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Case Study

Dic (17;20) (p11;q11) Preceded *MLL* Gene Amplification in a Patient with *de novo* Mixed-Lineage Leukemia

Ryuichi Amakawa,[1)] Nobuhiro Hiramoto,[1)] Seiji Kawano,[2)] Akira Hyo,[1)] Naoto Nakamichi,[1)] Kenichiro Tajima,[1)] Tomoki Ito,[1)] Shinichiro Mori,[1)] Yuji Kishimoto,[1)] and Shirou Fukuhara[1)]

We report a case of acute mixed-lineage leukemia, as seen in a 65 year-old female with *MLL* gene amplification and biallelic loss of wild type *p53* gene. The diagnosis was based on the findings that her bone marrow (BM) blasts expressed cytoplasmic CD3 (cyCD3), B-lineage antigens and myeloid antigens accompanied by clonal rearrangements of *IgH* gene. The BM blasts consisted of small-sized peroxidase-negative blasts (97%) and large-sized peroxidase-positive blasts (3%). The BM blasts showed a complex "karyotype," including dic(17;20) (p11;q11), -5 and add (11q23). Add (11q23) abnormality was found in sideline karyotypes as well as the stemline abnormality of dic(17;20) (p11;q11). For the *p53* gene, which is located at 17p13, fluorescence *in situ* hybridization analysis showed the loss of one of two p53 alleles. Furthermore, polymerase chain reaction-single-strand conformation polymorphism and following nucleotide sequencing showed that the *p53* gene was mutated at codon 215, leading to an amino acid substitution from Ser to Arg. For the *MLL* gene, southern blot analysis showed that the *MLL* gene locus was amplified but not rearranged at its breakpoint cluster region, which is usually rearranged in balanced translocations with many partner genes. These findings suggest that *MLL* gene amplification may in this case be based on the genetic instability caused by the preceding biallelic loss of the wild type *p53* gene. 〔*J Clin Exp Hematopathol 50(1) : 51-58, 2010*〕

Keywords: MLL amplification, leukemia, fluorescence *in situ* hybridization, p53

INTRODUCTION

Acute leukemia of ambiguous lineage is defined as a new entity in the new WHO classification.[1] This category of leukemia lacks the morphologic, cytochemical and immunophenotypic features sufficient to classify it as being of myeloid or lymphoid origin. This includes various types of leukemias that were formerly called such as acute undifferentiated leukemia, acute biphenotypic leukemia, mixed-lineage leukemia, or stem-cell leukemia, etc. Generally, the type of chromosomal aberration is closely related to the phenotype of leukemia. As for acute leukemia with ambiguous lineage, Ph1-abnormalities occupy a third of all cases, and cases with 11q23 abnormalities such as t(4;11) (q21;q23) are also frequently observed. On the other hand, acute leukemias with T/myeloid components or T/B components are infrequent and sometimes have complex karyotypes rather than Ph1 or 11q23 abnormalities.

Recently, a new clinicopathological entity in acute myeloblastic leukemia (AML)/myelodysplastic syndrome (MDS), 17p- syndrome, has been postulated.[2,3] This new entity is characterized by the strong correlation between unbalanced translocations involving 17p deletion, less often monosomy 17 or i (17p), and typical dysgranulopoiesis combining pseudo-Pelger-Huët hypolobulation and small vacuoles in neutrophils, and *p53* mutations. Notably, Soenen *et al*. reported that the deletion of one *p53* allele, which is located at 17q13, was found in all cases with 17p- syndrome and point mutation of the non-deleted *p53* allele in all but one.[2] Thus, loss of germ line *p53* might play an important role in 17p-syndrome.

Abnormalities in the *MLL* gene, located at 11q23, are among the most common recurring abnormalities in *de novo* and therapy-related hematologic disorders, including acute leukemias and MDS.[4-7] Various types of *MLL* translocations generating chimeric proteins are closely associated with

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[1)]First Department of Internal Medicine, Kansai Medical University, 10-15, Fumizono-Cho, Moriguchi-City, Osaka 570-8506, Japan.
[2)]Department of Clinical Laboratory, Kobe University Hospital, 7-5-2 Kusunoki-cho, Chuo-ku, Kobe, 650-0017, Japan.
Address correspondence and reprint request to Ryuichi Amakawa, M.D., Ph.D., Associate Professor, First Department of Internal Medicine, Kansai Medical University, 10-15, Fumizono-Cho, Moriguchi-City, Osaka 570-8506, Japan. e-mail : amakawa@takii.kmu.ac.jp

AML, acute lymphoblastic leukemia, or mixed lineage leukemia depending on the chimeric partners.[8] Currently, three types of *MLL* gene aberrations other than reciprocal translocation are recognized in leukemia/MDS : partial tandem duplication (PTD) within *MLL* gene locus, internal deletion, and gene amplification. *MLL* gene amplification has recently been implicated as a potential mechanism of leukemia development.[9-11] Moreover, it has been proposed that the precedent mutation of *p53* due to previous exposure to alkylating agents in therapy-related myeloid malignancies may contribute to the genetic instability of the *MLL* gene, resulting in the amplification of this gene.[12]

Here, we present a case of *de novo* mixed-lineage leukemia with *p53* mutation and *MLL* amplification. This is the first case-report of mixed-lineage leukemia with biallelic *p53* mutation and *MLL* amplification. This case provides new insights into the role of *MLL* amplification in leukemogenesis.

CASE REPORT

A 62-year-old woman was admitted to Kansai Medical University Hospital presenting pancytopenia (white blood cell count $1.0 \times 10^9/\mu L$, hemoglobin 6.6 g/dL, and platelet count $1.5 \times 10^9/\mu L$). A bone marrow (BM) aspirate showed 80% abnormal cells, 2% neutrophils, 16% lymphocytes and 2% erythroblasts. The abnormal cells in the BM consisted of 96% of small-sized blastic cells (less than three times the diameter of the erythrocytes) and 4% large-sized ones (more than three times the diameter of the erythrocytes). The small blasts were negative for myeloperoxidase (MPO) (< 0.1%), but the large blasts were MPO-positive (31%) (Fig. 1). Flow cytometric analysis revealed that the BM blasts were positive for cytoplasmic CD3 (cyCD3) and terminal deoxynucleotidyl transferase as well as CD13, CD19, CD22, CD24, but negative for surface CD3, CD4, CD20, CD33, CD41a/b, and CD56, as shown in Table 1. On gating, the large blasts were found to be also positive for cyMPO in addition to the antigens for which the small blasts were positive (data not shown). Southern blot analysis on BM blasts revealed that the *immunoglobulin heavy chain (IgH)* gene was monoclonally rearranged, whereas the *T cell receptor (TCR) β chain* gene was in germ-line configuration (Fig. 2). All these findings led to the diagnosis of mixed-lineage leukemia. The patient underwent induction chemotherapy with a combination of adriamycin, predonizolone, cyclophosphamide, L-asparaginase, and vincristine. However, she failed to achieve complete remission, and became refractory to succeeding therapies such as high-dose cytarabine. Her condition progressively worsened and she died 7 months after the diagnosis.

Table 1. Cell-surface and cytoplasmic antigen expression on bone marrow blasts

Cell-surface staining				Cytoplasmic staining	
CD1	< 1.0%	CD21	1.0%	cCD3	43.6%
CD2	< 1.0%	CD22	95.0%	cCD22	80.8%
CD3	1.3%	CD23	1.8%	TdT	98.6%
CD4	2.0%	CD24	35.3%	MPO	0.3%
CD5	< 1.0%	CD25	72.9	μ	< 1.0%
CD7	< 1.0%	CD30	1.5	κ	1.8%
CD8	< 1.0%	CD33	1.3	λ	1.0%
CD10	< 1.0%	CD34	99.0%		
CD11a	93.7%	CD38	91.5%		
CD11b	25.4%	CD41a	3.5%		
CD11c	1.2%	CD41b	< 1.0%		
CD13	94.0%	CD45	99.0%		
CD14	2.4%	CD56	< 1.0%		
CD15	73.1%	CD71	74.8%		
CD16a	< 1.0%	CD117	5.4%		
CD19	99.0%	HLA-DR	99.0%		
CD20	< 1.0%				

TdT, terminal deoxynucleotidyl transferase ; MPO, myeloperoxidase

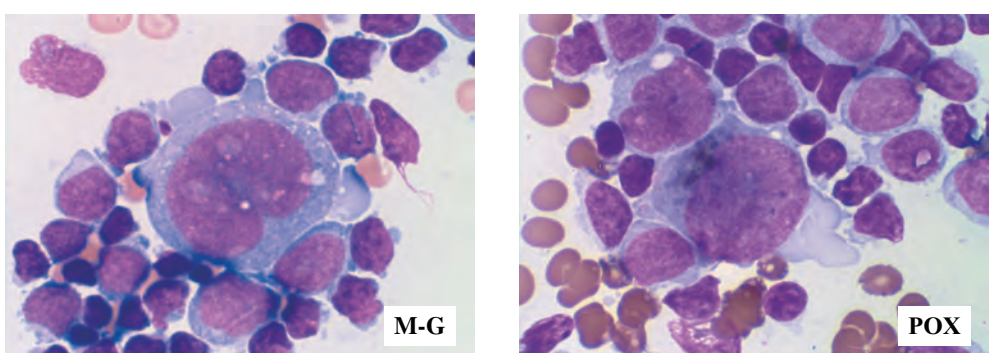

Fig. 1. Morphology of bone marrow (BM) blasts (×1000). The typical features on May-Giemsa (M-G) and peroxidase (POX) staining of bone marrow blasts are shown. Small blasts (< 3 × erythrocyte in diameter) were negative for POX, whereas 31% of large blasts (> 3 × erythrocyte in diameter) were positive for POX staining. (M-G) & (POX), ×1,000.

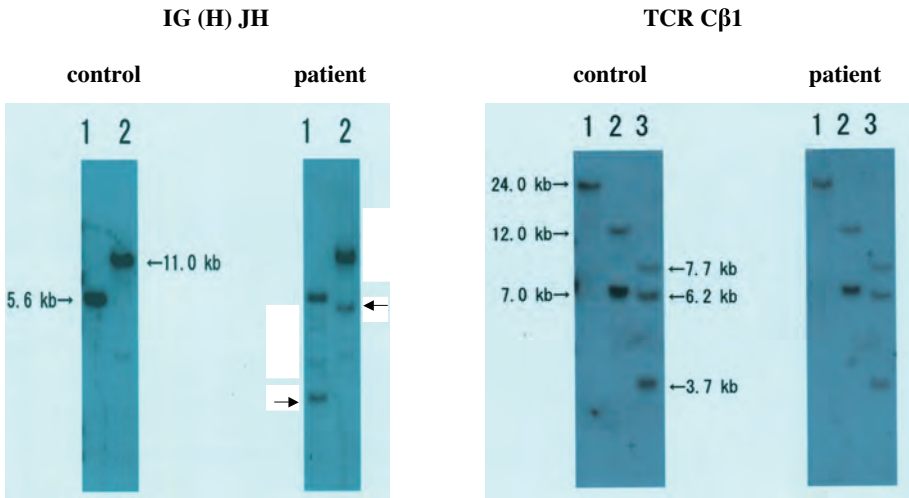

Fig. 2. Southern blot analysis for *IgH* and *TCRβ* genes. A quantity (5 μg) of restriction enzyme digests of patient DNA was loaded in each lane. (***2A***) JH probe. lane 1 ; BamHI plus HindIII, lane 2 ; HindIII. *Arrows* indicate rearranged bands. (***2B***) Cβ1 probe. lane 1 ; BamHI, lane 2 ; EcoRV, lane 3 ; HindIII.

MATERIALS AND METHODS

Karyotyping and fluorescence in situ *hybridization (FISH)*

Cells from BM samples were processed for chromosome analysis by standard techniques, with 24- to 48-hr unstimulated cultures. Air-dried chromosome preparations on glass slides were G-banded. At least 20 metaphase cells were analyzed in each sample. Karyotypes were designated according to the International System for Human Cytogenetic Nomenclature (ISCN, 2009).

FISH analysis was performed with D11Z1 (a marker probe for chromosome 11), CEP17 (a marker probe for chromosome 17), MLL, and p53 probes (Oncor, Gaithersberg, MD). All were hybridized and detected according to the manufacturers' directions. These probes were biotin-labeled by nick translation (Biotinick, GIBCO-BRL, Grand Island, NY). The hybridization solution for custom probes contained 0.2 μg labeled probe, 10 μg Cot-1 DNA (GIBCO-BRL), and 30 μg herring sperm DNA (GIBCO-BRL) in 15 μL of Hybrizol VII (Oncor) per slide. The probe cocktail was heat denatured at 70°C for 5 min and allowed to pre-anneal at 37°C for 2 hr. Chromosome preparations on slides were conditioned before hybridization by a 30-min 37°C bath in 2 × SSC, followed immediately by dehydration in 70%, 80%, and 95% EtOH (2 min each) at room temperature, and air-dried. The slides were then denatured in 70% formamide/2 × SSC at 70°C for 5 min, followed by serial dehydration at room temperature.

Hybridization was for 18 hr in a moist 37°C chamber. Slides were washed in 50% formamide/2 × SSC at 37°C for 30 min, followed by 2 × SSC at 37°C for 10 min. Slides were further washed three times at room temperature in a phosphate-buffered detergent before signal detection. Hybridized DNA was detected with Avidin-fluorescent isothiocyanate, followed by a single round of amplification according to the supplier's instructions (Oncor).

FISH signals were captured by using a monochromatic CCD camera mounted on a Zeiss epifluorescence microscope with a LUDL filter wheel and a fixed, multi-bandpass beam splitter with the use of Macprobe software (PSI, Houston, Tx).

Southern blot analysis

Southern blot analysis was performed according to the standard method. Briefly, 5 mg of DNA from the patient and normal control were digested separately or a combination of *Bam*HI, *Hind*III, *Eco*RI, or *Eco*RV (Takara, Kyoto, Japan), electrophoresed through 0.8% agarose gel, and transferred to a nylon membrane. After hybridization with the labeled probe, the nylon membranes were washed at an appropriate stringency, and autoradiographed.

For *TCRβ chain* gene, the *Hind*III-*Eco*RI 3.5 Kb fragment of the *TCRβ* constant region was used as a *Cβ1* probe. For *IgH*, the *Eco*RI -*Hind*III fragment of the *IgH* constant region was used as a *JH* probe. For testing the *MLL* gene, a 0.74 Kb *Bam*HI fragment of the *MLL* gene was used as a *MLL* probe, which detects all rearrangements (including PTD) within the *MLL* breakpoint cluster region.[8]

Polymerase chain reaction-single-strand conformation polymorphism (PCR-SSCP) and nucleotide sequencing

For detecting point mutations of the *p53* gene, one hundred nanograms of genomic DNA (gDNA) was used to amplify the exons 5 to 8 of *p53* gene. Primer sets for amplification of 4 exons of p53 were designed with fluorescence Cy-5 (Amersham Pharmacia Biotech, Little Chalfont, UK) at 5' site of primers according to benebank X54156. Table 2 shows the primer sequences used. PCR-SSCP analysis was performed according to Orita *et al*.[13] with the Expand™ High Fidelity PCR System (Roche Molecular Biochemicals Diagnostic, Manheim, Germany). The PCR conditions of exons 5, 6 and 8 were at 94°C for 30 sec (denature), 60°C for 60 sec (annealing), and 72°C for 60 sec (extension) for 35 cycles, and those of exon 7 were at 94°C for 30 sec, 70°C for 60 sec, and 72°C for 60 sec for 35 cycles. The PCR products were diluted fifty-fold with 95% formamide and denatured at 80°C for 5 min followed by rapid cooling on ice. Denatured products were separated on 5% DNA sequencer ALF express (Amersham) and analyzed with Allele link software (Amersham).

For direct sequencing of the PCR product, an interim sequencing primer for exon 6 (Table 2) was set to detect point mutations within exon 6 of p53. The PCR product was re-amplified with the Expand™ High Fidelity PCR System. The PCR product was purified using the High Pure PCR product purification kit (Roche) and directly sequenced using the Thermosequencing kit (Amersham) with ALF express. The sequence was finally compared with the wild-type *p53* gene.

Table 2. Primer sequences of *p53* for polymerase chain reaction-single-strand conformation polymorphism (PCR-SSCP) and nucleotide sequencing

Primers for PCR-SSCP		
Exon 5	E5SF	5'-TTCCTCTTCCTACAGTACTCC-3'
	E5SR	5'-GCCCCAGCTGCTCACCATCGC-3'
Exon 6	E6SF	5'-CACTGATTGCTCTTAGGTCTG-3'
	E6SR	5'-AGTTGCAAACCAGACCTCAGG-3'
Exon 7	E7SF	5'-CCAAGGCGCACTGGCCTCATC-3'
	E7SR	5'-TCAGCGGCAAGCAGAGGCTGG-3'
Exon 8	E8SF	5'-CCTATCCTGAGTAGTGGTAAT-3'
	E8SR	5'-GTCCTGCTTGCTTACCTCGCT-3'
Primers for direct sequencing of exon 6		
		5'-GAGGGCCACTGACAACCACCC-3'

RESULTS

Dic (17 ; 20) (p11 ; q11) and MLL amplification

Karyotype analysis of BM cells at the time of diagnosis (sample from June 27, 2002) demonstrated that 2 of 20 cells analysed had normal karyotypes while the remaining 18 cells had complex karyotypes with multiple structural and numerical abnormalities (Fig. 3). Four of the 18 cells showed the karyotype of 43, XX, -5, del(7) (q?), del(12) (p?), -16, dic(17; 20) (p11;q11) (Fig. 3A). The karyotype of the remaining 14 cells was 45, XX, -5, del(7) (q?), add(11) (q23), del(12) (p?), dic(17;20) (p11;q11), +mar (Fig. 3B). Thus, all the 18 cells with abnormal karyotypes had -5, del(7) (q?), del(12) (p?) and dic(17;20) (p11;q11) in common (Fig. 3A), suggesting that add(11) (q23) is a secondary chromosomal aberration. No double minute chromosomes, frequently accompanied by *MLL* gene amplification in AML/ MDS cases,[1,12] were detected in any of the 20 cells analyzed. Karyotype analysis on the refractory stage of disease (Dec 19, 2002) demonstrated

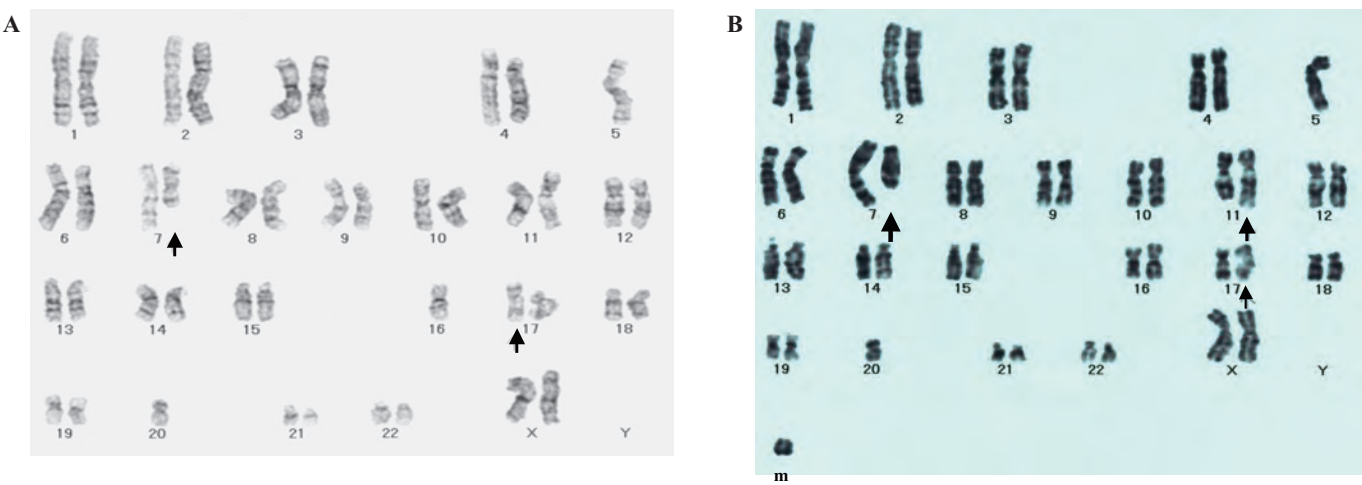

Fig. 3. Karyotype analysis (G-banding) of leukemic cells at the time of diagnosis (June 27, 2002). (***3A***) A stemline karyotype with no chromosome 11 abnormality is shown ; 43, XX, -5, del(7) (q?), del(12) (p?), -16, dic(17;20) (p11;q11). (***3B***) A sideline karyotype is shown ; 45, XX, -5, del(7) (q?), add(11) (q23), del(12) (p?), dic(17;20) (p11;q11), +mar.

the following karyotype : 48, XX, -5, del(7) (q?), add(11) (q23) × 2, del(12) (p?), dic(17;20) (p11;q11), +3mar, indicating further karyotypic evolution (data not shown).

Since the *MLL* gene is located on 11q23, we assumed that this gene might be involved in the 11q23 aberration observed in this case. FISH analysis demonstrated that some BM cells on the refractory stage of disease (Dec 19, 2002) had amplification of the *MLL* gene on chromosome 11 (Fig. 4A).

Southern blot analysis for the *MLL* gene on the refractory stage of disease revealed that this gene was of germ-line configuration (Fig. 4B). This result excluded the possibility of mutual translocations of the *MLL* gene with other partner genes. In addition, PTD of the *MLL* gene, which occasionally occurs in AML without cytogenetic aberrations at 11q23, is also unlikely to exist.[11] Interestingly, the density of the *MLL* band was ten times that of the germ line band of normal control as detected by image analyzer (Fig. 4B), demonstrating the amplification of the *MLL* gene locus without gene rearrangement.

Loss of wild type p53 alleles by dic(17;20) (p11;q11) and single nucleotide mutation

To demonstrate the deletion of the *p53* allele, which is located on 17p13, due to dic(17;20) (p11;q11), we performed FISH analysis using a probe specific for *p53* on BM blasts. FISH analysis on BM blasts at the time of diagnosis (sample of June 27, 2002) showed that all of the cells analyzed lost one of two *p53* alleles (Fig. 5). We further analyzed the status of another *p53* allele using PCR-SSCP. PCR-SSCP analysis on BM blasts at the time of diagnosis (sample of June 27, 2002) for exons 5 through 8 of the *p53* gene locus, which spans mutational hot spots in cancers, showed a shifted band on exon 6 (Fig. 6A). The direct sequencing of the PCR product for exon 6 revealed that AGT to AGG nucleotide substitution occurred at codon 215, resulting in amino acid conversion from Ser to Arg at least in some cells (Fig. 6B). On the other hand, the wild type codon 215 (AGT) was also detected, which may reflect the presence of cells with a normal karyotype. These results, combined with those from FISH analyses, demonstrated that both alleles of the wild type *p53* gene were lost because of the deletion of one allele resulting from unbalanced translocation, dic(17;20) (p11;q11), and one point mutation at codon 215 at the time of initial presentation.

DISCUSSION

Deletions of 17p or 20q are well-recognized abnormalities in myeloid malignancies. Deletions of 17p are found in 4% of AML/MDS cases and are strongly associated with loss and mutation of the *p53* gene,[1,2] while deletions of 20q are found in 5% of myeloid disorders including AML/MDS and myeloproliferative disorders such as polycythemia vera.[14] Recently, six cases of AML/MDS with a dicentric unbalanced translocation between chromosome 17 and 20, dic(17;20) (p11;q12), in which the segments distal to 17p11 and 20q12 are lost, have been reported.[15,16] These cases are characterized by myelodysplastic features and poor prognosis. It is therefore postulated that dic(17;20) is a rare but recurrent abnormality in

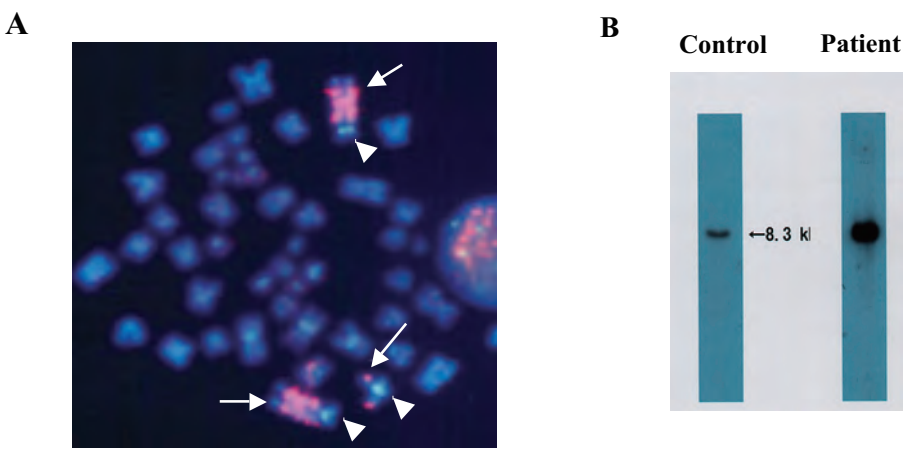

Fig. 4. Fluorescence *in situ* hybridization and southern blot analyses demonstrate multiple copies of *MLL*. (***4A***) Metaphase cells were double-stained with *MLL* and *D11Z1* probe, a marker probe for chromosome 11. Red signal (*arrow*) indicates *MLL*, and blue signal (*arrowhead*) indicates chromosome 11. Marked amplification of *MLL* gene at chromosome 11 is noted. (***4B***) Southern blot analysis for *MLL* gene. *MLL* probe was hybridized with *Bam*HI digest. *MLL* gene was of germ line configuration. Thick band for *MLL* gene segment shows the amplification of the *MLL* gene.

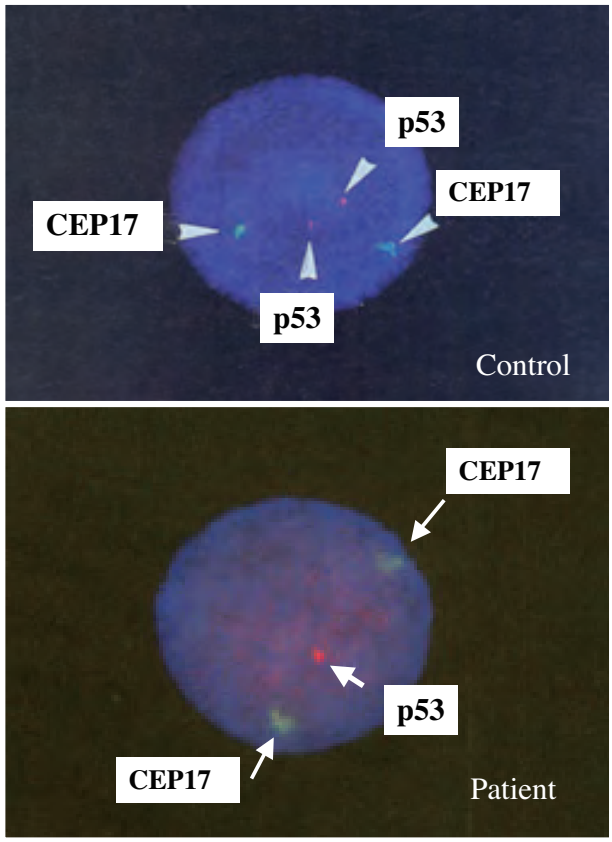

Fig. 5. Fluorescence *in situ* hybridization analysis demonstrates loss of a p53 locus. Metaphase cells were double-stained for *p53* and *CEP17* probe, a marker probe for chromosome 17. Red signal indicates *p53*, and blue signal indicates chromosome 17. One of the two *p53* signals is lost.

myeloid malignancies. Interestingly, the deletion of the *p53* gene on the dicentric chromosome with retention of the *p53* on the normal copy of chromosome 17 has been demonstrated by FISH analysis in 4 of 4 AML cases with dic(17;20).[16]

Loss of 17p is frequently associated with complete or partial monosomy of chromosome 5 and a poor prognosis in AML/MDS.[1-3] Moreover, a recurring abnormality of dic(5;17) (q11-13;p11) is reported in AML/MDS cases.[17] Therefore, a possible cooperation of *p53* gene located at 17p13 and a putative tumor suppressor gene at 5q13 has been proposed.[17,18] On the other hand, several cases of 17p- syndrome resulting from dic(17;20) in AML/MDS cases have also been reported.[15,16] This unique karyotypic combination of 17p- and monosomy 5 is detected at the initial presentation in our case. Since the reported cases carrying 17p- or dic(17;20) have been confined to AML/MDS cases, our case is the first, to the best of our knowledge, of *de novo* mixed-lineage leukemia with dic(17;20). Although clinical and phenotypic features may not be linked to 17p- syndrome in our case, the genetic features such as del(17p), del(5) and *p53* mutation of both alleles are quite similar to the genetic characteristics of 17p- syndrome.

A series of cytogenetical analyses of this case revealed that at the time of disease onset there was a stemline in which the 11q23 abnormality was absent but dic(17;20) (p11;q12) already existed. This suggests that the mutation of *p53* caused by dic(17;20) (p11;q12) may precede *MLL* amplification manifested as 11q23 abnormality in our case, although the possibility of *MLL* amplification without karyotypic change can not be completely discounted. Therefore, we assume that the biallelic loss of *p53* function was the earlier and crucial event, and that this event induced chromosomal instability that might trigger *MLL* amplification, although direct evidence is lacking.

The amplification of *MLL* is reported so far only in AML and MDS,[9] while PTD of *MLL* is reported only in AML[19-21] and internal deletion of *MLL* at exon 8 is reported only in T-acute lymphoblastic leukemia.[22] Gene amplification of *MLL* is cytogenetically manifested as either a homogenously staining region (hsr) or a double minute chromosome (dmin) in AML/MDS.[23] In our case, hsr(11q) but not dmin was detected in the advanced stages of the disease, and the intrachromosomal amplification of the *MLL* gene was confirmed by FISH analysis. The germ line configuration of the *MLL* gene in our case is in accordance with the reports that showed the duplicated or amplified *MLL* gene was in germ line configuration.[24] A recent report on myeloid malignancies with 11q23 amplification showed that the *MLL* and *DDX6* genes were identified as the most expressed genes among candidate oncogenes at 11q.[25] The transcription levels for the *MLL*-regulated genes such as *HoxA9* were also significantly enhanced. Furthermore, AML/MDS with 11q/MLL amplification shows a characteristic gene expression signature.[26] Therefore, *MLL* is considered to be the main target gene for 11q23 amplification, and the gain in *MLL* function is supposedly critical in leukemogenesis. Interestingly, *MLL* amplification has been reported almost exclusively in AML and MDS. This may imply that an abundance of the wild-type MLL protein may enhance the transcriptional activity of myeloid-specific genes in a hematopoietic precursor. Therefore, it is plausible that the myeloid phenotype on the large-sized blasts in our case are related to the *MLL* amplification, although we could not technically distinguish these blasts from the smaller and MPO-negative blasts based on cytogenetical analysis.

Taken together, we concluded that the mutated *p53* alleles, one by deletion and the other by point mutation, may have induced genomic instability and caused *MLL* amplification. We believe that our case is very instructive in understanding the roles of *p53* mutations and *MLL* amplification in hematologic malignancies.

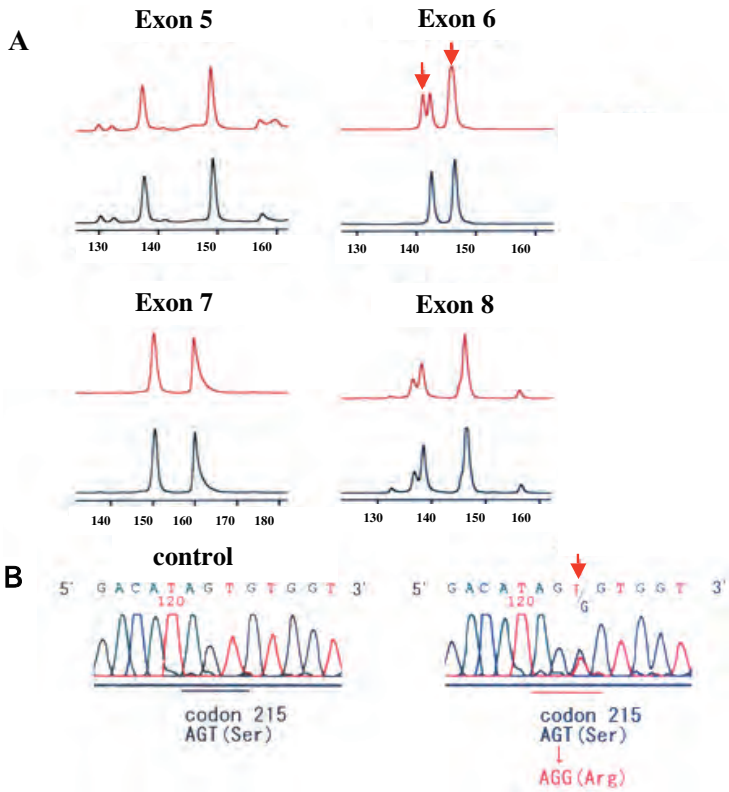

Fig. 6. Polymerase chain reaction-single-strand conformation polymorphism (PCR-SSCP) and direct sequencing of *MLL* gene. (***6A***) PCR-SSCP analysis for exon 5 through 8 of *p53* gene demonstrated a shifted peak in exon 6 (red *arrow*). (***6B***) Direct sequencing of exon 6 showed the single nucleotode mutation at codon 215 (red *arrow*, AGT to AGG), resulting in amino acid conversion from Ser to Arg.

References

1 WHO Classification of Tumours, Tumours of Haematopoietic and Lymphoid Tissues, Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, et al. (eds) : 4th ed, Lyon, IARC, 2008

2 Soenen V, Preudhomme C, Roumier C, Daudignon A, Laï JL, *et al.* : 17p deletion in acute myeloid leukaemia and myelodysplastic syndrome. Analysis of breakpoints and deleted segments by fluorescence *in situ*. Blood 91 : 1008-1015, 1998

3 Merlat A, Laï JL, Sterkers Y, Demory JL, Bauters F, *et al.* : Therapy-related myelodysplastic syndrome and acute myeloid leukemia with 17p deletion. A report on 25 cases. Leukemia 13 : 250-257, 1999

4 Thirman MJ, Gill HJ, Burnett RC, Mbangkollo D, McCabe NR, *et al.* : Rearrangement of the MLL gene in acute lymphoblastic and acute myeloid leukemias with 11q23 chromosomal translocations. N Engl J Med 329 : 909-914, 1993

5 Hunger SP, Tkachuk DC, Amylon MD, Link MP, Carroll AJ, *et al.* : HRX involvement in *de novo* and secondary leukemias with diverse chromosome 11q23 abnormalities. Blood 81 : 3197-3203, 1993

6 Pedersen-Bjergaard J, Philip P, Larsen SO, Andersson M, Daugaard G,, *et al.* : Therapy-related myelodysplasia and acute myeloid leukemia. Cytogenetic characteristics of 115 consecutive cases and risk in seven cohorts of patients treated intensively for malignant diseases in the Copenhagen series. Leukemia 7 : 1975-1986, 1993

7 Pedersen-Bjergaard J, Pedersen M, Roulston D, Philip P : Different genetic pathways in leukemogenesis for patients presenting with therapy-related myelodysplasia and therapy-related acute myeloid leukemia. Blood 86 : 3542-3552, 1995

8 Ayton PM, Cleary ML : Molecular mechanisms of leukemogenesis mediated by MLL fusion proteins. Oncogene 20 : 5695-5707, 2001

9 Streubel B, Valent P, Jager U, Edelhauser M, Wandt H, *et al.* : Amplification of the MLL gene on double minutes, a homogenous staining region, and ring chromosomes in five patients with acute myeloid leukemia or myelodysplastic syndrome. Genes Chromosomes Cancer 27 : 380-386, 2000

10 Sait SN, Qadir MU, Conroy JM, Matsui S, Nowak NJ, *et al.* :

Double minute chromosomes in acute myeloid leukemia and myelodysplastic syndrome : identification of new amplification regions by fluorescence *in situ* hybridization and spectral karyotyping. Genes Chromosomes Cancer 34 : 42-47, 2002

11 Pajuelo-Gámez JC, Cervera J, García-Casado Z, Mena-Durán AV, Valencia A, *et al.* : MLL amplification in acute myeloid leukemia. Cancer Genet Cytogenet 174 : 127-131, 2007

12 Andersen MK, Christiansen DH, Kirchhoff M, Pedersen-Bjergaard J : Duplication or amplification of chromosome band 11q23, including the unrearranged MLL gene, is a recurrent abnormality in therapy-related MDS and AML, and is closely related to mutation of the TP53 gene and to previous therapy with alkylating agents. Genes Chromosomes Cancer 31 : 33-41, 2001

13 Orita M, Suzuki Y, Sekiya T, Hayashi K : Rapid and sensitive detection of point mutations and DNA polymorphisms using the polymerase chain reaction. Genomics 5 : 874-879, 1989

14 Bench AJ, Nacheva EP, Hood TL, Holden JL, French L, *et al.* : Chromosome 20 deletions in myeloid malignancies : reduction of the common deleted region, generation of a PAC/BAC contig and identification of candidate genes. UK Cancer Cytogenetics Group (UKCCG). Oncogene 19 : 3902-3913, 2000

15 Watson N, Dunlop L, Robson L, Sharma P, Smith A : 17p- syndrome arising from a novel dicentric translocation in a patient with acute myeloid leukemia. Cancer Genet cytogenet 118 : 159-162, 2000

16 Patsouris C, Michael PM, Campbell LJ : A new nonrandom unbalanced t(17;20) in myeloid malignancies. Cancer Genet Cytogenet 138 : 32-37, 2002

17 Wang P, Spielberger RT, Thangavelu M, Zhao N, Davis EM, *et al.* : dic(5;17) : a recurring abnormality in malignant myeloid disorders associated with mutations of TP53. Genes Chromosomes Cancer 20 : 282-291, 1997

18 Castro PD, Liang JC, Nagarajan L : Deletions of chromosome 5q13.3 and 17p loci in myeloid neoplasms. Blood 95 : 2138-2143, 2000

19 Schnittger S, Kinkelin U, Schoch C, Heinecke A, Haase D, *et al.* : Screening for MLL tandem duplication in 387 unselected patients with AML identify a prognostically unfavorable subset of AML. Leukemia 14 : 796-804, 2000

20 Caligiuri MA, Strout MP, Lawrence D, Arthur DC, Baer MR, *et al.* : Rearrangement of ALL1 (MLL) in acute myeloid leukemia with normal cytogenetics. Cancer Res 58 : 55-59, 1998

21 Ariyama Y, Fukuda Y, Okuno Y, Seto M, Date K, *et al.* : Amplification on double-minute chromosomes and partial-tandem duplication of the MLL gene in leukemic cells of a patient with acute myelogenous leukemia. Genes Chromosomes Cancer 23 : 267-272, 1998

22 Lochner K, Siegler G, Fuhrer M, Greil J, Beck JD, *et al.* : A specific deletion in the breakpoint cluster region of the ALL-1 gene is associated with acute lymphoblastic T-cell leukemias. Cancer Res 56 : 2171-2177, 1996

23 Streubel B, Valent P, Jager U, Edelhauser M, Wandt H, *et al.* : Amplification of the MLL gene on double minutes, a homogeneously staining region, and ring chromosomes in five patients with acute myeloid leukemia or myelodysplastic syndrome. Genes Chromosomes Cancer 27 : 380-386, 2000

24 Cuthbert G, Thompson K, McCullough S, Watmore A, Dickinson H, *et al.* : MLL amplification in acute leukaemia : a United Kingdom Cancer Cytogenetics Group (UKCCG) study. Leukemia 14 : 1885-1891, 2000

25 Poppe B, Vandesompele J, Schoch C, Lindvall C, Mrozek K, *et al.* : Expression analyses identify MLL as a prominent target of 11q23 amplification and support an etiologic role for MLL gain of function in myeloid malignancies. Blood 103 : 229-235, 2004

26 Zatkova A, Merk S, Wendehack M, Bilban M, Muzik EM, *et al.* : AML/MDS with 11q/MLL amplification show characteristic gene expression signature and interplay of DNA copy number changes. Genes Chromosomes Cancer 48 : 510-520, 2009