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

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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.

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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.

Fig 1. Representative autoradiographs showing LOH in patients E, H, and S. Loss of one parental band was observed in the acute/lymphoma ATL samples (arrows). L, DNA samples isolated from the lymphoma cells; C, DNA samples isolated from the corresponding normal peripheral leukocytes after complete remission; A, DNA samples isolated from the leukemic cells in acute type.
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, 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, 6q23-24, 6q23-24, 6q23-24, 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.

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