stem cell transplantation (HSCT), including 11 *RUNX1*-mutated and 85 *RUNX1*-wild patients. Of the 96 allografts, 81 grafts were from related donors and 15 were from unrelated donors. HLA was matched in 87 and mismatched for at least one locus in 9. Forty-four patients were in first CR at the time of transplantation and 52, after relapse or refractory.

Cytogenetics

Bone marrow cells were harvested directly or after 1 to 3 days of unstimulated culture as described previously.[26] Metaphase chromosomes were banded by the trypsin-Giemsa technique and karyotyped according to the International System for Human Cytogenetic Nomenclature.

Immunophenotype analysis

A panel of monoclonal antibodies to myeloid-associated antigens, including CD13, CD33, CD11b, CD15, CD14, and CD41a, as well as lymphoid-associated antigens, including CD2, CD5, CD7, CD19, CD10, and CD20, and lineage nonspecific antigens HLA-DR, CD34, and CD56 were used to characterize the phenotypes of the leukemia cells as previously described.[27]

Mutation analysis

Mutation analysis of *RUNX1* exons 3 to 8 was performed by genomic DNA polymerase chain reaction (PCR) and direct sequencing as previously reported.[25] Abnormal sequencing results were confirmed by at least 2 repeated analyses. Sequential analysis of *RUNX1* mutation during the clinical course was performed in 294 samples from 133 patients. Mutation analysis of 11 other relevant molecular marker genes, *NPM1*,[28] *CEBPA*,[29] *FLT3/ITD and FLT3/TKD*,[27] *N-RAS*,[30] *K-RAS*,[30] *JAK2*,[30] *KIT*,[25] *MLL/PTD*,[31] *PTPN11*,[32] and *WT1*,[33] was performed as previously described.

TA cloning analysis

For patients with double mutations, TA cloning was performed to determine whether the 2 mutations were in the same or different alleles as previously described.[25] Briefly, the cDNA was amplified to cover both mutations, the PCR products were then cloned into the Taq polymerase-amplified (TA)–cloning vector pGEM-T Easy (Promega), and 10 clones were selected for sequencing.

Statistical analysis

The discrete variables of patients with and without gene mutation were compared using the χ^2 tests, but if the expected values of contingency tables were smaller than 5, Fisher exact test was used. If the continuous data were not normally distributed, Mann-Whitney tests were used to compare continuous variables and medians of distributions. To evaluate the impact of *RUNX1* mutation on clinical outcome, only the 330 patients who received conventional induction chemotherapy and then consolidation chemo-therapy if CR was achieved, as mentioned earlier, were included in analysis. OS was measured from the date of first diagnosis to the date of last follow-up or death from any cause, whereas disease-free status indicated that the patient achieved CR and did not relapse by the end of this study. Cox regression survival estimation was used to plot survival curves and to test the difference between groups. Multivariate Cox proportional hazard regression analysis was used to investigate independent prognostic factors for OS and disease-free survival (DFS). The proportional hazards assump-tion (constant hazards assumption) was examined using time-dependent covariate Cox regression before conducting multivariate Cox proportional hazard regression. The variables, including age, white blood cell count (WBC), lactic dehydrogenase (LDH), karyotype, *CEBPA*, *WT1*, *RUNX1*, and *NPM1/FLT3-ITD*, were used as covariates. Those patients who received HSCT were censored at the time of HSCT in survival analysis to ameliorate the influence of the treatment. A *P* value less than .05 was considered statistically significant. All statistical analyses were performed with SPSS 13 software (SPSS Inc) and Statsdirect.

Results

RUNX1 mutations in 470 adult patients with de novo non-M3 AML

Excluding the 4 single nucleotide polymorphisms (I87I, P157P, S362S, and P436P) that were detected in 10 patients but did not alter the amino acid residues, 68 mutations were identified in 70 patients (Figure 1). Among them, 4 mutations (G42R, D317N, V425G, and M439L) in 7 patients were supposed to be silent and insignificant for 2 reasons. First, these mutations did not disappear at CR (Table 1, cases 63-66, 68-70). Second, the 4 amino acid sites are located outside RHD region and are not conserved in RUNX proteins family among different species (supplemental Figure 1, available on the *Blood* website; see the Supplemental Materials link at the top of the online article). Another mutation Q370R, which was found in 2 patients, was also supposed to be insignifi-cant because it was still detectable at CR after consolidation chemotherapy and also after autologous HSCT in one child who had this mutation at diagnosis but was not recruited in this report (data not shown). Of the remaining 63 distinct mutations, 24 were missense mutations, 7 were nonsense mutations, 28 were frame-shift mutations, and 4 were in-frame mutations (Figure 1). Totally, 32 mutations were located at N-terminal (exons 3-5), including 31 at RHD, and 31 were at C-terminal (exons 6-8), including 17 at TAD. Among 24 missense mutations, 20 were in the RHD region. The 4 missense mutations (D255E, D305N, P323S, and P398L) located outside the RHD region were conserved during species evolution (supplemental Figure 1). Of the 28 frameshift mutations, 7 generated truncated peptides with complete or partial deletion of the RHD, 4 generated truncated peptides with intact RHD region, and the remaining 17 generated elongated proteins with intact RHD region (Figure 1); all were suggested to abolish transactivation potential resulting from complete or partial loss of TAD,[34] with the exception of P375fsX570, which retained the intact TAD. Totally, 62 patients (13.2%) had distinct *RUNX1* mutations (supplemental Table 1), including 1 patient (case no. 61) who had both distinct
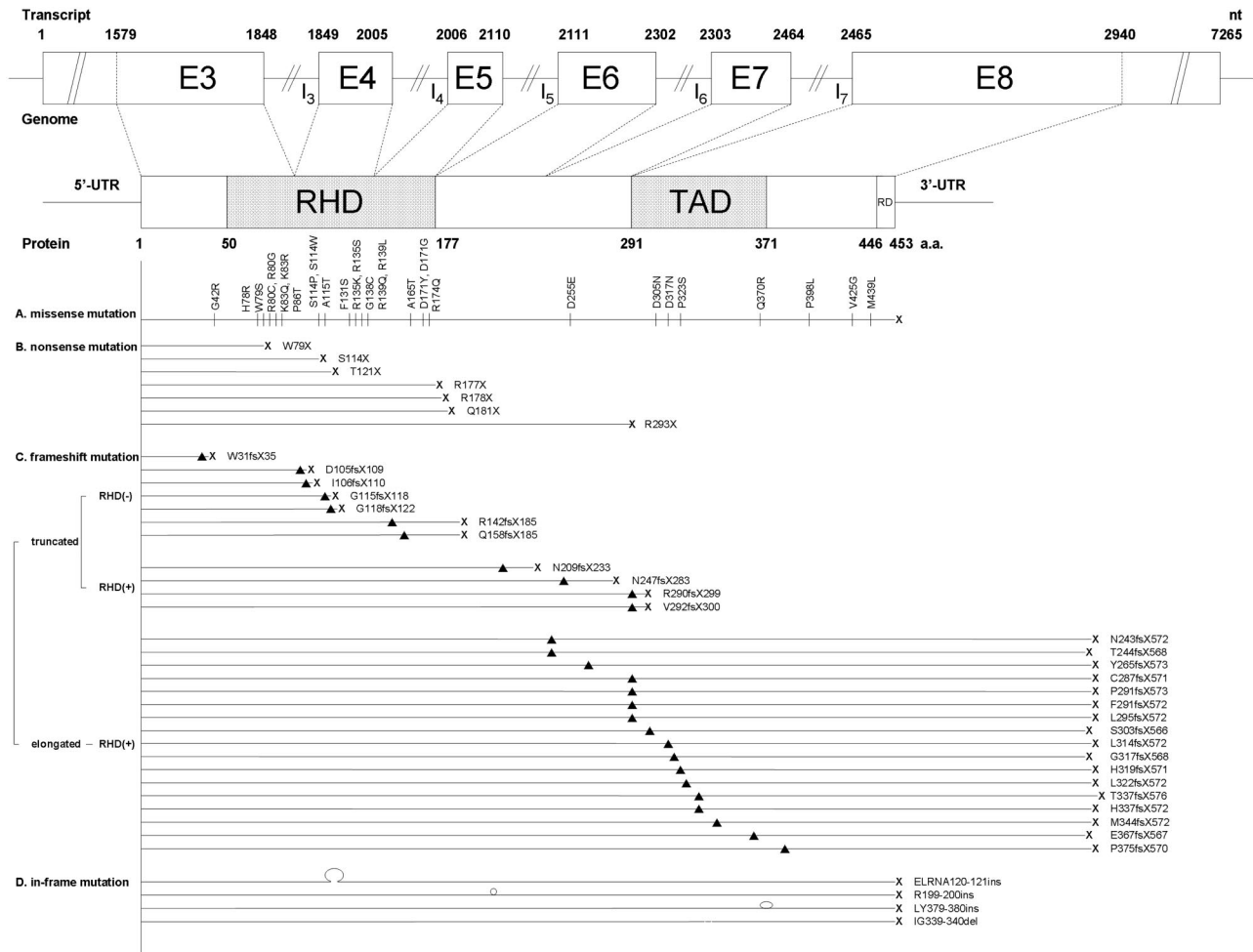
Figure 1. Patterns and locations of the 68 *RUNX1* mutations, including 63 distinct and 5 insignificant mutations. Four kinds of mutations were demonstrated: missense mutations, nonsense mutations, frameshift mutations, and in-frame mutations. Three kinds of frameshift mutations are noted: truncated mutations with defective RHD, truncated mutations with intact RHD, and elongated mutations with intact RHD. The numbering of nucleotide of transcript is according to the mRNA sequence from GenBank[42] accession number D43968 (AML1b); on the other hand, the numbering of amino acid of protein is according to the amino acid sequence from GenBank accession number Q01196. G42R, D317N, Q370R, V425G, and M439L are insignificant mutations. ▲ and | represent the sites of mutation; X, the site of the stop codon; Ω, the site of insertion; and ⸺ (hyphenated line), the site of deletion. RD indicates repression domain; E, exon; I, intron; nt, nucleotide; UTR, untranslated region; and a.a., amino acid.

mutation H78R on exon 3 and insignificant one Q370R on exon 8. The mutations in 6 patients (nos. 3, 4, 17, 35, 37, and 50) were double heterozygous with 2 *RUNX1* mutations; the 2 mutations were in different alleles in 3 patients (nos. 4, 35, and 50) and in the same allele in 1 patient (no. 37) who had available samples for TA cloning study. All others showed only 1 mutation; 3 (nos. 27, 34, and 44) were homozygous and the remaining 53 were heterozygous.

Correlation of *RUNX1* mutations with clinical features and immunophenotypes of leukemic cells

The comparison of clinical characteristics of patients with and without distinct *RUNX1* mutation is shown in Table 2. Those patients with insignificant mutations were included in the *RUNX1*-wild group. Male patients had a higher incidence of *RUNX1* mutations than female (18.4% vs 6.4%, $P < .001$). *RUNX1*-mutated patients were older (median, 62 years vs 48 years, $P = .010$) and had lower LDH levels than *RUNX1*-wild patients ($P = .003$). Patients with FAB M0 subtype of AML had the highest incidence (40%) of *RUNX1* mutation, whereas those with M2 subtype had the lowest incidence (6.3%, $P = .004$). *RUNX1* mutations were positively associated with the expression of HLA-DR ($P < .001$) and CD34 ($P = .012$) but inversely associated with the expression of

CD33 ($P < .001$), CD15 ($P = .003$), CD19 ($P = .014$), and CD56 ($P = .041$) on the leukemic cells (supplemental Table 2). There was no difference in the expression of other antigens between the patients with and without *RUNX1* mutation.

Association of RUNX1 mutations with cytogenetic abnormalities

Chromosome data were available in 452 patients at diagnosis, including 56 *RUNX1*-mutated and 396 *RUNX1*-wild patients (Table 3). *RUNX1* mutations occurred more frequently in patients with intermediate-risk (14.7%) or unfavorable cytogenetics (12.1%) than in those with favorable karyotype (0%, $P < .001$). There was no difference in the incidence of the *RUNX1* mutation among patients with normal karyotype (13.9%), simple abnormalities (11.2%), and complex abnormalities (9.4%, $P = .622$). None of the patients with t(8;21), inv(16), or 11q23 translocation showed *RUNX1* mutation. The same was also true for patients with t(15;17), who were not included in this study. There was close association of *RUNX1* mutation with +8, but not other chromosomal abnormalities, including +11, +13, +21, −5/del(5q), and −7/del(7q).

Table 1. Sequential studies in the AML patients with *RUNX1* mutations*

UPN	Date	Status	Karyotype	*RUNX1* mutation		Other mutations
Patients with distinct mutations						
3	01/26/1998	Initial	46,XY	Missense Frameshift	W79X G115fsX118	—
	05/16/1998	CR	—			—
	05/16/2000	Relapse	45,XY,t(3;5)(q26;q13),-7	Nonsense	W79X	—
4	04/30/1999	Initial	46,XY	Missense Frameshift	A115T C287fsX571	—
	10/09/2000	CR	—			—
8	07/19/1996	Initial	45,XY,-7	Nonsense	Q181X	—
	8/26/1996	CR	46,XY			—
9	06/15/1998	Initial	47,XY,+21	Missense	K83Q	*CEBPA*
	07/16/1998	CR	46,XY			—
11	05/20/1998	Initial	47,Y,inv(X)(p11q26),+del(8)(p21p23),del(8)(p22p23)	Nonsense	R178X	*FLT3/ITD* and *NRAS*
	10/20/1998	CR	46,XY			—
12	05/21/1997	Initial	46,XY	Frameshift	L314fsX572	—
	08/11/1997	CR	—			—
15	04/05/2000	Initial	46,XY,t(10;11)(q24;p15),add(6)(p25)	Frameshift	W31fsX35	*FLT3/ITD*
	10/03/2000	CR	46,XY			—
17	11/26/2001	Initial	46,XX	Nonsense Missense	R177X S114W	—
	04/01/2002	CR	—			—
	11/15/2002	Relapse	ND	Nonsense Missense	R117X S114W	*FLT3/ITD*
18	07/10/2002	Initial	47,XY,+8	Frameshift	Q158fsX185	—
	03/04/2003	CR	46,XY			—
23	10/21/2002	Initial	46,XY	Nonsense	T121X	*FLT3/ITD* and *FLT3/TKD*
	02/06/2003	CR	—			—
	04/30/2004	Relapse	46,XY			*FLT3/ITD*
28	11/20/2003	Initial	45,XY,-7del(12)(p12p13)	Missense	D171G	*PTPN11*
	01/28/2004	CR	46,XY			—
29	06/12/2001	Initial	47,XX,+8	Frameshift	L295fsX572	—
	01/17/2002	CR	46,XX			—
35	02/06/2004	Initial	46,XX	Missense Missense	S114P A165T	*FLT3/ITD*
	06/01/2004	CR	—			—
	03/07/2005	Relapse	ND	Missense	S114P	*FLT3/ITD*
41	12/20/2004	Initial	NM	Missense	R135K	—
	01/28/2005	CR	ND			—
44	11/15/2005	Initial	46,XY	Missense	R135K	—
	04/18/2006	CR	—			—
45	12/06/2005	Initial	46,XY	Frameshift	N209fsX233	—
	01/12/2006	CR	—			—
48	07/21/2006	Initial	46,XY	Frameshift	G118fsX122	*WT1* and *FLT3/TKD*
	09/21/2006	CR	—			—
	07/15/2008	Relapse	46,XY			*WT1* and *FLT3/TKD*
53	03/16/2006	Initial	46,XY	Frameshift	T244fsX568	*FLT3/ITD*
	06/21/2006	CR	—			—
	06/03/2008	Relapse	ND	Frameshift	T244fsX568	*FLT3/ITD*
Patients with insignificant mutations						
63	09/25/1998	Initial	46,XY,t(7;11)(p15;p15)		V425G	—
	11/20/1998	CR	46,XY		V425G	—
	03/18/1999	Relapse	46,XY,t(7;11)(p15;p15)		V425G	—
64	04/25/2000	Initial	46,XX,del(16q)		V425G	—
	01/07/2002	CR	ND		V425G	—
65	10/11/2002	Initial	46,XX		M439L	—
	06/09/2003	CR	—		M439L	—
66	10/11/2004	Initial	45,X,-Y, t(7;21;8)(q32;q22;q24)		G42R	*KIT(D816V)*
	12/22/2004	CR	46,XY		G42R	—
	07/28/2005	Relapse	45,X,-Y,t(7;21;8)(q32;q22;q24),t(8;16)(q11;p11),del(11)(q14q23)		G42R	*KIT(D816V)*
68	02/23/2006	Initial	46,XY, inv(16)(p13q22)		D317N	—
	04/03/2006	CR	46,XY		D317N	—
	09/05/2007	Relapse	46,XY, inv(16)(p13q22)		D317N	—
69	12/25/2006	Initial	47,XX,+8, inv(16)(p13q22)		V425G	*NRAS (Q61R)*
	01/25/2007	CR	46,XX		V425G	—
	12/20/2007	Relapse	46,XX		V425G	—
70	08/13/2007	Initial	46,XX		M439L	—
	09/13/2007	CR	—		M439L	—
	01/11/2008	Relapse	46,XX		M439L	ND

UPN indicates unique patient number; ND, not done; NM, no mitosis; and —, not applicable.

*The data of serial studies in another 108 patients who had no *RUNX1* mutation at diagnosis were not shown in this table. None of these 108 patients acquired *RUNX1* mutation at relapse after a median interval of 48 months (range, 5-160 months); 41.6% of them had karyotypic evolution at relapse.

Table 2. Comparison of clinical manifestation and laboratory features between AML patients with and without *RUNX1* mutation

Variable	Total (n = 470)	*RUNX1*-mutated* (n = 62, 13.2%)	*RUNX1*-wild (n = 408, 86.8%)	P
Sex, no. (%) of patients				< .001
Male	266	49 (18.4)	217 (81.6)	
Female	204	13 (6.4)	191 (93.6)	
Median age, y (range)	52.0 (15-90)	62.0 (15-89)	48.0 (15-90)	= .010
Laboratory data, median (range)				
WBC (/μL)	21 850 (120-627800)	15 225 (310-258900)	22 430 (120-627800)	= .184
Hemoglobin, g/dL	8.0 (2.9-16.2)	7.8 (3.8-11.9)	8.0 (2.9-16.2)	= .855
Platelets, × 1000/μL	45.0 (3-802)	49.5 (3-202)	43.0 (3-802)	= .584
Blasts, per μL	9863 (0-456 725)	7535 (102-246 602)	10 015 (0-456 725)	= .643
LDH, U/L	890 (206-15 000)	733 (299-8116)	918 (206-15 000)	= .003
FAB, no. (%) of patients				= .004
M0	10	4 (40.0)	6 (60.0)	
M1	114	20 (17.5)	94 (82.5)	
M2	174	11 (6.3)	163 (93.7)	
M4	126	19 (15.1)	107 (84.9)	
M5	25	4 (16.0)	21 (84.0)	
M6	12	3 (25.0)	9 (75.0)	
Undetermined	9	1 (11.1)	8 (88.9)	
Induction response*	330	37	293	= .006
CR	248 (75.2)	21 (56.8)	227 (77.5)	
PR	13 (3.9)	5 (13.5)	8 (2.7)	
Refractory	46 (13.9)	7 (18.9)	39 (13.3)	
Induction death	23 (7.0)	4 (10.8)	19 (6.5)	

*Only the patients with distinct *RUNX1* mutations were included in this group. Those with insignificant mutations, such as G42R, D317N, Q370R, V425G, and M439L, were excluded.

Association of RUNX1 mutation with other molecular abnormalities

To investigate the interaction of gene mutations in the pathogenesis of adult AML, a complete mutational screening of 11 other genes was performed in all 470 patients (Table 4). Among the 62 patients with *RUNX1* mutations, 31 (50%) showed additional molecular abnormalities at diagnosis (supplemental Table 3); 16 had 1 additional change, 14 had 2, and 1 had 3. Twenty-six of them (83.9%) had at least one concurrent class I mutation. The most common associated molecular event was *FLT3/ITD* (14 cases), followed by

MLL/PTD (9 cases), and *FLT3/TKD* (6 cases). Of note, 7 patients had concomitant *RUNX1*, *FLT3/ITD*, and *MLL/PTD* mutations (supplemental Table 3). Patients with *RUNX1* mutations had a significantly higher incidence of *MLL/PTD* (9 of 62, 14.5%) than those with *RUNX1*-wild-type (19 of 408, 4.7%, P = .006). On the contrary, *CEBPA* and *NPM1* mutations were rarely seen in patients with *RUNX1* mutations (3.2%, P = .006; and 4.8%, P < .001, respectively). However, there was no difference in the incidence of *FLT3/ITD* and *FLT3/TKD* between patients with and without *RUNX1* mutation. The same was also true for *N-RAS*, *K-RAS*, *PTPN11*, *KIT*, and *WT1* mutations.

Table 3. Association of *RUNX1* mutation with chromosomal abnormalities*

Variable	Total	*RUNX1*-mutated	*RUNX1*-wild	P
Karyotype†				< .001
Favorable	59	0 (0.0)	59 (100.0)	
Intermediate	327	48 (14.7)	279 (85.3)	
Unfavorable	66	8 (12.1)	58 (87.9)	
Unknown	18	6 (33.3)	12 (66.7)	
Normal	230	32 (13.9)	198 (86.1)	= .622
Simple	169	19 (11.2)	150 (88.8)	
Complex	53	5 (9.4)	48 (90.6)	
t(8;21)	40	0 (0.0)	40 (100.0)	= .009
inv(16)	19	0 (0.0)	19 (100.0)	.149
t(11q23)	16	0 (0.0)	16 (100.0)	= .239
−5/5q-‡	1	0 (0.0)	1 (100.0)	> .999
−7/7q-‡	10	3 (30.0)	7 (70.0)	= .115
+8‡	22	6 (27.3)	16 (72.7)	= .042
+11‡	3	0 (0.0)	3 (100.0)	> .999
+13‡	1	0 (0.0)	1 (100.0)	> .999
+21‡	11	2 (18.2)	9 (81.8)	.634

*A total of 452 patients, including 56 *RUNX1*-mutated and 396 *RUNX1*-wild patients, had chromosome data at diagnosis.

†Favorable consists of t(15;17), t(8;21), inv (16); unfavorable, −7, del(7q), −5, del(5q), 3q abnormality, complex abnormalities; and intermediate, normal karyotype and other abnormalities.

‡Includes only simple chromosomal abnormalities with 2 or fewer changes, but not those with complex abnormalities with 3 or more aberrations. There was no association of *RUNX1* mutations with any of these chromosome changes, including +8, when complex abnormalities were also included in the analysis.

Table 4. Association of RUNX1 **mutation with other genetic mutations**

Variable	Whole cohort, n = 470	*RUNX1*-mutated patients, n = 62	*RUNX1*-wild patients, n = 408	P
CEBPA	66 (14.0)	2 (3.2)	64 (15.7)	> .006
FLT3/ITD	110 (23.4)	14 (22.6)	96 (23.5)	> .999
FLT3/TKD	33 (7.0)	6 (9.7)	27 (6.6)	= .380
N-RAS	54 (11.5)	5 (8.1)	49 (12.0)	= .520
K-RAS	16 (3.4)	2 (3.2)	14 (3.4)	> .999
PTPN11	21 (4.5)	4 (6.5)	17 (4.2)	> .504
JAK2	4 (0.8)	0 (0.0)	4 (0.9)	> .999
NPM1	106 (22.6)	3 (4.8)	103 (25.2)	< .001
MLL/PTD	28 (6.0)	9 (14.5)	19 (4.7)	= .006
KIT	14 (3.0)	0 (0.0)	14 (3.4)	.233
WTI	32 (6.8)	2 (3.2)	30 (7.4)	= .290

Values are no. (%) of patients with alteration.

Impact of RUNX1 mutation on response to therapy and clinical outcome

Of the 330 AML patients undergoing conventional induction chemotherapy, 248 patients (75.2%) achieved a CR. *RUNX1* mutation was associated with inferior response (CR rate, 56.8% vs 77.5%, $P = .009$). The multivariate analysis also showed that *RUNX1* mutation was an independent poor-risk factor for patients to achieve CR (hazard ratio [HR] = 0.443; 95% confidence interval [CI], 0.196-0.998, $P = .050$) independent from other covariates. With a median follow-up of 53 months (range, 1.0-160 months), patients with *RUNX1* mutation had significantly poorer OS than those without *RUNX1* mutation (median, 10.5 months vs 30.5 months, $P < .001$, Figure 2A). Subgroup analysis of patients with normal karyotype showed that *RUNX1*-mutation was also closely associated with worse OS ($P = .001$, Figure 2C). Similar
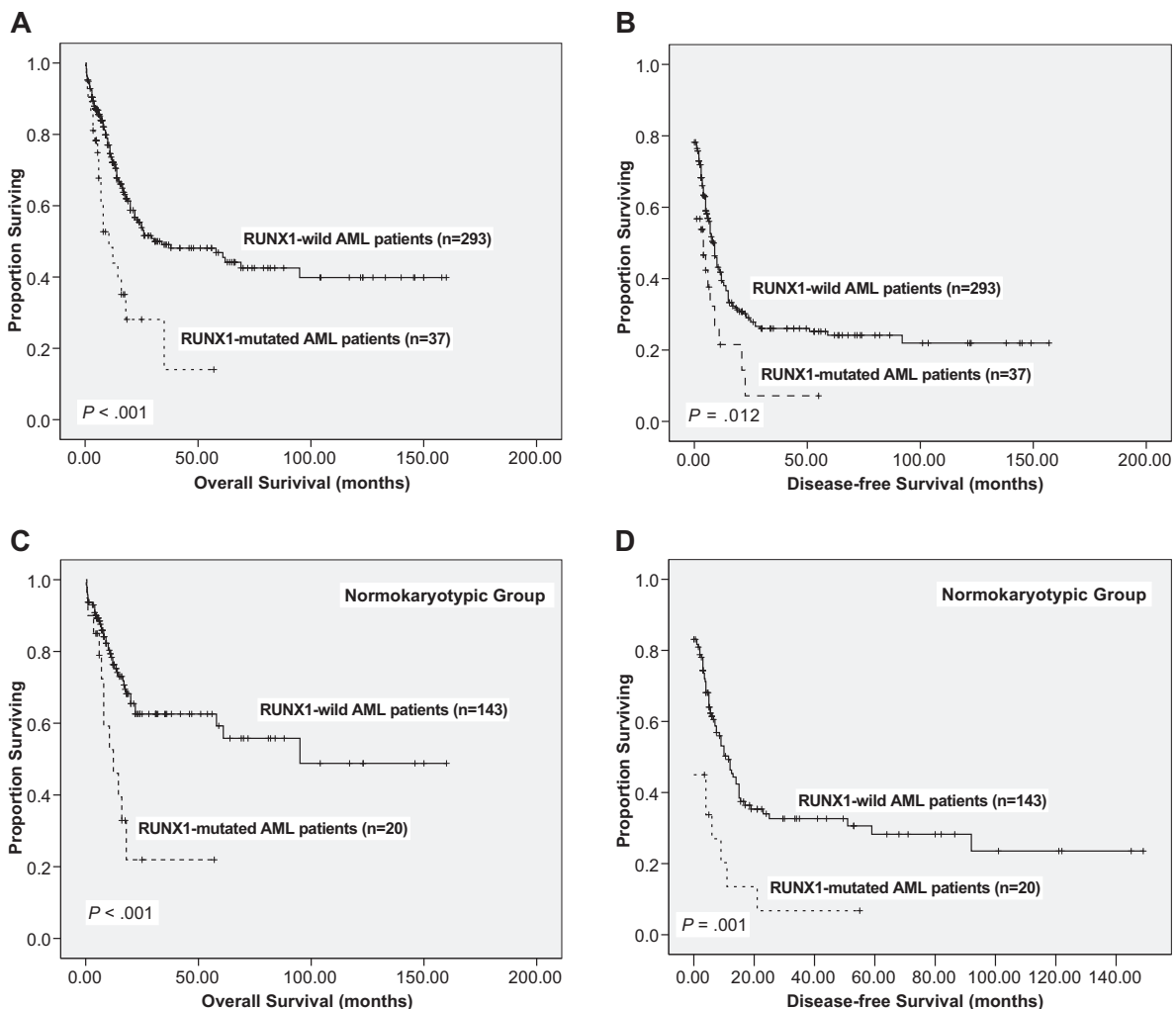

Figure 2. Kaplan-Meier survival curves according to *RUNX1* mutation status. (A-B) A total of 330 AML patients receiving standard chemotherapy. (C-D) Those patients with normal karyotype. Data are shown for OS (A,C) and DFS (B,D). Tick marks represent patients whose data were censored at the last time they were known to be alive (A) or in CR (B) or at the time of HSCT.

Table 5. Multivariate analysis (Cox regression) on the DFS and OS

Variable	DFS, 95% CI				OS, 95% CI			
	RR	Lower	Upper	P	RR	Lower	Upper	P
Age†	1.520	1.145	2.017	.004*	2.682	1.867	3.853	< .001*
Karyotype	2.104	1.625	2.724	< .001*	2.325	1.638	3.300	< .001*
LDH‡	1.203	0.895	1.616	.221	1.487	1.018	2.173	.040*
WBC§	1.336	0.973	1.834	.073	1.583	1.077	2.325	.019*
RUNX1	1.426	0.922	2.207	.111	1.878	1.109	3.179	.019*
CEBPA	0.420	0.274	0.644	< .001*	0.423	0.240	0.746	.003*
NPM1/FLT3-ITD‖	0.282	0.156	0.510	< .001*	0.276	0.132	0.578	.001*
WT1	1.861	1.204	2.877	.005*	2.155	1.219	3.809	.008*

*Statistically significant (*P* < .05).
†Age older than 50 years relative to age 50 years or younger (reference value).
‡LDH greater than 920 U/L versus less than 920 U/L.
§WBC greater than 50×10^9/L versus less than 50×10^9/L.
‖*NPM1*mutant/*FLT3-ITD−* versus other subtypes.

results could be demonstrated for DFS (Figure 2B,D). There was no significant difference in survival between patients with mutations within RHD and those with mutations outside it (*P* = .863), and between patients with frameshift mutations and others (*P* = .308).

In multivariate analysis (Table 5), the independent poor risk factors for OS were age older than 50 years, unfavorable karyotype, *RUNX1* mutation, *WT1* mutation, WBC count more than 50×10^9/L, and high LDH level. *CEBPA* mutation and *NPM1*^mutant^/*FLT3-ITD−* were independent favorable prognostic factors. Multivariate analysis for DFS revealed that the independent poor risk factors for DFS included unfavorable karyotype, *WT1* mutation, and age older than 50 years. There was a trend of poorer DFS in patients with *RUNX1* mutation (HR = 1.426; 95% CI, 0.922-2.207, *P* = .111). *CEBPA* mutation and *NPM1*^mutant^/*FLT3-ITD−* were independent favorable factors for DFS.

To confirm the prognostic significance of *RUNX1* mutation in AML, we also did multivariate analysis on the subgroup of patients (n = 234) who did not undergo allogeneic HSCT. *RUNX1* mutation was still an independent poor prognostic factor for OS (HR = 1.874; 95% CI, 1.075-3.269, *P* = .027) and a borderline poor prognostic factor for DFS (HR = 1.580; 95% CI, 0.940-2.653, *P* = .084) in this rather homogeneous cohort. However, among patients (n = 96) receiving allogeneic HSCT, the poor prognostic impact of *RUNX1* mutation in OS and DFS was lost, and it seemed that there was a trend of better outcome in patients with *RUNX1* mutation (HR = 0.465, *P* = .090 and HR = 0.317, *P* = .061, respectively). It implies that HSCT may ameliorate the poor survival impact of *RUNX1* mutations. However, only 11 patients with *RUNX1* mutations received HSCT in our cohort. Further study in more patients may be needed to clarify this point.

Sequential studies of *RUNX1* mutations in AML patients

RUNX1 mutations were serially studied in 294 samples from 133 patients, including 18 patients with distinct *RUNX1* mutations, 7 patients with insignificant *RUNX1* mutations, and 108 patients without mutation at diagnosis. Among the 18 patients with distinct *RUNX1* mutations, all lost their mutations at remission status (Table 1). In the 6 patients who had available samples for serial study at relapse, 2 patients (nos. 17 and 53) regained the same mutations as those detected at diagnosis. In the other 2 patients (nos. 3 and 35), one of the mutations detected at diagnosis remained, whereas the other one was lost at relapse. The remaining 2 patients (nos. 23 and 48) lost *RUNX1* mutation at relapse but retained *FLT3/ITD* (case no. 23) or *FLT3/TKD* and *WT1* mutation (case no. 48). In all 7 patients

with insignificant mutations (nos. 63-66 and 68-70), the mutations remained the same at CR and also at relapse in the 5 patients studied, suggesting that these mutations were germline origin and might not be relevant to leukemogenesis. Among the 108 patients who had no *RUNX1* mutation at diagnosis, none acquired *RUNX1* mutation at relapse, whereas karyotypic evolution was noted at relapse in 41.6% of them (data not shown).

Discussion

Our results showed that *RUNX1* mutation could be detected in 13.2% of adult patients with de novo non-M3 AML and was correlated with distinct clinical and biologic features. Furthermore, *RUNX1* mutation was an independent risk factor for poor prognosis.

The reported incidence of *RUNX1* mutation in AML varied from 2.9% to 46% depending on the patient population selected (eg, age range, history of MDS, FAB subtypes, and karyotype), the regions of *RUNX1* screened, and the methods used.[11,19,21,23,24,35-38] In most of the previous studies, the N-terminal exons 3 to 6 covering the RHD were selected for mutation screening based on the observation that mutations were clustered within this region in AML M0 and on the assumption that only approximately 10% of mutations were outside this region, and were seen mainly in MDS or in AML after MDS.[11,20,23,24] In the present series, 31 mutations (49%) were located in the C-terminal region. This frequency was significantly higher than previously assumed. Recently, Gaidzik et al reported that 34.4% (11 of 32) of *RUNX1* mutations were located in exon 8, 34.4% (11 of 32) in exon 3, and 31.2% (10 of 32) in other regions in adult AML.[38] In this report, they did not distinctly mention the incidence of mutations in exons 6 and 7. More studies are necessary to clarify the true incidence of *RUNX1* mutations in the C-terminal region.

Four kinds of *RUNX1* mutations were demonstrated in this study: missense mutations, nonsense mutations, frameshift mutations, and in-frame mutations (Figure 1). Most RHD mutations were missense mutations (20 of 31, 64.5%), whereas most TAD mutations were of frameshift (13 of 17, 76.5%). Excluding the 5 insignificant mutations, 24 missense mutations were noted. Previous reports have verified that several residues were important for DNA binding and heterodimerization with CBF-β.[39] The mutations occurring in these residues, such as

R80C, R80G, K83Q, K83R, R135K, R135S, R139Q, R139L, D171Y, D171G, and R174Q detected in this study, would result in loss of hydrogen bonding interaction to DNA or disruption of the folding architecture of RUNX1; additional S114P and S114W were supposed to abolish heterodimerization with CBF-β.[39] The remaining 7 RHD mutations and the 4 mutations outside the RHD region have not been reported previously but were supposed to be distinct mutations because these mutations involved residues that were highly conserved in the RUNX family (supplemental Figure 1). Of the 7 nonsense mutations, 4 mutations generated truncated RHD. The remaining 3 mutations (R178X, Q181X, and R293X), retaining the RHD region for DNA and CBF-β binding, would have a dominant negative effect on wild-type RUNX1.[34] Twenty-eight mutations were frameshift mutations (Figure 1); with the exception of P375fsX570, all resulted in partial or total deletion of the TAD and loss of transactivation potential.[34]

In this study of a large cohort of adults with de novo non-M3 AML, we have identified distinct clinical characteristics in patients with *RUNX1* mutation that either were not reported before or were different from previous studies in selected cohorts (eg, AML-M0 subtype, therapy- or MDS-related AML). We found that *RUNX1*-mutated patients were predominantly male, with a male-to-female ratio of 3.8, and were older than *RUNX1*-wild patients. The higher frequency of the leukemic cells to express CD34 and HLA-DR, but lower frequency to express CD15 in AML with *RUNX1* mutations, might reflect the association of this mutation with immature subtypes of AML, M0, and M1. In previous reports, *RUNX1* mutation was frequently associated with specific cytogenetic abnormalities, such as trisomies 8 (+8), +13, or +21[21]; however, we failed to find any association between *RUNX1* mutation and −5/5q-, −7/7q-, +11, +13, or +21, except for +8 (Table 3). Further, *RUNX1* mutation was never found in patients with t(15;17), t(8;21), or inv(16).

The role of *RUNX1* mutation in the leukemogenesis of AML remains to be defined. *RUNX1* mutation, a class II mutation, has been implicated as the initiating event to block differentiation of hematopoietic cells, and the subsequent class I gene mutation would synergistically provide growth advantages of these cells and lead to the development of AML.[11,40] As shown in Table 4 and supplemental Table 3, 31 of the 62 *RUNX1*-mutated patients (50%) concurrently had other gene mutations and the majority (26 of 31, 83.9%) simultaneously showed class I mutations, most commonly *FLT3/ITD*, *FLT3/TKD*, and *N-RAS*, which might result in hyperactivation of the receptor tyrosine kinase-RAS signaling pathways.[41] This finding was consistent with the current hypothesis of 2-hit model of leukemogenesis.[5,6] However, *MLL/PTD*, another class II mutation, was also found in a significantly higher frequency in *RUNX1*-mutated patients (9 of 62, 14.5%) than in *RUNX1*-wild patients (19 of 408, 4.7%, *P* = .006). Intriguingly, 8 of the 9 patients with concurrent *RUNX1* mutation and *MLL/PTD* also had simultaneously *FLT3/ITD* (7 cases) or *FLT3/TKD* (1 case) (supplemental Table 3). The molecular interaction among *RUNX1*, *FLT3/ITD*, and *MLL/PTD* deserves further investigation. Matsuno et al[40] reported a higher frequency of *FLT3* mutation in AML M0 patients with *RUNX1* mutation (5 of 8, 63%) than in those with *RUNX1*-wild type (5 of 41, 12%, *P* = .005). Dicker et al,[21] however, found that *FLT3* mutations were equally distributed between *RUNX1*-mutated and -wild patients. In this study,

although *FLT3* mutation was the most frequent gene alteration cooperative with *RUNX1* mutation, it occurred with similar incidence in *RUNX1*-mutated and -wild patients.

To the best of our knowledge, this is the first report of sequential study of *RUNX1* mutation in a large number of AML patients during the clinical course. It showed that all 18 patients with distinct *RUNX1* mutations studied lost the mutations at CR, indicating that these mutations were acquired in the leukemogenesis. On the other hand, all 108 patients without *RUNX1* mutation at diagnosis remained *RUNX1*-wild at relapse. Furthermore, 2 of the 6 patients who had *RUNX1* mutations at diagnosis lost the mutations at relapse (Table 1). These results suggested that, although *RUNX1* mutations are important for the development of AML, they may play a small role in disease progression. Further studies are warranted to confirm the result.

So far, there has been no large cohort study to address the impact of *RUNX1* mutation on treatment response and clinical outcome in primary AML. In the present study, the adult AML patients with *RUNX1* mutation had inferior response to conventional chemotherapy and poorer OS and DFS than those without the mutation. The same was also true for the subgroup of patients with normal karyotype. The multivariate analysis confirmed that *RUNX1* mutation was a poor prognostic factor for OS independent from other risk factors, such as age, karyotype, WBC, LDH, *NPM1/FLT3-ITD*, *WT1*, and *CEBPA* mutation. These results suggest that *RUNX1* can be used as a new candidate molecular marker, along with *NPM1/FLT3-ITD* and *CEBPA* mutations, to stratify patients in the management of AML.

In this study, 5 types of missense mutations (G42R, D317N, Q370R, V425G, and M439L) could be detected both at diagnosis and at CR. None of these sequence alterations was recorded as polymorphisms or variants in the GenBank database,[42] except for G42R, which was never reported in healthy Japanese persons.[19,43] Comparison of RUNX proteins family among different species revealed that these amino acid changes might have no effect on functional alteration (supplemental Figure 1). Sequence variation in *RUNX1* gene has been found not infrequently before. In normal British persons, 2 *RUNX1* variations, L29S and S21syn, could be found in 5% and 3%, respectively.[37] Although it was suggested that these mutations were germline mutations or variations that were functionally insignificant and might not be relevant to leukemogenesis, germline mutations of *RUNX1* gene have been reported in familial platelet disorder with propensity to myeloid leukemia.[44-46] However, patients with the 5 mutations mentioned did not have other hematologic diseases before, nor did their families. To clarify whether these mutations were involved in leukemogenesis, further studies, such as mutation analysis on a large cohort of normal persons or functional analyses of these mutations, need to be done.

In conclusion, this study demonstrated that *RUNX1* mutations could be detected in a substantial proportion of patients with de novo AML and were closely associated with male sex, older age, immature FAB subtypes, and trisomy 8. They were mutually exclusive with *CEBPA* and *NPM1* mutations but were closely associated with *MLL/PTD*. Furthermore, the *RUNX1* mutation was an independent poor risk factor for OS. Sequential study during the clinical course for 133 patients showed that none of these patients acquired novel *RUNX1* mutation at relapse, indicating that the mutation may play a small role in disease progression.

Authorship

Contribution: J.-L.T. and H.-A.H. were responsible for literature collection, data management and interpretation, and manuscript writing; L.-I.L. was responsible for mutation interpretation and manuscript writing; C.-Y.L was responsible for statistical analysis and interpretation of the statistical findings; C.-Y.C., W.-C.C., M.Y., S.-Y.H., B.-S.K., S.-C.H., S.-J.W., W.T., and Y.-C.C. contributed patient samples and clinical data; M.-H.T., C.-F.H., F.-Y.L., and M.-C.L. performed the laboratory research and chromosomal studies; and H.-F.T. planned, designed, wrote the manuscript, and coordinated the study over the entire period.

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References

1. Lowenberg B. Acute myeloid leukemia: the challenge of capturing disease variety. *Hematology.* 2008:1-11.

2. Renneville A, Roumier C, Biggio V, et al. Cooperating gene mutations in acute myeloid leukemia: a review of the literature. *Leukemia.* 2008;22(5):915-931.

3. Mrozek K, Marcucci G, Paschka P, Whitman SP, Bloomfield CD. Clinical relevance of mutations and gene-expression changes in adult acute myeloid leukemia with normal cytogenetics: are we ready for a prognostically prioritized molecular classification? *Blood.* 2007;109(2):431-448.

4. McCormack E, Bruserud O, Gjertsen BT. Review: genetic models of acute myeloid leukaemia. *Oncogene.* 2008;27(27):3765-3779.

5. Frohling S, Scholl C, Gilliland DG, Levine RL. Genetics of myeloid malignancies: pathogenetic and clinical implications. *J Clin Oncol.* 2005;23(26):6285-6295.

6. Gilliland DG. Molecular genetics of human leukemias: new insights into therapy. *Semin Hematol.* 2002;39[suppl 3]:6-11.

7. Gaidzik V, Dohner K. Prognostic implications of gene mutations in acute myeloid leukemia with normal cytogenetics. *Semin Oncol.* 2008;35(4):346-355.

8. Thiede C, Koch S, Creutzig E, et al. Prevalence and prognostic impact of NPM1 mutations in 1485 adult patients with acute myeloid leukemia (AML). *Blood.* 2006;107(10):4011-4020.

9. Schlenk RF, Dohner K, Krauter J, et al. Mutations and treatment outcome in cytogenetically normal acute myeloid leukemia. *N Engl J Med.* 2008;358(18):1909-1918.

10. Ito Y. RUNX genes in development and cancer: regulation of viral gene expression and the discovery of RUNX family genes. *Adv Cancer Res.* 2008;99:33-76.

11. Osato M. Point mutations in the RUNX1/AML1 gene: another actor in RUNX leukemia. *Oncogene.* 2004;23(24):4284-4296.

12. Yamagata T, Maki K, Mitani K. Runx1/AML1 in normal and abnormal hematopoiesis. *Int J Hematol.* 2005;82(1):1-8.

13. Kitabayashi I, Aikawa Y, Nguyen LA, Yokoyama A, Ohki M. Activation of AML1-mediated transcription by MOZ and inhibition by the MOZ-CBP fusion protein. *EMBO J.* 2001;20(24):7184-7196.

14. Yoshida H, Kitabayashi I. Chromatin regulation by AML1 complex. *Int J Hematol.* 2008;87(1):19-24.

15. Friedman AD. Cell cycle and developmental control of hematopoiesis by Runx1. *J Cell Physiol.* 2009;219(3):520-524.

16. Niebuhr B, Fischer M, Tager M, Cammenga J, Stocking C. Gatekeeper function of the RUNX1 transcription factor in acute leukemia. *Blood Cells Mol Dis.* 2008;40(2):211-218.

17. Michaud J, Wu F, Osato M, et al. In vitro analyses of known and novel RUNX1/AML1 mutations in dominant familial platelet disorder with predisposition to acute myelogenous leukemia: implications for mechanisms of pathogenesis. *Blood.* 2002;99(4):1364-1372.

18. Osato M, Yanagida M, Shigesada K, Ito Y. Point mutations of the RUNx1/AML1 gene in sporadic and familial myeloid leukemias. *Int J Hematol.* 2001;74(3):245-251.

19. Taketani T, Taki T, Takita J, et al. AML1/RUNX1 mutations are infrequent, but related to AML-M0, acquired trisomy 21, and leukemic transformation in pediatric hematologic malignancies. *Genes Chromosomes Cancer.* 2003;38(1):1-7.

20. Harada H, Harada Y, Niimi H, Kyo T, Kimura A, Inaba T. High incidence of somatic mutations in the AML1/RUNX1 gene in myelodysplastic syndrome and low blast percentage myeloid leukemia with myelodysplasia. *Blood.* 2004;103(6):2316-2324.

21. Dicker F, Haferlach C, Kern W, Haferlach T, Schnittger S. Trisomy 13 is strongly associated with AML1/RUNX1 mutations and increased FLT3 expression in acute myeloid leukemia. *Blood.* 2007;110(4):1308-1316.

22. Silva FP, Lind A, Brouwer-Mandema G, Valk PJ, Giphart-Gassler M. Trisomy 13 correlates with RUNX1 mutation and increased FLT3 expression in AML-M0 patients. *Haematologica.* 2007;92(8):1123-1126.

23. Roumier C, Eclache V, Imbert M, et al. M0 AML, clinical and biologic features of the disease, including AML1 gene mutations: a report of 59 cases by the Groupe Francais d'Hematologie Cellulaire (GFHC) and the Groupe Francais de Cytogenetique Hematologique (GFCH). *Blood.* 2003;101(4):1277-1283.

24. Preudhomme C, Warot-Loze D, Roumier C, et al. High incidence of biallelic point mutations in the Runt domain of the AML1/PEBP2 alpha B gene in Mo acute myeloid leukemia and in myeloid malignancies with acquired trisomy 21. *Blood.* 2000;96(8):2862-2869.

25. Chen CY, Lin LI, Tang JL, et al. RUNX1 gene mutation in primary myelodysplastic syndrome: the mutation can be detected early at diagnosis or acquired during disease progression and is associated with poor outcome. *Br J Haematol.* 2007;139(3):405-414.

26. Tien HF, Wang CH, Lin MT, et al. Correlation of cytogenetic results with immunophenotype, genotype, clinical features, and ras mutation in acute myeloid leukemia: a study of 235 Chinese patients in Taiwan. *Cancer Genet Cytogenet.* 1995;84(1):60-68.

27. Chou WC, Tang JL, Lin LI, et al. Nucleophosmin mutations in de novo acute myeloid leukemia: the age-dependent incidences and the stability during disease evolution. *Cancer Res.* 2006;66(6):3310-3316.

28. Falini B, Mecucci C, Tiacci E, et al. Cytoplasmic nucleophosmin in acute myelogenous leukemia with a normal karyotype. *N Engl J Med.* 2005;352(3):254-266.

29. Lin LI, Chen CY, Lin DT, et al. Characterization of CEBPA mutations in acute myeloid leukemia: most patients with CEBPA mutations have biallelic mutations and show a distinct immunophenotype of the leukemic cells. *Clin Cancer Res.* 2005;11(4):1372-1379.

30. Chen CY, Lin LI, Tang JL, et al. Acquisition of JAK2, PTPN11, and RAS mutations during disease progression in primary myelodysplastic syndrome. *Leukemia.* 2006;20(6):1155-1158.

31. Shiah HS, Kuo YY, Tang JL, et al. Clinical and biological implications of partial tandem duplication of the MLL gene in acute myeloid leukemia without chromosomal abnormalities at 11q23. *Leukemia.* 2002;16(2):196-202.

32. Hou HA, Chou WC, Lin LI, et al. Characterization of acute myeloid leukemia with PTPN11 mutation: the mutation is closely associated with NPM1 mutation but inversely related to FLT3/ITD. *Leukemia.* 2008;22(5):1075-1078.

33. Paschka P, Marcucci G, Ruppert AS, et al. Wilms' tumor 1 gene mutations independently predict poor outcome in adults with cytogenetically normal acute myeloid leukemia: a cancer and leukemia group B study. *J Clin Oncol.* 2008;26(28):4595-4602.

34. Harada Y, Harada H. Molecular pathways mediating MDS/AML with focus on AML1/RUNX1 point mutations. *J Cell Physiol.* 2009;220(1):16-20.

35. Schnittger S, Dicker F, Wendland N, et al. RUNX1 mutations can be found in 34% of de novo AML with normal karyotype or single chromosomal imbalances and are associated with good prognosis but turn to unfavourable outcome if further cytogenetic or molecular mutations are acquired [abstract]. *Blood.* 2008;112(11):146a.

36. Osato M, Asou N, Abdalla E, et al. Biallelic and heterozygous point mutations in the runt domain of the AML1/PEBP2alphaB gene associated with myeloblastic leukemias. *Blood.* 1999;93(6):1817-1824.

37. Langabeer SE, Gale RE, Rollinson SJ, Morgan GJ, Linch DC. Mutations of the AML1 gene in acute myeloid leukemia of FAB types M0 and M7. *Genes Chromosomes Cancer.* 2002;34(1):24-32.

38. Gaidzik VI, Schlenk RF, Wittke K, et al. RUNX1 mutations in acute myeloid leukemia (AML): correlation with distinct cytogenetic subgroups and clinical outcome. Results of the AML Study Group (AMLSG) [abstract]. *Blood.* 2008 2008;112(11):145a.

39. Tahirov TH, Inoue-Bungo T, Morii H, et al. Structural analyses of DNA recognition by the AML1/Runx-1 Runt domain and its allosteric control by CBF-βeta. *Cell.* 2001;104(5):755-767.

40. Matsuno N, Osato M, Yamashita N, et al. Dual mutations in the AML1 and FLT3 genes are associated with leukemogenesis in acute myeloblastic leukemia of the M0 subtype. *Leukemia.* 2003; 17(12):2492-2499.

41. Niimi H, Harada H, Harada Y, et al. Hyperactivation of the RAS signaling pathway in myelodysplastic syndrome with AML1/RUNX1 point mutations. *Leukemia.* 2006;20(4):635-644.

42. NIH genetic sequence database, GenBank. http://www.ncbi.nlm.nih.gov/Genbank. Accessed March 12, 2009.

43. Harada H, Harada Y, Tanaka H, Kimura A, Inaba T. Implications of somatic mutations in the AML1 gene in radiation-associated and therapy-related myelodysplastic syndrome/acute myeloid leukemia. *Blood.* 2003;101(2):673-680.

44. Owen CJ, Toze CL, Koochin A, et al. Five new pedigrees with inherited RUNX1 mutations causing familial platelet disorder with propensity to myeloid malignancy. *Blood.* 2008;112(12):4639-4645.

45. Preudhomme C, Renneville A, Bourdon V, et al. High frequency of RUNX1 biallelic alteration in acute myeloid leukemia secondary to familial platelet disorder. *Blood.* 2009;113(22):5583-5587.

46. Kirito K, Sakoe K, Shinoda D, Takiyama Y, Kaushansky K, Komatsu N. A novel RUNX1 mutation in familial platelet disorder with propensity to develop myeloid malignancies. *Haematologica.* 2008;93(1):155-156.