MISSENSE MUTATION AND DEFECTIVE FUNCTION OF ATM IN A CHILDHOOD ACUTE LEUKEMIA PATIENT WITH MLL GENE REARRANGEMENT

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Abstract

The possible involvement of germline mutation of the *ataxia telangiectasia mutated* (*ATM*) gene in childhood acute leukemia with mixed lineage leukemia (*MLL*) gene rearrangement (MLL+) was investigated. Of the seven patients studied, one showed a germline missense *ATM* mutation (8921C→T; Pro to Leu at codon 2974), located in the PI-3 kinase domain. In reconstitution assays, the ATM mutant 8921T could only partially rescue the radiosensitive phenotype of AT fibroblasts, and in *in vitro* kinase assay, it showed a defective phosphorylation of p53-Ser15. Furthermore, the introduction of 8921T in U2OS cells, characterized by a normal ATM/p53 signal transduction, caused a significant reduction of *in vivo* p53-Ser15 phosphorylation, suggesting a dominant negative effect of the mutant ATM over the wild-type protein. Our finding in this patient suggests that altered function of ATM plays some pathogenic roles in the development of MLL+ leukemia.

Introduction

Ataxia telangiectasia (AT) is an autosomal recessive disorder characterized by cerebellar ataxia, oculocutaneous telangiectasia and immunodeficiency. The responsible gene, *ATM* (*ataxia telangiectasia mutated*), is located on chromosome 11q22-23 and encodes a 350 kDa nuclear protein with a carboxy terminal domain
similar to the catalytic subunit of the phosphatidylinositol-3 kinases (PI-3 kinases).\textsuperscript{1-3} PI-3 kinase-related proteins are known to function in the maintenance of genomic stability, cell cycle control, and cellular responses to DNA damage.\textsuperscript{4,5} AT cells exhibit hypersensitivity to ionizing radiation and are defective at multiple cell cycle checkpoints.\textsuperscript{4-7}

It is well established that AT patients are at increased risk of cancer,\textsuperscript{8} in particular, neoplasms of lymphoid origin.\textsuperscript{9,10} Somatic mutations in the \textit{ATM} gene have been identified in T-cell prolymphocytic leukemia (T-PLL)\textsuperscript{11} and B-cell chronic lymphocytic leukemia (B-CLL) patients with no family history of AT.\textsuperscript{12-14} Mutations observed in these reports are missense mutations and most occur in the PI-3 kinase domain. Heterozygous germline missense mutations were also found among B-CLL patients, indicating that such genetic alterations might act as predisposing factors for the development of lymphoid tumors.\textsuperscript{12,13} The germline missense \textit{ATM} mutations have been reported in breast carcinoma patients with early onset disease and positive family history.\textsuperscript{15}

The chromosomal translocation of the mixed lineage leukemia (\textit{MLL}) gene at 11q23 is involved in a subset of childhood leukemia, most frequently in infantile leukemia.\textsuperscript{16-18} The \textit{MLL} gene rearrangement is also involved in treatment-related leukemias secondary to chemotherapy using topoisomerase II (topo II) inhibitors. This has led to the hypothesis that \textit{in utero} exposure to chemicals, such as certain antibiotics, laxatives, podophyllin resins, flavonoids, herbal medicines and benzene
metabolites, may cause infantile leukemia via an effect on topo II.\textsuperscript{19,20} Recent epidemiological data indicate that maternal alcohol exposure and exposure to recreational drugs, pesticides and anti-inflammatory drugs increase the risk of infantile leukemia.\textsuperscript{21-23} Thus, environmental factors appear to play an important role in the development of this disease.

Previous studies have shown that AT cells are hypersensitive to topo II inhibitors.\textsuperscript{24-28} It is plausible that MLL+ leukemias such as infantile leukemia could arise in individuals with hypersensitivity to topo II inhibitors, and this hypothesis led us to investigate \textit{ATM} gene mutation in patients who had developed MLL+ leukemia. In this study, we attempted to determine whether the germline \textit{ATM} gene mutation could represent one genetic factor for predisposition to MLL+ leukemia in children, and whether it could play a role in the pathogenesis of this condition.

\textbf{Materials and Methods}

\textit{Patients}

The study included seven patients diagnosed as having de novo acute lymphoblastic (4 patients) or myeloid (3 patients) leukemia with \textit{MLL} gene rearrangement. The patients were aged from 2 month to 1 y 11 month old, two of whom were less than one year old and classified as infantile leukemia. Normal peripheral blood samples were obtained from these patients during their complete hematological remission. The clinical features of these patients are summarized in Table 1. None of the patients
exhibited clinical evidence of AT, or a family history of AT or Li-Fraumeni syndrome. Normal control samples (wt1, wt2) were obtained from two independent healthy volunteers. Cord blood samples were obtained from local cord blood bank. Samples were collected after informed consent was obtained, based on the approval of our local ethics committee.

*MLL* gene rearrangement was determined by the method described previously.²⁹

### Table 1. Patient clinical data.

<table>
<thead>
<tr>
<th>LCL</th>
<th>Sex</th>
<th>Age</th>
<th>*Diagnosis of leukemia</th>
</tr>
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<tbody>
<tr>
<td>L-1</td>
<td>M</td>
<td>1y</td>
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<td>L-2</td>
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</tr>
<tr>
<td>L-5</td>
<td>M</td>
<td>1y11m</td>
<td>AML(M5)</td>
</tr>
<tr>
<td>L-6</td>
<td>F</td>
<td>1y3m</td>
<td>AML(M5a)</td>
</tr>
<tr>
<td>L-7</td>
<td>F</td>
<td>1y4m</td>
<td>AML(M5b)</td>
</tr>
</tbody>
</table>

*MLL* gene rearrangement was seen in leukemia samples of all the patients in this study.

*ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia. AML was classified according to FAB classification.

LCL, lymphoblastoid cell line

*Cells*
EBV-immortalized lymphoblastoid cell lines (L-1-L-7, LCL-wt1, LCL-wt2) were established by infecting normal lymphocytes obtained from the patients and two normal volunteers with EBV strain B95-8 as previously described.\(^\text{30}\) Immortalized LCLs were maintained in RPMI 1640 (GIBCO BRL Life Technologies, Gaithersburg, MD) with 15% fetal calf serum (FCS) (GIBCO BRL) at approximately 5 x 10\(^5\) cells/ml at 37 °C in 5% CO\(_2\). The SV40-transformed AT cell line GM05849C (7009 del TG) was obtained from Coriell Cell Repositories (Camden, NJ). The osteosarcoma cell line U2OS and human embryonic kidney cell line 293 were obtained from Health Science Research Resources Bank (Osaka, Japan).

**PCR/DNA sequencing**

Mutation analysis of the \(ATM\) coding sequence was undertaken by reverse transcription PCR (TAKARA LA RT-PCR kit; Takara, Shiga, Japan) and direct sequencing of PCR products. Direct sequencing of PCR products was performed by cycle sequencing using ABI Big Dye Terminator chemistry (Applied Biosystems, Foster City, CA) followed by capillary electrophoresis on an ABI 310 automated sequencer. Mutations identified in RT-PCR products were confirmed by sequencing of genomic DNA-based PCR products from fresh peripheral blood lymphocytes.

**Microsatellite analysis**

Microsatellite analysis at chromosome 11q neighboring or intragenic to \(ATM\) was
performed as described\textsuperscript{31}. Briefly the microsatellite markers D11S2178, D11S1294, D11S1778, D11S2179, D11S2366, D11S1787 were selected. The forward primer of each pair was 5’-labeled with HEX or 6-FAM dye. Template DNA was amplified by touchdown PCR using AmpliTaq Gold (Applied Biosystems).

\textit{PCR/RFLP analysis}

Allele frequency of the mutation was analyzed using the PCR/RFLP method. For the 8921 C\textrightarrow{}T nucleotide change, digestion with AlwNI (New England BioLabs) yielded two bands for exon 64 of mutant ATM. For the -787A\textrightarrow{}G nucleotide change, digestion with SacII (Takara) yielded two bands and three bands for exon 2 of wild-type ATM and mutant ATM, respectively. This analysis was also used to detect the expression level of 8921T ATM mRNA in transfectants and L-4 LCL.

\textit{Subcloning of the ATM allele}

RNA was obtained from 1 x 10\textsuperscript{6} EB-transformed cells using the Dynabeads RNA extraction kit (Dynabeads, Oslo, Norway). ATM partial sequence (7012-9402) was amplified using LA taq (Takara) followed by cDNA synthesis using reverse transcriptase XL (Takara). Amplified cDNA was subcloned into the pGEM-T Easy TA cloning system (Promega, Madison, WI). The plasmid, which contained the corresponding nucleotide change of the ATM gene, was digested with PflMI (New England BioLabs) and SalI (Takara), and the insert was subcloned into PflMI and XhoI (Takara) sites of
pcDNA3-YZ5 using Ligation Kit Ver. II (Takara). The expression vector thus constructed (pcDNA3/8921T) was subjected to DNA sequencing analysis and was found to have wild-type sequence except for the 8921T change.

**Western blot analysis**

Cells (1 x 10^6) were washed with PBS and lysed in 150mM NaCl, 1.0% NP-40, 0.1% SDS, 0.1% sodium deoxycholate, 5mM EDTA, and 10mM Tris-HCl (pH 7.4) containing protease inhibitors. Protein concentrations were measured using the DC protein assay (BioRad, Hercules, CA). After boiling with sample buffer, 30µg of protein were subjected to SDS-PAGE. After transfer to PVDF membrane (Millipore, Bedford, MA), the blots were probed with an anti-p53-Ser15 phosphospecific antibody (New England BioLabs, Beverly, MA), anti-ATM antibody or anti-α-tubulin (Oncogene Science, Cambridge, MA) antibody. Horseradish peroxidase (HRP)-conjugated anti-rabbit or -mouse antibody (Amersham, Buckinghamshire, England) was used as the secondary antibody.

**Clonogenic assay**

The plasmid pcDNA3, pcDNA3-YZ5 or pcDNA3-YZ5/8921T was transfected into GM05849C (Effecten; QIAGEN, Hilden, Germany) and stable clones were selected with 500µg/ml neomycin (Sigma, St. Louis, MO). Cells were trypsinized, plated onto 60mm dishes at a density of 7~10x10^3 cells/dish and were incubated for 17h. Then cells were
exposed to a range of doses of X-IR (0 to 5 Gy) followed by incubation for 10 days at 37°C in 5% CO₂. Prior to counting colonies, the culture medium was decanted and the cells were fixed in 95% methanol and stained with 0.5% crystal violet, and the numbers of colonies (>50 cells) from triplicate dishes were counted. Mean colony numbers relative to unirradiated colony numbers were plotted.

**Kinase assay**

293 cells were transiently transfected by FLAG-tagged pcDNA3-YZ5 or pcDNA3-YZ5/8921T. Cells (2 x 10⁷) were washed with PBS and lysed in TGN buffer (150 mM NaCl, 0.3% NP-40, 1% Tween, and 50 mM Tris-HCl [pH 7.5] containing protease inhibitors). Cell lysate (500 µg) was precleared by constant mixing for 1 hour with protein A-Sepharose beads (Pharmacia Biotech, Uppsala, Sweden). The beads were removed by centrifugation, and the supernatant was mixed for 1 hour with anti-FLAG M2 monoclonal antibody (Sigma). Immune complexes were adsorbed onto protein A-Sepharose and then washed twice with TGN buffer, twice with TGN buffer plus 0.5 M LiCl and twice with kinase buffer (20 mM HEPES (pH 7.5), 50 mM NaCl, 10 mM MgCl₂, 1mM dithiothreitol, and 10 mM MnCl₂) and incubated with GST-p53 (1-100) in 20 mM Tris HCl buffer (pH 7.6; 10 mM MgCl₂, 10 mM MnCl₂, 50 mM ATP), containing 100 µM ATP for 30 min at 30°C. Anti-p53 phosphoserine 15 antibody was used to detect phosphorylation of Ser15 of GST-p53 (1-100).
Results

Nucleotide change of the ATM gene

In order to search for ATM mutations, we sequenced the open reading frame (ORF) of ATM cDNA using RT-PCR-based direct sequencing. L-4 was identified to have a nucleotide change of 8921C→T on one allele (Figure 1). The 8921C→T transversion results in a Pro to Leu amino acid change at codon 2974 within the PI-3 kinase domain. Thus L-4 was determined to be heterozygous (wt/8921T) at ATM gene.

Figure 1. Missense nucleotide change of ATM gene at 8921C→T in L-4. The heterozygous missense nucleotide change at 8921C→T in exon 64 in L-4 is shown. The arrow indicates the position of the nucleotide change. The first codon of the ORF was designated +1 (GenBank no. U33841).

The same nucleotide change was confirmed in fresh peripheral lymphocyte genomic DNA from patient L-4 (data not shown). Furthermore, the father of patient L-4 was demonstrated to have the same nucleotide change on one of the alleles (data not shown), indicating paternal inheritance of this nucleotide change.
We also analyzed for the presence or absence of wild-type allele in leukemic cells of patient L-4 by microsatellite analysis, and no loss of heterozygosity (LOH) was identified (supplement data).

We screened 166 samples from an archive of DNA stock of unselected neonatal cord blood for detection of this nucleotide change using RT-PCR/RFLP analysis and direct sequencing. One sample was heterozygous for 8921C→T, resulting in an allele frequency of 0.3%.

**Biological activity of 8921T ATM**

It is well established that AT cells are hypersensitive to ionizing radiation.\(^6,^7,^{33}\) To determine whether the 8921T nucleotide change, which is located within the PI-3 kinase domain, can rescue the radiosensitive phenotype of an AT cell line (ATM-/-), we transfected pcDNA3 (vector alone), pcDNA3-YZ5 (wild-type ATM) or pcDNA3-YZ5/8921T (mutant ATM) into the AT fibroblast cell line GM05849C. Stable clones expressing comparable amounts of wild-type or 8921T ATM protein were isolated (Figure 2B) and analyzed for clonogenic cell survival after exposure to variable doses of irradiation. pcDNA3-YZ5/8921T showed only partial rescue of the radiosensitive phenotype of GM05849C, when compared with pcDNA3-YZ5 (Figure 2A).
Figure 2. Radiosensitivity of AT fibroblast cell transfectants.

(A) Radiosensitivity of GM05849C cells transfected with pcDNA3-YZ5 and pcDNA3-YZ5/8921 was determined by clonogenic assay. Cells were exposed to 0, 1, 2, 3, 4, or 5 Gy of X-IR and then cultured for 10 days prior to fixation, staining and assessment of colony formation. The assay was performed in triplicate; mean colony numbers relative to unirradiated controls were plotted and the SE was shown as an error bar.

(B) Expression of ATM protein was equivalent between GM05849C cells transfected with pcDNA3-YZ5 and pcDNA3-YZ5/8921T. Western blot analysis was performed with anti-ATM antibody. α-tubulin was used as a control for protein loading.

ATM directly phosphorylates p53 at Ser15 in response to XIR-induced DNA damage. Ser15 phosphorylation is a necessary step for p53 accumulation and p53-dependent transactivation. Rapid phosphorylation of p53 at Ser15 in response
to X-IR is reduced in AT cells and cells from some obligate AT heterozygous carriers.\textsuperscript{32} To examine the ability of the 8921T ATM to phosphorylate p53-Ser15, we transiently transfected 293 cells with pcDNA3-YZ5 or pcDNA3-YZ5/8921T and \textit{in vitro} kinase activity was assessed using anti-FLAG-tagged immunoprecipitates. The 8921T exhibited about 60\% less p53-Ser15 phosphorylation activity (Figure 3), suggesting that this is a genuine mutation rather than a polymorphic variant.

\textbf{Figure 3.} \textit{In vitro} phosphorylation of p53-Ser15 by immunoprecipitated ATM. The catalytic activity of ATM protein immunoprecipitated with anti-FLAG antibody from 293 cells transiently transfected with pcDNA3-YZ5 or pcDNA3-YZ5/8921T was assayed \textit{in vitro} against the GST-p53 (aa1-100) substrate. Kinase reactions were western blotted and tested with an anti-p53-Ser15 phosphospecific antibody, or with an anti-ATM antibody, to verify the amount of immunoprecipitated ATM protein per lane. Equal amounts of GST-p53 were loaded. Representative data from three independent experiments are shown (upper panels). Phosphorylation of recombinant GST-p53 (1-100) by pcDNA3-YZ5/8921T was standardized for kinase activity of pcDNA3-YZ5 (=1), and was shown as fold increase. Data were expressed as the mean (±SE) of three independent experiments (bottom panel).
Dominant negative effect of the 8921T mutation

An issue of great interest was to determine whether the 8921T mutant protein interferes with the normal ATM function. We transfected U2OS cells, which exhibit normal ATM-dependent p53-Ser15 phosphorylation activity, with a mock or pcDNA3-YZ5/8921T mutant ATM expression vector, and in vivo p53-Ser15 phosphorylation was examined by Western blotting. We isolated two independent U2OS clones expressing wild-type and 8921T ATM at a ratio of 1:0.1 (clone 1) and 1:0.8 (clone 2), and assayed for p53-Ser15 phosphorylation 30 min after X-IR. The phosphorylation level was reduced by approximately 30% and 50% in clone 1 and clone 2, respectively, when compared with mock transfectant (Figure 4).
Figure 4. Dominant negative effect on p53-Ser15 phosphorylation in U2OS transfectants.

(A) Western blot analysis of p53-Ser15 phosphorylation after X-IR in clone 1 (left panel) and clone 2 (right panel). Cells were harvested at 0 and 30 time after 5Gy X-IR and analyzed by Western blotting with anti-p53-Ser15 phosphospecific antibody. α-tubulin was used as a control for protein loading.

(B) Fold decrease of p53-Ser15 phosphorylation in clone 1 and clone 2, standardized for the level in irradiated/non-irradiated U2OS-mock (= 1.00). Data were expressed as the mean (±SE) of two independent experiments. U2OS cells were transfected with vector alone or pcDNA3-YZ5/8921T. Two independent clones (1 and 2) were isolated and analyzed for expression of wt and mt ATM mRNA by RT-PCR/RFLP analysis using the AlwNI restriction site specific for the 8921T mutation. Clones 1 and 2 expressed wt and mt ATM at a ratio of 1:0.1 and 1:0.8, respectively.
In vivo phosphorylation of p53 at Serine 15 in L-4

The finding that 8921T has a dominant negative activity in transfection assay lead us to study in vivo p53-Ser15 phosphorylation in L-4 immediately after X-IR.

RT-PCR/RFLP analysis allowed us to determine that the expression level of 8921T allele was comparable to that of wild-type allele in L-4 (Figure 5A). At 15 and 30 min post-5 Gy X-IR, L-4 exhibited 40-80% reduction in phosphorylation compared with two normal control LCLs (LCL-wt1, LCL-wt2) and L-6 with no ATM mutation (Figure 5B and C). We noted an unexpected finding that L-2, with no detectable mutation within the open reading frame of ATM (data not shown), also demonstrated a significant decrease in p53-Ser15 phosphorylation (Figure 5C). ATM and Rad-3-related (ATR) kinase also phosphorylates p53-Ser15 1 hour post-X-IR, whereas ATM acts immediately. Therefore, the level of p53-Ser15 phosphorylation at 60 min in this assay was less informative with regard to ATM activity.
A

B

C

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Figure 5. Reduction of X-IR-induced phosphorylation of p53 in L-4.
(A) PCR products of exon 64 (338bp) from L-4, LCL-wt1 and pcDNA3-YZ5/8921T plasmid were digested with AlwNI. 249bp and 89bp fragments are expected in the 8921C→T mutation, but not in the wild-type sequence. 
(B,C) Cells were harvested at various time intervals after 5Gy X-IR and analyzed by Western blotting with anti-p53-Ser15 phosphospecific antibody. α-tubulin was used as a control for protein loading. Levels of phosphorylated p53-Ser15 as detected by Western blotting were quantified by scanning densitometry and were corrected for α-tubulin content, and standardized to those of LCL-wt1 (=1)(B) and LCL-wt2(C). Data were expressed as the mean (±SE) of two independent experiments (bottom panels in C). In (C), the histogram was shown only for the data at 30 min, because the data at 60 min is less informative due to the activity of ATR.

Normal expression of ATM protein in L-4
In most heterozygous AT carriers, the ATM protein level is reduced due to truncation mutations. However, in the case of missense mutation (7271T→G), the ATM protein is expressed at normal level. Thus, ATM protein expression was studied by Western blotting in L-4 and L-2, the latter showing no mutation of ATM gene but poor p53 phosphorylation activity. The level of ATM protein expression in L-4 was comparable with those seen both in normal LCL-wt1 and in the remaining LCLs, except for L-2 (data not shown for L-5, -6, -7). In L-2, the ATM protein was approximately 37% of normal LCL-wt1 (Figure 6A and B). ATM mRNA expression in L-2 was reduced by 30% as analyzed by semiquantitative RT-PCR assay (data not shown). L-2, however, showed no mutation in ORF of ATM gene. Thus we sequenced the 5’ and partial 3’ UTR of the ATM gene and identified the -787A→G nucleotide change (data not shown). Subsequent analysis in 100 Japanese cord blood samples revealed a 61% allele frequency of -787A→G and this was considered as a normal polymorphism (data not shown). Thus, the reason of the decreased ATM expression in L-2 requires further investigation.
Figure 6. Normal expression of ATM protein in L-4

(A) The levels of ATM protein present in the lysates from the indicated cell lines were determined by Western blotting using anti-ATM antibody and normalization with an α-tubulin antibody. Lanes 1-4, LCLs from patients L-2, L-4, L-3, L-4, respectively. Lane 5, normal control LCL(LCL-wt1).

(B) The histograms were obtained by the densitometric analysis of the western blot autoradiographs, after correction of each lane for α-tubulin content. Values are normalized to those of the normal control LCL-wt1(= 1). Although not shown, ATM protein levels in L-5, -6 and -7 were comparable to those of LCL-wt1 and of another control(LCL-wt2). Data was expressed as the mean (±SE) of three independent experiments.

Discussion

AT homozygotes and heterozygotes are reportedly hypersensitive to topo II inhibitors, such as etoposide (VP16). The finding that the plant isoflavenoid genistein (that has topo II inhibitor activity), activates p53 and Chk2 in an
ATM-dependent manner further suggests an important link between topo II inhibitors and ATM. Epidemiological data indicate a significant association between infantile leukemia and maternal exposures to topo II inhibitors or topo II inhibitor-like chemicals, which are known to cause intracellular rearrangement of the MLL gene. These findings lead us to hypothesize that at least some leukemias with MLL rearrangement may develop in individuals with a combination of hypersensitivity to topo II inhibitors as characterized by genetic ATM dysfunction and environmental effects, like exposure to topo II inhibitors.

In the present study, a heterozygous germline mutation (8921C>T) in the PI-3 kinase domain of the ATM gene was identified in a case (L-4) of MLL+ leukemia. L-4 expressed apparently normal level of ATM protein. 8921T is associated with amino acid substitution of 2974 Pro to Leu, which leads to a change in the secondary structure of the ATM protein, namely loss of random coil between α-helices. The in vitro kinase assay showed that 8921T ATM was defective in mediating p53-Ser15 phosphorylation following X-IR. Thus, this change in the secondary structure of the PI-3 kinase domain may disrupt its enzymatic function. Expression of 8921T ATM in AT fibroblasts rescued the radiosensitive phenotype only partially. Furthermore, its expression in U2OS cells revealed an interfering effect with the normal function of ATM. DNA damage-induced in vivo p53-Ser15 phosphorylation activity was reduced in L-4, which was compatible with dominant negative activity against p53-Ser15 phosphorylation in U2OS cell transfected with 8921T. Thus it is strongly suggested...
that 8921T is not just a polymorphism but a pathogenic mutation. LOH was not identified in leukemia cells of L-4. This finding is not incompatible with dominant negative activity of this mutation, and suggests an activity distinct from the tumor suppressor function which has been suggested in some B-CLL patients with ATM mutation-associated p53 dysfunction.44

The ATM protein is known to multimerize and to associate with other proteins, forming a functional complex.45 Gatti et al. has hypothesized that there are two types of ATM heterozygotes, those with truncating mutations making no protein, and those with missense mutations making the mutant protein.46 Presence of the mutant protein in such a complex may disrupt its function, leading to a dominant negative effect against wild-type ATM protein.46 47 In addition, it has been reported that overexpression of a kinase dead ATM cDNA has dominant interfering effect on ATM kinase activity.48 During the preparation of this manuscript, Scott et al. have shown that a missense mutation (7775C→G) of ATM gene identified in a breast cancer patient has a dominant negative effect against wild-type ATM45. Furthermore, Spring et al. reported mice heterozygous for Atm mutation (7636 del 9) but not for truncation mutation, showed an increased susceptibility to developing tumors.49

In a segregation analysis, the father of patient L-4 was heterozygous for the 8921T mutation, excluding the possibility that the alteration arose de novo in the patient. The father, however, remains free from malignant disease to date. These evidences suggest that the 8921T mutation has a relatively low penetrance, and the
environmental stresses, such as exposure to topo II inhibitors at certain developmental stages, played a critical role in the development of MLL+ leukemia in this case. This is important under the light of the finding that allele frequency of 8921T was 0.3% in normal cord blood samples.

Thus although further extensive studies are required to justify our hypothesis, our findings suggest that dysregulation of ATM/p53 signal transduction cascade in response to environmental stresses may be one of the possible genetic factors underlying the pathogenesis of MLL+ leukemia.

Further study is required to understand better of the role of ATM dysfunction with regard to the disease susceptibility and exposures to DNA damaging agents.

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Supplement data. Example of an electropherogram showing no LOH in L-4

Microsatellite analysis of the D11S2179 marker intragenic to ATM. PCR products from normal DNA sample from patient L-4 (A), LCL-wt1(C) and tumor DNA sample from patient L-4 (B).
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