Rapid Communication

Homzygous Deletions of the p15 (MTS2) and p16 (CDKN2/MTSI) Genes in Adult T-Cell Leukemia

By Yoshihiro Hatta, Toshiyasu Hirama, Carl W. Miller, Yasuaki Yamada, Masao Tomonaga, and H. Phillip Koeffler

Adult T-cell leukemia (ATL) is associated with prior infection with human T-cell leukemia virus type I (HTLV-I). Twenty to 40 years often elapse from viral infection to overt ATL, suggesting that other genetic events must occur to produce frank leukemia. The p15 (MTS2) and p16 (CDKN2/MTSI) genes located on chromosome 9p have been implicated as candidate tumor-suppressor genes in several types of tumors. We examined for alterations of these genes in ATL using Southern blot and polymerase chain reaction–single-strand conformation polymorphism analyses. Both p15 and p16 genes were homozygously deleted in 8 of 23 acute/lymphomatous ATL (35%). An additional 3 (13%) and 4 (17%) acute/lymphomatous samples had hemizygous deletions in at least one exon of p15 and p16, respectively. One of 14 chronic ATL samples had a homozygously deleted p16 gene and another had a hemizygous deletion of p16. Neither homozygous nor hemizygous deletions of the p15 gene were found in chronic ATL. In total, 10 of 37 (27%) ATL samples had loss of the p15 and/or p16 genes. No point mutations of the p15 and p16 genes were found. The ATL patient with a homozygously deleted p16 in the chronic phase rapidly progressed to acute ATL and died within 6 months of the initial diagnosis. One instructive patient had no detectable deletion of the p15 and p16 genes during the chronic phase of ATL but had a homozygous deletions of both genes when she progressed to acute ATL. Our results suggest an association of p15/p16 deletions with development of acute ATL.

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Materials and Methods

Samples. Thirty-seven samples from 35 patients were examined in this study. Samples were collected predominantly from Kyushu, Japan, where ATL is epidemic. They consisted of 23 acute-phase, 14 chronic-phase, and 2 lymphomatous ATL samples. Three sequential samples in chronic and acute phase from the same patient (patient no. 19) were included in this study.

Mononuclear cells from patients with ATL were obtained by density gradient separation from the peripheral blood of chronic and acute ATL patients, lymph nodes from lymphomatous ATL samples, and pleural effusions from patients with acute and lymphomatous...
ATL. Most samples had greater than 75% CD4+ cells. Bone marrow DNA from a normal individual was used as a standard for normal p15 and p16 genes. DNA from the K562 cell line, which has a homozygous deletion of the p16 gene, was also used as a negative control. DNA was extracted by a standard methodology using phenol and chloroform extractions.

Southern blot hybridization. Southern blotting was performed for 37 tumor samples (23 in acute/lymphomatous phase and 14 in chronic phase of ATL). Five micrograms of each DNA was digested with EcoRI (GIBCO-BRL, Gaithersburg, MD), separated on 0.7% agarose gels, and transferred to nylon membranes (Hybond-N; Amersham, Amersham, UK). The p15 exon 1 was detected with a 466-bp DNA fragment that was prepared by PCR using primers 4BS0 (GGAGGGTGGACGGTGCCCA) and 4BA1 (CCTCCCGAA-AAGGAATGTGACTCC) designed to amplify exon 2s of both p15 and p16 were detected simultaneously by probing with a 212-bp probe, which is specific for exon 2 of p16 and does not detect the p16 cDNA representing the 3' coding portion of p16 exon 2.

The rationale for using EcoRI was to assure that equal amounts of DNA were applied to each lane. The intensities of signal were assessed by densitometry, because the intensities of signals of these probes at 68°C overnight and then washed sequentially with decreasing concentrations of SSC with a final wash of 0.1X SSC at 68°C for 60 seconds. The PCR condition for p16 exon 1 was 35 cycles of 94°C for 45 seconds, 59°C for 30 seconds, and 72°C for 60 seconds. The PCR condition for p16 exon 1 was 35 cycles of 95°C for 45 seconds, 52.5°C for 30 seconds, and 72°C for 60 seconds. The condition for p16 exon 2 was 35 cycles of 95°C for 40 seconds, 55°C for 30 seconds, and 72°C for 35 seconds. The MgCl2 concentrations were 1.0 mmol/L and 0.75 mmol/L for those of p16, respectively.

Each reaction contained 100 ng of sample DNA, 0.7 pmol of each primer, 5 pmol of each of the four deoxyribonucleotide triphosphates (Pharmacia, Stockholm, Sweden), 0.5 U of Taq DNA polymerase (GIBCO-BRL), and 3 μCi of [α-32P]dCTP (ICN) in 20 μL of the specified buffer. The conditions for exons 1 and 2 of p15 were 30 cycles of 94°C for 30 seconds, 57°C for 30 seconds, and 72°C for 60 seconds. The PCR condition for p16 exon 1 was 35 cycles of 95°C for 45 seconds, 52.5°C for 30 seconds, and 72°C for 60 seconds. The condition for p16 exon 2 was 35 cycles of 95°C for 40 seconds, 55°C for 30 seconds, and 72°C for 35 seconds. The PCR products were denatured and electrophoresed through a non-denaturing 5% polyacrylamide Mutation Detection Enhancement (MDE; J.T. Baker Inc, Phillipsburg, NJ) gel at 400 V for 24 hours, dried, and exposed to Kodak X-OMAT AR film (Kodak, Rochester, NY) at −80°C.

RESULTS

Table 1 and 2 summarize the results. Using Southern blot analysis, we examined 23 acute/lymphomatous-phase ATL samples and 14 chronic-phase ATL samples. Four of 23 (17%) acute/lymphomatous-phase samples and 1 of 14 (7%) chronic-phase samples were homozgyously deleted in both exons 1 and 2 of the p16 gene (Fig 1). The chronic ATL patient with homozygous p16 deletion progressed to acute ATL and died within 6 months (sample no. 33). All acute/lymphomatous-phase samples with homozgyously deleted p16 also had homozygous deletions of p15. However, the chronic-phase ATL sample with homozgyous loss of p16 had an intact p15 gene. One instructive case had no detectable deletions of the p15 and p16 genes in the ATL cells during the chronic phase of the disease (samples no. 19C1 and 19C2), but, during the acute phase of the disease, the ATL cells from the same patient had homozygous deletions of these genes (sample no. 19A; Fig 2). This sample had homozgyous deletions of both p15 and p16 genes as determined by densitometry, because the intensities of signals of these genes were less than one eighth of those of the control.

Hemizygous deletion of the p16 gene in at least one exon was found in 4 of 23 (17%) acute/lymphomatous-phase sam-

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**Table 1. Deletions of the p15 and p16 Gene in ATL**

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Disease/Stage</th>
<th>Exon 1</th>
<th>Exon 2</th>
<th>Exon 1</th>
<th>Exon 2</th>
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<tbody>
<tr>
<td>1</td>
<td>Acute Homo</td>
<td>4 (17)</td>
<td>4 (17)</td>
<td>4 (17)</td>
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<tr>
<td>2</td>
<td>Acute Homo</td>
<td>3 (13)</td>
<td>3 (13)</td>
<td>4 (17)</td>
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<td>3</td>
<td>Acute Homo</td>
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<td>0 (0)</td>
<td>1 (7)</td>
<td>1 (7)</td>
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<tr>
<td>4</td>
<td>Acute Homo</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>1 (7)</td>
<td>1 (7)</td>
</tr>
</tbody>
</table>

Abbreviations: Homo, homozygous deletion; Hemizygous deletion.

**Table 2. Southern Blot Analysis: Samples With Altered p15 and p16 Genes in ATL**

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Exon 1</th>
<th>Exon 2</th>
<th>Exon 1</th>
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<tr>
<td>Acute</td>
<td>Hemizygous deletion</td>
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Abbreviations: Homo, homozygous deletion; Hemizygous deletion.
transferred to nylon membranes, with various patients were digested. The cross-hybridizing bands with exons 1 and 2 of p16, lower blot) show hemizygous deletions of exons 1 and 2, respectively. The asterisk shows a band of similar size on Southern blots after EcoRI digestion has been also noted by other investigators.15

To detect the presence of small deletions or point mutations, we performed PCR-SSCP analysis for p15 and p16. None of the 37 samples had detectable mutations or small deletions of these genes (data not shown).

**DISCUSSION**

The close association between ATL and HTLV-I virus has been established. Nevertheless, the mechanisms of development of ATL after HTLV-I infection have not been clarified. Although HTLV-I does not have an oncogene,26,36 it contains the *tax* region in addition to the genes common to all retroviruses.30 The *Tax* protein encoded from this region is required for viral replication30 and activates the expression of several cytokines and cytokine receptors including interleukin-2 (IL-2) and the α subunit of IL-2 receptor (IL-2Rα).32 The *Tax*-mediated induction of NF-κB protein that binds to the IL-2Rα promoter may contribute to polyclonal or oligoclonal expansion of HTLV-I–infected T cells.33 In addition, the overexpression of *tax* can transform RAT-1 cells.34 These in vitro results strongly implicate the involvement of *Tax* in the progression toward development of ATL. However, this does not explain the 20 to 40 years of latency from infection to development of ATL. In addition, ATL cells usually do not express *tax* in vivo.35 Therefore, additional genetic changes are probably required for progression to ATL.

In this study, we determined for the first time that p15 and p16 genes are altered in ATL. Four of 23 (17%) acute/lymphomatous ATL samples had homozygous deletions of both the p15 and p16 genes. Among 14 chronic-phase samples, homozygous deletions of p16 were found in 1 case (14%), but deletions of p15 were not found. This chronic ATL patient rapidly progressed to acute ATL and died, suggesting that the sample obtained from the patient in the “chronic” phase was in a “pre-acute” phase. Another instructive patient had no deletions of the p15 and p16 genes in the ATL cells in the chronic phase, but had homozygous deletions of both genes in the acute phase, indicating that the deletions were acquired during the transition from chronic to acute ATL. The association of these genetic events with acute but not chronic ATL suggests to us that homozygous deletions of these genes may be frequently acquired abnormalities during the development of the acute phase of ATL.

We have found that all samples with homozygous p15 deletions also had homozygous p16 deletions; however, one sample had a homozygous deletion and two samples had a hemizygous deletion of p16 with a normal appearing p15 gene. These data suggest that p16 rather than p15 may be the target of these deletion. Potentially, homozygous deletions of the p15/p16 genes coupled with point mutations of the undeleted allele could also inactivate these genes. This is the frequent mechanism of inactivation of the p53 gene.36,37 However, we were unable to detect any point mutations of the p15 and p16 genes in our samples. Because we did not analyze the promoter region and exon 3 of p16, which is only 14 bp,38 we do not know if this part of the gene is altered.

![Southern blot analysis of the p16 gene in ATL](image-url)
The $p15$ and $p16$ genes have been shown to be involved in the development of various types of cancer. In acute and chronic myeloid leukemias, such as T- and B-ALL, p15 and p16 point mutations are rare, but instead, homozygous and hemizygous deletions of these genes are found.

In contrast, p16 deletions are more common in T-cell lymphomas and ATL, with a frequency of about 40%.

We hypothesize that the inactivation of the $p15$ and $p16$ genes in ATL occurs through a combination of mechanisms, including point mutations and deletions. This hypothesis is supported by the observation that in ATL, p16 is often mutated or deleted, whereas it is usually intact in cells containing wild-type p53.

Further studies are required to determine if this hypothesis is correct.

ACKNOWLEDGMENT

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