Mutations of the p53 Gene in Adult T-Cell Leukemia

By Akiko Sakashita, Toshio Hattori, Carl W. Miller, Hitoshi Suzushima, Norio Asou, Kiyoshi Takatsuki, and H. Phillip Koeffler

The p53 tumor suppressor gene was examined by direct sequencing of polymerase chain reaction-amplified DNA from fresh tumor cells of 10 patients with adult T-cell leukemia (ATL). Samples included nine patients with acute or lymphomatous ATL, and one patient in whom samples were examined in both his acute and chronic stages of ATL. Four missense mutations and one silent point mutation in the coding region of the p53 gene were found in cells from five patients with either acute or lymphomatous ATL. The missense mutations were homozygous and occurred in evolutionarily highly conserved regions of p53. One patient had no p53 mutation in his leukemic cells during chronic phase of ATL, but had a homozygous point mutation at codon 273 (Arg to His) when he progressed to acute ATL. In summary, we show that p53 is frequently mutated in the acute phase of ATL and one informative case suggests that p53 mutations may be associated with the transition from chronic to acute ATL.

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ADULT T-CELL leukemia (ATL) is a distinct clinical entity found throughout the world, but especially prevalent in Southwestern Japan. Acute or lymphomatous phase of ATL is a fulminant disease rapidly resulting in death. Isolation of human T-cell leukemia virus type I (HTLV-1) from cell lines derived from patients with ATL suggested that HTLV-1 was the etiologic agent for ATL. However, only 2% to 5% of individuals infected with HTLV-1 develop acute ATL and this usually occurs after a latency of 20 to 30 years from the time of infection. Therefore, infection with HTLV-1 probably is not sufficient for leukemogenesis; other secondary mutations are probably required for transformation.

The p53 gene is a nuclear phosphoprotein that can function as a tumor suppressor gene. Allelic loss of human chromosome 17p13, site of the p53 gene, has been found in several malignancies, including cancers of breast, colon, and lung, as well as astrocytomas and blast crisis of chronic myeloid leukemia. Mutations of the p53 gene have often been found when the gene has been sequenced in these tumors. In the present study, we report that fresh ATL cells from patients with the acute phase of the disease often have alteration of the p53 gene.

MATERIALS AND METHODS

ATL samples were from the Kyushu area of Japan, where ATL is endemic. Mononuclear cells (MC) from patients with ATL were prepared by density gradient separation from peripheral blood (chronic and acute ATL patients), lymph nodes (two patients with lymphomatous ATL), and pleural effusion (one patient with lymphomatous ATL). The surface phenotype of the separated MC fractions was determined by indirect immunofluorescence (IF) by laser flow cytometry (FACSter; Becton Dickinson Monoclonal Center, Mountain View, CA) with mouse monoclonal antibodies (MoAbs) that recognize T-lymphocyte-associated antigens (anti-CD4, -CD8, -CD5; purchased from Ortho Diagnostics, Raritan, NJ).

Total DNA was prepared by lysis of cells with sodium dodecyl sulfate (SDS), and digestion with proteinase K followed by phenol extraction. Genomic DNA was amplified using polymerase chain reaction (PCR). Two p53 fragments were amplified: one was 1.2 kb encompassing exons 4, 5, and 6; and the other was 700 bp including exons 7 and 8. The primers used for these two amplifications were sense 5′AAAACCTACGAGGAGCTAC3′ and antisense 5′CTCCTAGTGTGGCTCTGACTG3′ (exon 4); sense 5′CAGGGCGTGGTTGCCCAGGGTCCCCA3′ and antisense 5′GCCCCAGCTGCTACCATGCTG3′ (exon 5); sense 5′CAAGCTGCTGGTCCAGGGTCCCCA3′ and antisense 5′ATGGGAAACCCAGAAGCTG3′ (exon 6); sense 5′CTCCTAGTGTGGCTCTGACTG3′ and antisense 5′AACAGGTGCTTCCTGAGG3′ (exon 7); and sense 5′TCTGCTGGTACCTGCTGTTATT3′ and antisense 5′CTGCTGGTACCTGCTGTTAG3′ (exon 8).

RESULTS

Cell samples from individuals with ATL were isolated from blood, lymph node, and pleural fluid. After the isolation of mononuclear cells by density sedimentation, cell populations were phenotyped by flow cytometry (Table 1). CD4 cells were more than 87% of the cell population in eight samples and 57% to 76% of the population in the other three cases. ATL cells are usually CD4-positive and CD8-negative.

From the Division of Hematology/Oncology, Department of Medicine, Cedars-Sinai Medical Center, UCLA School of Medicine, Los Angeles, CA; and the Second Department of Internal Medicine, Kumamoto University Medical School, Kumamoto, Japan.

Submitted July 8, 1991; accepted September 17, 1991.

Supported in part by US Public Health Service Grants No. DK41936, CA26038-11, CA33936, and CA42710, the Weitz Family Foundation, the Leukemia Fund in memory of Marilyn Levine, and the Realtors of Real Estate Industry Division to H.P.K.

Address reprint requests to Akiko Sakashita, MD, Division of Hematology/Oncology, UCLA School of Medicine, 11-543 Factor Building, 10833 Le Conte Ave, Los Angeles, CA 90024-1678.

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DNA was isolated from these cell populations. Sequencing of p53 was performed between codons 110 to 308 (exons 4, 5, 6, 7, and 8). In addition, at least 25 nucleotides of the introns surrounding these regions were determined. In sequencing 11 samples from 10 patients, mutations were found in five (Table 1). Four of these were missense mutations involving regions of the p53 previously shown to be prone to mutate: patient 1, codon 273 (Arg to His); patient 2, codon 197 (Val to Gly); patient 3, codon 245 (Gly to Asp); patient 4, codon 245 (Gly to Ser) (Table 1, Fig 1). The ATL cells from patient 5 had a mutation at codon 125, the last nucleotide of exon 4; this was a silent mutation (Table 1). Furthermore, ATL cells of one instructive patient (patient 1) had no detectable p53 mutation during the chronic phase of the disease, but during acute phase of the disease, the ATL cells had a homozygous p53 mutation (Fig 2). We believe that this result does not represent a sampling error because 95% of the cells in the chronic-phase ATL sample were CD4-positive, consistent with most of these cells being involved in the disease. The sensitivity of direct sequencing should allow the detection of a heterozygous mutation in a population in which tumor cells comprise about 30% of the population.\(^9\) Also, the population of CD4-positive cells was more than 57% in all the other samples that had no detectable p53 mutations by direct sequencing. All mutations were reproducibly detected in both sense and antisense strands from several preparations of either two or more independently amplified DNA samples, ruling out spurious PCR-induced mutations.

**DISCUSSION**

In the present study, a point mutation of the p53 gene was found in 5 of 10 ATL patients. Four of the mutations were missense and mapped to the highly evolutionarily conserved regions of the p53 gene, which previously were identified as hot spots for mutations.\(^11,19\) The missense mutations from all four samples were either homozygous or hemizygous. This finding probably resulted from a point mutation of the p53 gene on one allele and loss of the other,
normal p53 allele. The fifth mutation was at the last nucleotide of exon 4 and was a heterozygous silent mutation, outside of the mutational hot spots. Recently, the p53 gene was also found to be mutated in non-HTLV-1 infected T-lymphoid leukemic cells from several patients and cell lines at a frequency of about 20% and 50%, respectively.

Despite the accumulated information about HTLV-1, a clear understanding of the pathogenesis of ATL has not emerged. HTLV-1 does not contain an oncogene, probably does not activate proto-oncogenes by insertional activation, nor does it inactivate tumor suppressor genes by disrupting them. In addition to the genes common to all retroviruses, HTLV-1 contains a X region; the tax product, which is encoded from this region, is required for viral replication. Cotransfection studies showed that tax can activate other genes such as those coding for interleukin-2 receptor (IL-2R), granulocyte-macrophage colony-stimulating factor (GM-CSF), as well as c-fos and c-sis. Overexpression of tax can transform RAT-1 cells. However, ATL cells in vivo usually do not express viral proteins, including tax. Furthermore, the natural history of development of acute ATL suggests that HTLV-1 infection alone is not sufficient to cause leukemia. Many people infected with the virus do not develop ATL; those who do, often have a 20- to 30-year latency period. Our data suggest that alterations of the p53 gene are frequently acquired abnormalities in acute ATL and may occur in the transition to the more aggressive leukemic phenotype. Leukemic cells of 4 of 10 of our acute ATL cases had homozygous p53 mutations. In addition, we studied an informative patient whose ATL cells had no detectable p53 mutations in chronic-phase ATL. A novel clone emerged that quickly expanded to acute ATL and rapidly led to the death of the patient. Cells of this novel clone had a homozygous missense mutation of p53.

A possible hypothesis concerning the development of ATL can be stated as follows. HTLV-1 infection results in acute expression of tax, perhaps resulting in autocrine growth stimulation through production and growth stimulation by IL-2. This stimulation may cause a polyclonal expansion of these HTLV-1–infected T lymphocytes. These cells may periodically expand by various stimuli such as infections that might enhance tax production. This polyclonally expanded population of cells may have a slight growth advantage over normal cells. Evolution from chronic to acute ATL may result in part from homozygous mutational alteration of p53. If our thesis is accurate, ATL will provide a valuable model for analyzing the role of p53 in the clonal development of cancer.

ACKNOWLEDGMENT

We thank Kim Burgin, Elisa Weiss, Margery Goldberg, and Elaine Epstein for their excellent secretarial support.

REFERENCES


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