CONCISE REPORT

Identification of a Restriction Fragment Length Polymorphism Involving the Oncogene ETS-1 on Chromosome 11q23

By Paul D. Savage, Curtis A. Hanson, and John H. Kersey

Twenty four samples of DNA from 23 unrelated individuals were analyzed for the presence of a novel restriction fragment length polymorphism (RFLP) involving the proto-oncogene ETS-1 at an Xba I site. Four samples from unrelated individuals lacked an Xba I site, giving rise to a longer restriction fragment detectable by Southern analysis; two samples were from normal tissue, and two were from acute myelogenous leukemic blasts. Thus, no association could be found between the RFLP and disease among the individuals studied. Pedigree analysis of another cohort demonstrated Mendelian inheritance consistent with a somatic polymorphism. The practical applications of RFLP analysis in clinical and research settings, and the usefulness of this Xba I RFLP in the study of hematologic malignancies because of its location in 11q23, are discussed.

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VARIATION IN PHENOTYPIC characteristics between individual members of a species is often the result of minor base sequence differences between the genes responsible for that phenotype. Those differences that occur at particular restriction endonuclease sites are detected following restriction endonuclease digestion and Southern analysis by the presence of fragments of varying lengths. These fragment length differences are referred to as restriction fragment length polymorphisms (RFLPs), and their analysis has been shown to be useful for a number of studies, including assistance in the mapping of genes to certain regions of the genome, serving as markers for certain diseases or phenotypes, and determination of engraftment in allogeneic bone marrow transplantation.

Many types of malignancies, both hematologic and nonhematologic, are associated with cytogenetic abnormalities of chromosome 11 band q23 (11q23). A number of genes have been mapped to this area, including the proto-oncogene ETS-1. ETS-1 has been of interest because proto-oncogene activation, which occurs as a result of translocations, deletions, or amplifications, can be important in the pathogenesis of these malignancies.

Using the genomic ETS-1 probe described by de Taisne et al., we have looked at many cases of acute lymphocytic leukemia (ALL), acute nonlymphocytic leukemia (ANLL), and a leukemic cell line with a known translocation of 11q23. A number of genes have been mapped to this area, including the proto-oncogene ETS-1. ETS-1 has been of interest because proto-oncogene activation, which occurs as a result of translocations, deletions, or amplifications, can be important in the pathogenesis of these malignancies.

RESULTS

In the course of experiments to evaluate possible ETS-1 rearrangements in leukemias, we found a nongermline restriction fragment on an Xba I digest of cells from a patient with ANLL with FAB-M4 morphology. Since multiple other enzyme digests revealed germline configuration, we sought to determine whether this was an RFLP or a pathophysiological point mutation. In order to evaluate this question, DNA

MATERIALS AND METHODS

Leukemic tissues used in this study included excess peripheral blood and/or bone marrow obtained during diagnostic work-up or relapse studies; materials were either fresh or quick frozen in DMSO and stored in liquid nitrogen. Leukemias were analyzed by routine histopathologic studies and cell marker analysis and classified according to the French-American-British (FAB) system. Cell lines were representative of human lymphoid and myeloid lineages and were used because they represented leukemias that are cytogenetically normal at 11q23; the cell line RS(4;11) was also used because it contains a known t(4;11)(q21;q23) that was described elsewhere. Normal DNA was obtained either from placentas or excess bone marrow (obtained during harvesting for bone marrow transplantation); the cell line HF-26 was also used as a source of normal DNA.

The use of all human material was approved by the University of Minnesota Committee on the Use of Human Subjects in Research; the methods and facilities used followed NIH guidelines for recombinant DNA studies.

Genomic DNA was extracted from tissue using the nuclei isolation technique as described by Bahkshi et al. Ten micrograms were then digested with the indicated enzyme under conditions as outlined by the enzyme manufacturer for four hours (Bethesda Research Laboratories, Gaithersburg, MD). The restriction fragments were size fractionated on a 0.8% agarose gel, then transferred to an activated nylon membrane (Zetabind; AMF Cuno, Meriden, CT) with 20X SSC (standard saline citrate) using the Southern technique.

The probe used in this study is the 5.4 kb Eco R1 human genomic ETS-1 probe described by de Taisne et al. Radiolabeled probes were synthesized to high specific activity using the random hexamer primer method and 32P-dCTP.

Hybridization was performed for 48 hours in the presence of 50% formamide at 42°C. Posthybridization was performed at high stringency, the final washes being done with 0.1X SSC, 0.1% SDS at 60°C, for one hour. The blots were air dried for six hours, and exposed to Kodak XAR-5 film (Rochester, NY) at −70°C for one-half to three days.

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was studied from a number of normal individuals. A Southern blot of an Xba I digest of total genomic DNA from 19 unrelated individuals studied with the ETS-1 genomic probe is shown in Fig 1. This probe hybridizes to restriction fragments of 6.6 kb and 1.15 kb in length in most individuals; however, four samples demonstrated a 2.4 kb restriction fragment in addition to the other two. The presence of only one additional band (2.4 kb) in the four individuals, combined with a probe size of 5.4 kb, can best be explained by loss of the Xba I site flanking the 1.15 kb fragment, with extension of this fragment to the next Xba I site, 1.25 kb away. Thus, the 2.4 kb fragment and the 1.15 kb fragment appear to be alleles. In no case did we find a homozygote who lacked the 1.15 kb fragment. This appears to represent an RFLP, in that germline configuration is found on digestion of these DNAs with other enzymes (at least three [Bam HI, Eco RI, Hind III], and up to eight in some cases [including Bgl II, Kpn I, Pst I, Sal I, and Sst I]). Further evidence that this is an RFLP resulted from demonstration that it is transmitted in a Mendelian co-dominant inheritance pattern on analysis of family members (data not shown); here, too, no homozygote for the 2.4 kb allele was found. Thus, the two alleles may be represented as follows:

<table>
<thead>
<tr>
<th>Allele no.</th>
<th>Size (kb)</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.15</td>
<td>0.913</td>
</tr>
<tr>
<td>2</td>
<td>2.4</td>
<td>0.087</td>
</tr>
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</table>

DISCUSSION

RFLPs are useful as unique markers for certain regions of the genome; as such, they are useful to both laboratory and clinical investigators for a variety of studies. We have described a new RFLP that involves an Xba I site within the ETS-1 locus; it is interesting to note that the size of this other restriction fragment is approximately the same as that of the rearranged Xba I fragment reported by Rovigatti et al. This site is important as 11q23 is involved more than any other region of the genome in ANLL; abnormalities of 11q23 are also reported for ALL, non-Hodgkin's lymphoma (NHL) (both 1 and 2*), solid tumors, congenital disorders, and most recently, as the most common site of involvement when progressive cytogenetic abnormalities are seen in childhood acute leukemia.

The role of the proto-oncogene ETS-1 in the pathogenesis of many of the above malignancies is unclear, although there is evidence that the ETS-1 proto-oncogene moves to chromosome 4 in at least some translocations associated with these leukemias. We have not to date demonstrated rearrangement in the cases that we have studied. The significance of this RFLP that we report here is not clear. Proto-oncogene activation may occur following point mutation, as has been demonstrated with two other proto-oncogenes, and it is interesting to note that we did not detect a homozygote for allele no. 2, although this latter point is most likely secondary to the small sample size. The significance of an ETS-1 point mutation can only be definitively answered once the normal ETS-1 cDNA sequence, as well as the sequences of the RFLPs in both normal and pathologic tissues, has been determined. Care must be taken in analyzing this region of DNA with Xba I, or any restriction endonuclease whose site may partially overlap the Xba I site, until the exact base changes are known, since the variant length restriction fragments can be mistaken as evidence for gene rearrangement if further analysis with other enzymes is not performed.

This RFLP may be useful as a marker for a predilection to a disease or group of diseases. Our data do not support nor refute this at present, although the numbers of normal specimens and malignant subtypes are small; further studies involving larger numbers of cases are needed in order to address this issue.

We feel it is of interest to note that this Xba I RFLP is the fourth RFLP that has been identified within the ETS-1 locus; the other three are Ava II and two for Sst I. All four are detectable with the same probe, and three RFLPs (Ava II, Sst I, Xba I) lie within a few kilobases of each other. To date no other RFLPs have been reported within 11q23 that are not detected by ETS-1 probes.

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AN RFLP 11q23 INVOLVES ETS-1

REFERENCES

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