行政院國家科學委員會補助專題研究計畫成果報告

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※　急性骨髓性白血病 MLL 基因部分縱列複製　※
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計畫主持人：田蕙芬

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行政院國家科學委員會專題研究計畫成果報告

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中文摘要:

染色體的異常已是臨床上治療急性骨髓性白血病(AML)很重要的指標，它不但可做為白血病診斷及分類的參考，不同的染色體變化，也影響治療的選擇，同時這些變化在白血病的發生上也扮演著重要角色。但約有 30%-40%的 AML 病人沒有染色體的異常，這些病人可能存在著由傳統染色體檢驗法偵測不到的各種不同的基因異常，因此這些病人雖籠統的歸屬於 intermediate cytogenetic risk group，但卻極可能包含著由好至壞，各個不同預後的族群。如何將這些染色體檢查屬 normal karyotype 的病人，做更適當的分類，將是很重要的課題。最近這類病人中有一部分被發現有 MLL (mixed-lineage leukemia)基因的 partial tandem duplication。MLL 基因位於染色體 11q23 上，最初是在具 11q23 轉位(translocation)的病人中發現有此基因重組，之後在部分染色體正常的病人或有 11q23 以外染色體異常的病人，也偵測到此基因的重組，後者經進一步 PCR (polymerase chain reaction)的檢驗，發現他們 MLL 的重組是由於此基因的 partial tandem duplication 所造成，與 11q23 translocation 不同。零星的報告顯示，染色體檢查正常，但帶有 MLL tandem duplication 的病人預後較差，但尚未得到共識。MLL tandem duplication 對帶有 11q23 以外染色體異常之 AML 病人預後的影響更是不清楚。

我們在中華民國兒童癌症基金會的資助下，曾對 113 例 AML 病人進行 MLL 基因重組的研究，發現 14%的病人有 MLL 基因重組，包括 13%染色體正常及 15%有 11q23 以外染色體異常的病人，後二者基因重組的機轉，在前項計畫中並未進一步研究。在這次研究計畫中，我們希望對 AML 病人進行 MLL 基因 tandem duplication 的偵測，除了原先 113 例病人外，目前又新增了一百多例 AML 病人，預期總共約一百例染色體正常的病人及約 50 例具 11q23 以外染色體異常的病人。除了 cytogenetic study 外，我們將進行 Southern blotting, reverse transcriptase PCR (RT-PCR)，及 genomic DNA 之 PCR，相互對照，如此可測知有無 MLL 基因重組，其基因重組是否來自 tandem duplication，及造成 tandem duplication 是在 DNA 層次或在 RNA。所得結果將與病人的臨床表現、血液學及染色體變化作一對照，MLL 基因 tandem duplication 在臨床上的意義應可得到釐清，有助於臨床上對病人治療的決定，這種變化亦可用以偵測 minimal residual disease。

關鍵詞：急性骨髓性白血病，混雜型白血病基因，部分縱列複製

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Revision I

Title: Clinical and biologic implications of partial tandem duplication of the MLL gene in acute myeloid leukemia without chromosomal abnormalities at 11q23

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Running Title: Implications of MLL duplication in AML

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ABSTRACT

The clinical and biological features of acute myeloid leukemias (AML) with 11q23/MLL translocations are well known, but the characteristics of AML with partial tandem duplication of the MLL gene have not been explored comprehensively. In this study, MLL duplication was analyzed on 81 AML patients without chromosomal abnormalities at 11q23 using Southern blotting, genomic DNA polymerase chain reaction (PCR), reverse-transcription PCR and complementary DNA sequencing. Nine patients showed partial tandem duplication of the MLL gene, including eight (12%) of the 68 with normal karyotype. Seven patients showed fusion of exon 6/exon 2 (e6/e2), one, combination of differentially spliced transcripts e7/e2 and e6/e2, and the remaining one, combination of e8/e2 and e7/e2. Among the patients with normal karyotype, children aged 1 to 15 had a trend of higher frequency of MLL duplication than others (2/5 or 40% vs 6/62 or 10%, P = 0.102). The patients with tandem duplication of the MLL gene showed a significantly higher incidence of CD11b expression on the leukemic cells than those without in the subgroup of patients with normal karyotype (75% *vs* 28%, $P = 0.017$). There were no significant differences in the expression of lymphoid antigens or other myeloid antigens between the two groups of patients. In adults, the patients with MLL duplication had a shorter median survival time than those without (4.5 months *vs* 12 months, $P = 0.036$). In conclusion, partial tandem duplication of the MLL gene is associated with increased expression of CD11b on leukemic blasts and implicates poor prognosis in adult AML patients. The higher frequency of MLL duplication in children older than one year than in other age groups needs to be confirmed by further studies.

Keywords: MLL gene; partial tandem duplication; acute myeloid leukemia.

INTRODUCTION

The prognostic impact of chromosomal aberrations in acute leukemia has been well recognized and the non-random cytogenetic abnormalities are increasingly applied to treatment planning and monitoring of the disease.[1] The chromosomal alterations at 11q23 are among the most common cytogenetic abnormalities in acute leukemia and may involve in leukemogenesis.[2-4] Recently, MLL (for mixed-lineage or myeloid-lymphoid leukemia) gene that spans the 11q23 breakpoints has been cloned, which encodes a protein with several regions of homology to the *Drosophila* trithorax protein and is a putative transcription factor.[5,6] Rearrangements of the MLL gene can be demonstrated not only in most acute leukemias with various 11q23 translocations[7-9] but also in some acute myeloid leukemia (AML) without cytogenetic abnormalities at 11q23.[10-12] Molecular characterization of the MLL gene in the latter condition has revealed that the gene is rearranged by partial tandem duplication.[13] The duplication involved a portion of the gene corresponding to the

amino-terminus of the MLL protein which contains the AT hook and a region of homology to DNA methyltransferase (MT) motifs, two domains suggested to be critical for leukemogenesis.[14]

AML with 11q23/MLL translocation are well characterized that they occur more frequently in infants than in adults and children older than 1 year, usually show M4/M5 phenotype, and coexpress myeloid- and lymphoid-associated antigens.[8,15,16] However, the clinical and biological characteristics of AML with partial tandem duplication of the MLL gene have not been studied comprehensively. These patients were found to have poor prognosis,[17,18] and almost all patients who achieved a complete remission relapsed, but the treatments of choice for these patients are not clear. In addition, the age distribution and immunophenotype of the leukemic cells in this subgroup of AML patients are totally unknown. In this study, partial tandem duplication of the MLL gene was studied on 81 AML patients without 11q23 abnormalities; 68 of them had normal karyotype. It was found that children older than one year tend to have a higher incidence of partial tandem duplication of the MLL gene than infants and adults, and the patients with the gene change had a higher frequency of CD11b expression on the leukemic cells than others. The adult AML patients with the gene rearrangement had a shorter median survival time than those without the gene change. Two of the three patients with MLL duplication who received allogeneic stem cell transplantation remained in continuous complete remission (CR) at 27 and 41 months respectively, suggesting that this procedure may reverse the poor outcome of this subgroup of patients.

PATIENTS AND METHODS

Patients

Partial tandem duplication of the MLL gene was studied on 81 newly diagnosed patients with *de novo* AML at presentation in National Taiwan University Hospital. Patients with normal karyotype were selectively recruited, and those with chromosomal abnormalities involving 11q23 were excluded. No patients had a history of prior exposure to chemotherapy or radiotherapy, and none had a history of other underlying hematological or malignant disorders. The median age was 46 years, ranging from 4 months to 80 years. There were three infants, seven children (> 1 years, ≤ 15 years), and 71 adults. Forty-two were males and 39, females. The diagnosis and classification of AML were based on the criteria established by the French-American-British (FAB) Cooperative Study Group.[19-21] Sixty-two patients received conventional induction chemotherapy with one of the anthracyclines (doxorubicin 30 mg/m^2/day or idarubicin 12 mg/m^2/day) for 3 days and cytosine arabinoside (cytarabine) 100-200 mg/m^2/day for 7 days. Nineteen patients did not receive any chemotherapy or were only treated with low dose cytarabine. Among the patients who received conventional induction treatment, forty-two patients obtained a complete remission (CR); one of them did

not received further treatment, eight patients received allogeneic hematopoietic stem cell transplantation, and the remaining 33 patients underwent either standard dose of consolidation chemotherapy with cytarabine and one of the anthracyclines (22 patients), or high dose cytarabine (1-3 gm/m^2 every 12 hours) for 8 doses with or without VP-16 or one of the anthracyclines (11patients).

Cytogenetic study

Chromosome analysis was performed as described previously.[22] Briefly, bone marrow (BM) cells were harvested either directly or after 1-3 days of nonstimulated culture. Metaphase chromosomes were banded by the conventional trypsin-Giemsa banding technique and karyotyped according to the International System for Human Cytogenetic Nomenclature.[23]

Immunophenotyping

A panel of monoclonal antibodies (Immunotech Inc., Marseille Cedex 9, France) to myeloid-associated antigens including CD13, CD33, CD11b, CD14, CD15, glycophorin A and CD41a, as well as to lymphoid-associated antigens including CD2, CD5, CD7, CD10, CD19 and CD20, and to lineage-nonspecific antigens CD34 and HLA-DR were selected to characterize immunologic phenotypes of leukemic cells. An indirect immunoalkaline phosphatase method was applied to detect the expression of surface antigens on leukemic cells.[24] The cut-off value for positive result of the markers was more than 20%.

Nucleic acid preparation

Mononuclear cells obtained from bone marrow aspirates were isolated by Ficoll-Hypaque gradient centrifugation and cryopreserved on the same day of sample collection with a few exceptions. Genomic DNAs were extracted by using a conventional isolation method.[24] Total cellular RNAs were extracted from cryopreserved cells by a modified one-step guanidinium thiocyanate-phenol-chloroform method using RNAzol B commercial reagent (Biotecx Laboratories, Houston, TX, USA) as described previously.[25]

Detection of partial tandem duplication of the MLL gene

Partial tandem duplication of the MLL gene was detected by Southern blot analysis, genomic DNA polymerase chain reaction (PCR), reverse-transcription PCR (RT-PCR) and complementary DNA (cDNA) sequencing.

Southern blot analysis was performed as described previously.[12] Genomic DNAs were digested with restriction enzymes *Bam*HI and *Hind*III. After being size-fractionated by electrophoresis and transferred to Hybond membrane (Amersham Pharmacia Biotech, England, UK), they were hybridized with ^{32}P-dCTP-labelled probe. The probe used was a 740-bp cDNA fragment of the MLL gene spanning exons 5 through 11 (kindly provided by J.R. Downing, St Jude Children's Research Hospital, Memphis, TN, USA).

PCR of the genomic DNAs was done according to the method described previously.[22] The primer set used was the same as that designed by Caligiuri *et al*[26]: 6.1

(5'-GTCCAGAGCAGAGCAAACAG-3') from exon 6 (sense orientation) and 2.0R (5'-CGCACTCTGACTTCTTCATC-3') from exon 2 (antisense orientation).

Reverse transcription was performed at 37℃ for 1.5 hours in a volume of 20 μl including 1 to 5 μg of total cellular RNA, 50 ng of random hexamer, 25 units rRNasin, 200 units M-MLV reverse transcriptase (Promega, Madison, WI, USA), deoxynucleotides (final concentration 0.5 mM each), and 4 μl of 5x RT buffer as described previously.[25] The cDNA was then amplified by nested PCR.[25] The PCR primer set for the first round amplification was 5.3 (5'-GGAAGTCAAGCAAGCAGGTC-3') from exon 5 (sense) and 654c (5'-AGGAGAGAGTTTACCTGCTC-3') from exon 3 (antisense), as reported by Caligiuri *et al*[26] and that for the second round amplification was 6.1 from exon 6 (sense) and 400c (5'-ACACAGATGGATCTGAGAGG-3') from exon 3 (antisense). PCR was run in a volume of 50 μl containing 5 μl of 10x PCR buffer, 2.5 μl of each primer (final concentration 0.5 μM each), deoxynucleotides (final concentration 125 μM each) and 1 unit Taq DNA polymerase (Qiagen Inc., Valencia, CA, USA). The mixture was denatured at 95℃ for 1 minute, annealed at 60℃ for 1 minute and extended at 72℃ for 1 minute for 35 cycles. All the PCR products were analyzed by electrophoresis on 2.0% agarose gel and visualized with ethidium bromide under an ultraviolet lamp.

The presence of partial tandem duplication of the MLL gene was also confirmed by direct cDNA sequencing using a method described previously.[25] The PCR-amplified cDNA was gel purified, and then sequenced using the Applied Biosystems 373A DNA sequencer (Perkin-Elmer Corp., Foster City, CA, USA) with data-processing programs from the Genetics Computer Group system (IntelliGenetics, Campbell, CA, USA).

Statistics

Continuous variables were compared by Wilcoxon rank-sum test and discrete ones, by χ^2 or Fisher's exact test. Curves of survival and complete remission duration were plotted by the Kaplan-Meier method; differences between curves were analyzed by the log-rank test. The patients who received allogeneic hematopoietic stem cell transplantation were censored at the date of the procedure. The significance of results were defined as a level of $P < 0.05$ at both tails. All statistical analyses were done using the SPSS for Windows Release 10.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

Partial tandem duplication of the MLL gene and its correlation with clinical and hematological features

Nine (11%) of the 81 patients were found to have partial tandem duplication of the MLL gene (Table 1 and Figures 1 to 3). Seven patients showed fusion of MLL exon 6/exon 2 (e6/e2), one revealed combination of differentially spliced transcripts e7/e2 and e6/e2 (case 3), and the remaining one, combination of e8/e2 and e7/e2 (case 8). One patient who showed

positive result in RT-PCR but negative in Southern blotting and genomic DNA PCR was not considered to have partial tandem duplication of the MLL gene and was excluded from the study.

The clinical and hematological features of the patients with partially duplicated MLL gene were compared with those of the patients without the change (Table 2). Among the 81 patients, 68 showed normal karyotype of the BM cells, 12 had chromosomal abnormalities, including two t(8;21), two t(7;11), one 3q abnormality and 7 nonspecific aberrations. One patient did not have cytogenetic study. Eight (12%) of the 68 patients with normal karyotype were found to have partial tandem duplication of the MLL gene. Cytogenetic data was not available for the remaining one case with partially duplicated MLL gene.

The median age was 34 years (range 13-79 years) in the group of patients with partial tandem duplication of the MLL gene, compared with 47 years (range 0.3-80 years) in the group without the change ($P = 0.266$). Partial tandem duplication of the MLL gene was found in 2 (29%) of the 7 children aged between one to 15 years, compared with that in 8 (11%) of the 71 adults and none of the 3 infants; but the difference did not reach statistical significance ($P = 0.266$). The trend became more prominent when the analysis was performed only on the patients with normal karyotype (2/5 or 40% in children older than one year, *vs* 6/62 or 10% in adults, and 0/1 in infants, $P = 0.102$). There was a trend of lower hemoglobin (Hb) level in the group of patients with MLL duplication than in others (Hb 6.4 g/dl *vs* 8.5 g/dl, $P = 0.056$). Other clinical and hematological features were not significantly different between the two groups of patients. Similar results could be demonstrated when the analyses were done separately on the adults and children (Table 2) or on the subgroup of patients with normal karyotype (data not shown).

Correlation of partial tandem duplication of MLL gene with FAB subtype and immunophenotype

Among the 81 newly diagnosed AML, 24 were M1, 32 M2, 19 M4, 4 M5, 1 M7 subtypes, and 1 undetermined. Three of the M4 patients (16%), 4 of the M2 patients (13%), 2 of the M1 patients (8%) and none of the M5 and M7 patients showed partial tandem duplication of the MLL gene; the difference was not statistically significant *($P = 0.865$). The patients with partial tandem dup*lication of the MLL gene had a trend of higher frequency of CD11b expression than those without the gene rearrangement (67% *vs* 28%, $P = 0.051$, Table 3); the difference became statistically significant when the analysis was restricted to the patients with normal karyotype (75% *vs* 28%, $P = 0.017$). There were no much differences in the expression of other surface antigens between the two groups of patients whether the analyses were performed on all patients who had the study or only on those with normal karyotype (Table 3).

Correlation of partial tandem duplication of the MLL gene with outcome

Sixty-two patients, including 7 with partially duplicated MLL gene and 55 without,

underwent conventional combination chemotherapy, which consisted of cytarabine and one of the anthracyclines; these patients were the subjects of the outcome analysis. The CR rate was not significantly different between the patients with partial tandem duplication of the MLL gene and those without (57% with 95% confidence interval, CI: 8-107% *vs* 69% with 95% CI: 56-82%, $P = 0.671$, Table 2). Three of the four patients who had partial tandem duplication of the MLL gene and achieved CR received allogeneic hematopoietic stem cell transplantation; two of them, one child and one adult, remained in continuous CR at 41 and 27 months respectively, and the third one, a child, relapsed 8 months after the procedure, but were still alive with a survival time of 25 months at the date of analysis. The remaining one patient was treated with conventional consolidation chemotherapy and died of complication during the treatment. Among the 38 patients without the MLL duplication who achieved CR, 5 received allogeneic hematopoietic stem cell transplantation; 3 of them were in continuous CR at 96, 36 and 21 months respectively, one relapsed, and the remaining one died of chronic hepatitis B with acute exacerbation while in CR. Among the remaining 33 patients, except the one without further treatment after obtaining CR, 32 patients were treated with either standard dose of consolidation chemotherapy (21 patients) or high dose of cytarabine with or without one of anthracyclines or VP-16 (11 patients). Six of the 33 patients were alive and remained in the first CR, 3 died of complication during consolidation chemotherapy, 2 were loss of follow-up while in first CR, and 22 relapsed. The median CR duration and survival time between the two groups of patients with partial tandem duplication of the MLL gene and those without were not different (not reached *vs* 10 months, $P = 0.561$ and 4.5 months *vs* 12 months, $P = 0.141$, respectively, Table 2). Similar results could be demonstrated when the analyses were performed only on the patients with normal karyotype (median CR duration: not reached vs 10 months, $P = 0.490$ and median survival time 4.5 months vs 12 months, $P = 0.164$). However, when the analysis was performed only on the adults, the patients with partial tandem duplication of the MLL gene had a significantly shorter median survival time than those without (4.5 months *vs* 12 months, $P = 0.036$, Table 2 & Figure 4), but the CR rate and CR duration were not statistically different between the two groups of patients.

DISCUSSION

In this study, partial tandem duplication of the MLL gene was found in 11% of all AML patients and 12% of those with normal karyotype, incidences similar to those reported in literature.[17,18,27] Although infant AML was found to have high frequency (around 50%) of the MLL gene rearrangements, they usually involved reciprocal translocation of the MLL gene with its partner genes.[8,12,16,28] Little is known about the frequency of partial tandem duplication of the MLL gene in infant as well as other pediatric AML. In this study, we did not observe such abnormality in any infant, but children more than one year tended to have a higher frequency of MLL duplication than patients in other age groups, especially among the

patients with normal karyotype. Because the number of cases studied is limited, these results need to be confirmed by further studies on more pediatric patients. All other studies regarding the MLL gene duplication were performed on adults[17,18,27] with one exception, in which a slightly higher incidence of MLL duplication in pediatric AML than in adult AML was suggested.[29] The only child, aged 9, recruited in the study of Schnittger *et al*[18] also showed tandem duplication of the MLL gene. Similar to other reports, we did not observe a significantly higher incidence of the MLL duplication in the FAB M4/M5 subtypes than others, in contrast to the finding that translocation of the 11q23/MLL gene occurred more frequently in monocytic leukemia than in other AML subtypes.[16]

This is the first report that demonstrated a significantly higher frequency of CD11b expression on the leukemic blasts in the AML patients with partial tandem duplication of the MLL gene than in those without the abnormality in the subgroup of patients with normal karyotype (75% *vs* 28%, $P = 0.017$). Expression of other markers including monocyte–associated antigen CD14 and lymphoid-associated antigens was not much different between the two groups of patients. The animal studies have shown that the MLL gene is required for differentiation of hematopoietic cells, especially along the myeloid and macrophage pathways, which is accompanied by expression of the myeloid-associated antigens CD11b and CD14.[30,31] On the other hand, human leukemic cell line expressing AT hook domain of the amino-terminus of the MLL gene alone, the region involved in tandem duplication, expressed CD11b, but not CD14.[32] The finding might explain the difference in the incidence of CD11b expression, but not CD14 expression, between the patients with partial tandem duplication of the MLL gene and those without. To our knowledge, no other study regarding surface marker expression in AML with the MLL gene duplication has been reported.

The portion most frequently involved in MLL duplication in this study was exons 2 to 6, similar to that reported by others.[17,18,27] Duplication of exons 2 to 7 or exons 2 to 8, with or without alternative splicing, was also commonly seen.[17,18,27] The exon 3 and exons 4 through 6, involved in almost all cases with partial tandem duplication of the MLL gene, encode AT hook and MT motifs of the MLL gene protein respectively, and duplication of these critical regions may play important roles in leukemogenesis. [14,33,34]

Partial tandem duplication of the MLL gene has been detected in peripheral blood and bone marrow cells of healthy donors by nested RT-PCR.[35] However, in contrast to leukemic cells with partial tandem duplication of the MLL gene, in normal cells with fusion transcript, a corresponding genomic rearrangement of the MLL gene could not be demonstrated by Southern blotting or genomic DNA PCR.[36] It is suggested that the MLL fusion transcript detected in normal cells results from differential mRNA splicing rather than true genomic rearrangement.[36] All nine patients with MLL duplication in this study showed genomic rearrangement of the gene.

In the limited number of reports, AML patients with partial tandem duplication of the MLL gene were shown to have shorter CR duration and/or overall survival time, compared with those without the abnormality.[17,18,37] Almost all patients with MLL duplication who achieved a CR relapsed.[17,18] The only one patient remaining in continuous CR reported by Caligiuri *et al*[17] received eight intensive cycles of combination chemotherapy. In another report, 8 of 10 AML patients with MLL duplication who obtain a CR relapsed, one died in aplasia of the bone marrow, and the remaining one died of complications of allogeneic bone marrow transplantation.[18] In this study, three of the four patients with partial tandem duplication of the MLL gene who got a CR received allogeneic hematopoietic stem cell transplantation, and two of them remained in continuous CR at 27 and 41 months respectively. Although the data are limited, it is suggested that hematopoietic stem cell transplantation or very intensive consolidation chemotherapy may be indicated to reverse the poor prognosis of AML patients with tandem duplication of the MLL gene. In one study,[36] in which 17 of the 41 recruited AML patients with normal karyotype received autologous or allogeneic hematopoietic stem cell transplantation, the patients with MLL self fusion showed similar outcome to those without the gene change. Prospective studies on more patients are needed to find out the most proper treatment for the patients with tandem duplication of the MLL gene.

In conclusion, partial tandem duplication of the MLL gene occurs in substantial number of the AML patients without cytogenetic aberrations at 11q23, especially in those with normal karyotype. MLL self fusion is associated with the expression of certain surface antigens on the myeloid leukemic blasts. Whether the frequency of MLL duplication in children older than one year is higher than other age groups needs to be confirmed by further studies on more patients. In adult AML patients, MLL duplication implicates poor prognosis. The necessity of allogeneic hematopoietic stem cell transplantation in this subgroup of AML patients remains to be elucidated.

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Table 1 Results of molecular analysis in the patients with partial tandem duplication of the MLL gene

Case No.	Sex/Age (years)	Southern blot	RT-PCR	Genomic PCR	Fusion exons
1	M/61	+	NA	+	e6/e2
2	F/34	+	+	+	e6/e2
3	M/47	+	+	–	e7/e2; e6/e2
4	F/18	+	+	+	e6/e2
5	F/13	+	+	+	e6/e2
6	F/14	+	+	+	e6/e2
7	M/53	+	+	+	e6/e2
8	F/79	+	+	+	e8/e2; e7/e2
9	M/18	+	NA	+	e6/e2

Abbreviations: F, female; M, male; NA, not available; +, positive finding; -, negative finding; e6/e2, exon 6/exon 2; e7/e2, exon 7/exon 2; e8/e2, exon 8/exon2.

Table 2 Correlation of partial tandem duplication of the MLL gene with clinical and hematological features

	Adult			Children[a]			Total		
	PTD (+)	PTD (-)	*P* value	PTD (+)	PTD (-)	*P* value	PTD (+)	PTD (-)	*P* value
No. of cases	7	64		2	8		9	72	0.266
Median age (year)	47	55	0.298	14	7	0.103	34	47	0.237
Sex (Male:Female)	4:3	31:33	0.710	0:2	7:1	0.067	4:5	38:34	0.732
Hepatomegaly (%)	29	17	0.604	100	88	1.000	44	25	0.245
Splenomegaly (%)	14	6	0.414	50	50	1.000	22	11	0.307
CNS involvement (%)	0	2	1.000	0	13	1.000	0	3	1.000
Skin involvement (%)	14	0	0.099	0	13	1.000	11	1	0.211
Hemoglobin (median, g/dl)	6.4	8.5	0.116	6.0	7.2	0.267	6.4	8.5	0.056
WBC (median, 10^9/l)	200.6	31.2	0.123	5.3	158.3	0.148	30.9	35.9	0.484
Platelet (median, 10^9/l)	70	45	0.361	116	62	0.948	79	48	0.311
LDH (median, U/l)	1769	1028	0.432	647	1179	0.163	1176	1050	0.718
CR rate (%)	40	74	0.148	100	43	0.444	57	69	0.671
CR duration (median, months)	NA[b]	10	0.644	NA[b]	7	NA[b]	NA[b]	10	0.561
Survival time (median, months)	4.5	12	0.036	NA[b]	11	0.432	4.5	12	0.141

[a]Including infants (≤ 1 year) and children older than 1 year but younger than 15 years. All three infants were negative for partial tandem duplication of the MLL gene.

[b]NA, data not available because median time was not reached yet or too few patients were analyzed. Two, one adult and one child, of the three patients with partial tandem duplication of the MLL gene who received allogeneic bone marrow transplantation remained in continuous CR at 41 and 27 months respectively.

Abbreviations: PTD, partial tandem duplication of the MLL gene; CNS, central nervous system, WBC, white blood cell; LDH, lactate dehydrogenase; CR, complete remission.

Table 3 Comparison of immunophenotype between AML with partial tandem duplication of the MLL gene and those without[a]

Marker[b]	Partial tandem duplication (+)		Partial tandem duplication (-)		*P* value
	No. studied	No. positive (%)	No. studied	No. positive (%)	
HLA-DR	9	9 (100)	48	36 (75)	0.180
CD34	8	4 (50)	43	18 (42)	0.713
CD13	9	8 (89)	48	40 (83)	1.000
CD33	9	8 (89)	48	37 (77)	0.667
CD11b	9	6 (67)	46	13 (28)	0.051[a]
CD14	9	4 (44)	49	9 (18)	0.102
CD15	8	5 (63)	43	28 (65)	1.000
CD2	9	2 (22)	48	2 (4)	0.113
CD7	9	0 (0)	47	14 (30)	0.093

[a]The data in this table were from the analysis on all patients who were studied. When only the patients with normal karyotype were included in the analysis, 6 (75%) of 8 patients with MLL duplication and 11 (28%) of the 40 patients without the change showed CD11b expression on the leukemic cells; the difference was statistically significant ($P = 0.017$). But there were still no differences in the expression of other surface antigens between the patients with and without MLL duplication.

[b]The results of CD41a, CD5, CD10, CD19 and CD20 were not shown in this Table. There was no difference in the expression of these markers between the two groups of patients.

Figure Legends

Figure 1. A representative film of genomic DNA PCR of the MLL gene showing amplified bands in lanes 1 to 6 (corresponding to cases 1, 9, 7, 2, 6 and 8 respectively). Case 8 (lane 6) had duplication spanning exons 2-8, and others had duplication spanning exons 2-6. M, marker; N, normal control.

Figure 2. A representative film of RT-PCR of the MLL duplication transcript showing amplified bands in lanes 1 to 5 (upper panel, corresponding to cases 6, 5, 3, 8, and 9 respectively). RT-PCR of the β-actin mRNA was used as internal control (lower panel). M, marker; N, normal control; W, water.

Figure 3. A representative film of cDNA sequencing from case 8 showing fusion of exon 8 and exon 2.

Figure 4. Kaplan-Meier survival curves of the adult patients with partial tandem duplication of the MLL gene (PTD+, solid line) and those without (PTD-, dashed line) among the 53 patients who received conventional induction chemotherapy. The 5 patients receiving allogeneic hematopoietic stem cell transplantations, 1 PTD+ and 4 PTD-, were censored on the day of the transplantation procedure in the analysis.

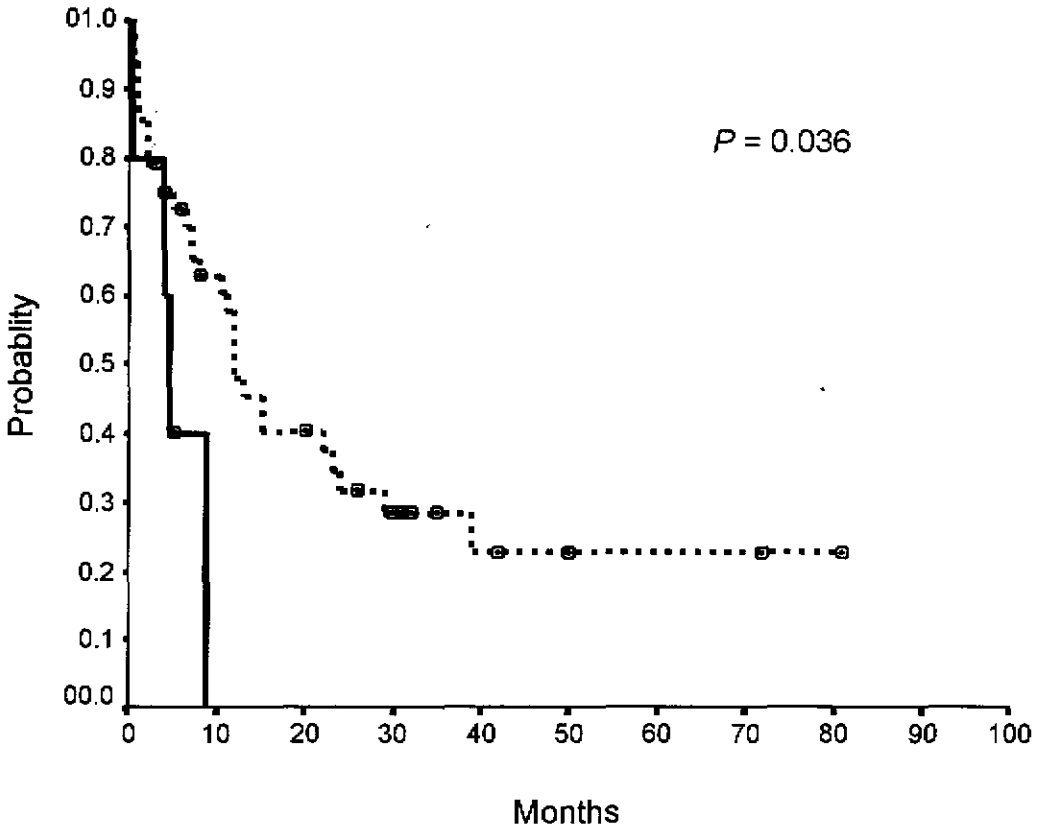

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Figure 4 ↑