Detailed Deletion Mapping of the Long Arm of Chromosome 6 in Adult T-Cell Leukemia

By Yoshihiro Hatta, Yasuaki Yamada, Masao Tomonaga, Isao Miyoshi, Jonathan W. Said, and H. Phillip Koeffler

Previously, we have found that the loss of heterozygosity (LOH) was frequently observed on chromosome 6q in acute lymphoma-type adult T-cell leukemia (ATL), suggesting a putative tumor-suppressor gene for ATL may be present on chromosome 6q. To further define a region containing this gene, we performed fine-scale deletional mapping of chromosome 6q in 22 acute/lymphomatous ATL samples using 24 highly informative microsatellite markers. LOH was found in 9 samples (40.9%) at 1 or more of the loci examined. Of the 9 samples, 8 shared the same smallest commonly deleted region flanked by D6S1652 and D6S1644 (6q15-21). The genetic distance between these two loci is approximately 4 cM. These results suggest that a putative tumor-suppressor gene on chromosome 6q15-21 probably plays a very important role in the evolution of acute/lymphomatous ATL. Our map provides key information toward cloning the gene.

© 1999 by The American Society of Hematology.

Materials and Methods

Samples. Twenty-two paired genomic DNA samples were obtained from the patients with acute/lymphoma-type ATL after their informed consent. All the ATL samples were ascertained by determining the monoclonal integration of the HTLV-I proviral genome. The clinical subtypes of ATL were based on the diagnostic criteria proposed by the Lymphoma Study Group of Japan.15 The percentage of containing normal cells in the acute/lymphoma-phase samples was at most 30% and usually less than 10%. The corresponding control DNAs were obtained from either their peripheral blood after complete remission (n = 17) or during their chronic phase (n = 5). DNA was extracted by a standard technique with proteinase K digestion and phenol/chloroform extraction.

Allelic loss analysis. The LOH analysis was performed by PCR-amplification of microsatellite sequences as described before.18 The genetic map of chromosome 6 and chromosome 6–specific microsatellite markers, including their primer sequences and sizes used in this study, were compiled from the Genent human genetic linkage map.17 Some markers have been assigned to the same location in a 0 cM cluster. Primers for polymerase chain reaction (PCR) amplification of microsatellite markers were obtained from Research Genetics (Huntsville, AL). PCR was performed in a final volume of 20 µL containing 25 ng DNA, 1.5 mmol/L MgCl2, 10 pmol/L of each of the primers, 2 nmol/L of each of the four deoxynucleotide triphosphates (dNTP; Pharmacia, Stockholm, Sweden), 1 unit of Taq DNA polymerase (GIBCO-BRL, Gaithersburg, MD), and 2 µCi 32 P-labeled deoxyctydine triphosphates (dCTP) (3000 µCi/mmol; New England Nuclear/Dupont, Boston, MA) with specified buffer provided by the supplier. PCR consisted of 40 seconds at 94°C, 30 seconds at 55°C to 75.5°C, and 1 minute at 72°C for 27 to 33 cycles in a Programmable Thermal Controller (MJ Research Inc, Water Town, MA) to examine the products in the linear range of signals. For some of the markers, PCR reaction was performed in a multiplex fashion to ascertain either LOH or duplication of the region;
two primer sets were mixed under the conditions described above. PCR products were mixed with a formamide gel-loading solution, heat denatured at 94°C, separated on a denaturing 5% to 8% polyacrylamide gel containing 8.3 mol/L urea, and visualized by autoradiography. Allelic losses were defined by visual comparison of the relative allelic ratios of the normal and tumor samples on the autoradiographs. In some cases of weak radiographic intensity, differences in the alleles in the tumor versus control DNA were analyzed with respect to the number of normal cells compared with malignant cells in representative slides from the tumors. In such cases, the ratio of allele intensities was classified as LOH if it roughly agreed with the percentage of the tumor cells in the sample. When visible reduction of radiographic signal was equivocal, a radioanalytic imaging detector (Ambis; Ambis Inc, San Diego, CA) was used to confirm our interpretation. All positive results were repeated for confirmation.

RESULTS

We screened 22 paired ATL samples for LOH with a panel of 24 highly informative microsatellite markers spanning chromosome 6q. All patients were informative at multiple loci on chromosome 6q. Allelic loss was observed in 9 of 22 cases (40.9%); 4 (samples D, H, L, and T) of the 15 acute leukemias and 5 (samples E, F, G, P, and S) of the 7 lymphoma type. The most frequent LOH (5 of 11 informative cases; 45.5%) was observed at the D6S1601 locus. Figure 1 shows examples of allele loss.

Figure 2 shows the deletional map on chromosome 6q as composed from the nine cases that had LOH on the arm. Of the 9 samples, 8 shared the same smallest consensus region, which was approximately 4 cM between markers D6S1652 and D6S1644 located at the 6q15-21 chromosomal band. Allelic loss of the smallest commonly deleted region on 6q was observed in both acute (3 of 15, 30.0%) and lymphoma type (5 of 7, 71.4%) of ATL.

DISCUSSION

In ATL, chromosomal regions of nonrandom deletions have been identified by cytogenetics including 6q, especially at band 6q21. Similarly, we have previously identified by allelotyping using microsatellite markers that chromosomal arm 6q is one of the most frequent sites of LOH in acute/lymphoma-type ATL.
The aim of the present study was to delineate precisely the critical region that is deleted on the long arm of chromosome 6 to localize further the tumor-suppressor gene involved in ATL. To narrow this region, the LOH on the arm 6q in ATL was mapped using 24 polymorphic markers. We have found that the frequency of LOH on 6q (40.9%) was higher than that reported by cytogenetic analysis (23%). Thus, cytogenetic studies have probably missed some cases of small interstitial deletions on 6q. Our study showed that eight of the nine tumors with interstitial losses or partial losses of chromosome 6q had a commonly deleted region between D6S1652 and D6S1644 at 6q15-21. The distance between these two loci corresponds to 4 cM of physical distance.

From several LOH studies, chromosome 6q appears to be involved in the pathogenesis of a number of solid tumors including ovarian carcinoma, breast carcinoma, malignant melanoma, renal cell carcinoma, hepatocellular carcinoma, salivary gland adenocarcinoma, small-cell lung carcinoma, prostate carcinoma, and parathyroid adenoma. However, the precise nature of these molecular deletions has so far not been analyzed in detail. In hematological malignancies, deletions involving the long arm of chromosome 6 are observed primarily in lymphoid malignancies, i.e., acute lymphoblastic leukemias (ALL), lymphoproliferative disorders (LPD), and non-Hodgkin’s lymphomas (NHL). Several commonly deleted regions along 6q have been reported in lymphoma and lymphoblastic leukemia including 6q12-21, 6q14-21, 6q21, 6q21-22, 6q21-23, 6q23, 6q23-24, 6q23-27, and 6q25-27. However, to date, no altered tumor-suppressor gene responsible for these tumors has been determined. Cloning of the candidate gene(s) will define whether a single or multiple tumor-suppressor gene(s) is clustered on 6q and is commonly involved in these types of tumors. Deletions of chromosome 6q are correlated with a poor prognosis in NHL. Although all of the patients in our series were not treated uniformly, we did not find any significant association between LOH of 6q and the observed proportion of treatment failures probably because the survival time of all the individuals with acute/lymphoma-type ATL was very short. Taken together, we have identified a commonly deleted region of LOH on chromosome 6q15-21 that may play a pivotal role in development of ATL. Studies are in progress to investigate further this region of interest.

ACKNOWLEDGMENT

The authors thank Kim Burgin and Marge Goldberg for their excellent secretarial help.

REFERENCES


Detailed Deletion Mapping of the Long Arm of Chromosome 6 in Adult T-Cell Leukemia

Yoshihiro Hatta, Yasuaki Yamada, Masao Tomonaga, Isao Miyoshi, Jonathan W. Said and H. Phillip Koeffler