Breakpoints and Junctional Regions of Intragenic Deletions in the HPRT Gene in Human T-Cells

Irene R. Rainville, Richard J. Albertini, and Janice A. Nicklas

IVCC Genetics Laboratory, University of Vermont, 32 North Prospect Street, Burlington, Vermont 05401

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Abstract—DNA sequences of the deletion breakpoints of 24 human T-lymphocyte hprt gene mutations are reported. These independent deletions ranged in size from 18 to 15655 base pairs. Seven of the 21 in vivo mutations arose in normal adults, three in normal children, eight in radioimmunotherapy patients and three in platinum chemotherapy patients. One in vitro mutation was isolated after 93cGy radon exposure and two after 300cGy γ radiation. The breakpoints were found to be non-random and a cluster of small deletions in exon 6 is reported. Ten of the mutations had 2–5bp direct repeats at the breakpoints. There was no excess of “deletion-associated” motifs over that expected by chance. Some breakpoints do occur at consensus topoisomerase II cleavage sites and the centromeric end of a Donehower sequence occurs exactly at a telomeric breakpoint. Three mutants had breakpoints at hairpins expected by the model of Glickman and Ripley (1).

INTRODUCTION

Deletions of specific genes are frequently disease-associated genetic events. Numerous examples have been studied in detail, including the sil-tal fusions, i.e. del (1) p32–34, in T-cell acute lymphocytic leukemia (2, 3), deletions of the retinoblastoma gene, del (13) q14, in retinoblastoma (4, 5) and deletions within the dystrophin gene in muscular dystrophy (6). The pathogenic relevance of deletions and related mutations, such as DNA translocations, makes it important to characterize the molecular bases of this class of genetic events.

Deletions of tumor suppressor genes are common in solid tumors (7–9). While frequently identified as loss of heterozygosity (LOH), most are poorly characterized at the DNA sequence level. By contrast, DNA deletions and rearrangements have been studied at the molecular level in hematological malignancies. Breakpoint motifs and clusterings have been identified (10, 11), but it is difficult to determine if these represent frequencies of the genetic events, or selection in vivo for the resultant malignant cells. The deletions resulting in human genetic diseases also have been studied in detail, and widely different frequencies have been observed in different genes. For example, in the factor VIII gene (26 exons, 186 kb), 2.5% of the disease related mutations are deletions while for the steroid sulfatase gene (10 exons, 146 kb), the LDL receptor gene (18 exons, 60kb) and the dystrophin gene (> 60 exons, 2.3 Mb), the relative frequencies are 90%, 2–6% and 70%, respectively (12).
Maximum deletion sizes are similarly variable, e.g. in the LDL gene it is > 11 kb (13) while in the steroid sulfatase gene it is > 146 kb (12). Breakpoint clustering also occurs, e.g. the dystrophin gene has one 200 kb region with 55 breakpoints observed (6) and, in the steroid sulfatase gene, total deletion breakpoints cluster at positions with low copy repetitive elements (12).

Numerous mechanisms have been proposed for the creation of large scale changes in DNA. These include: slipped mispairing at tandem repeats (14), errors of mismatch repair (15), strand switch and migration (15), breakage in DNA hairpins (1), illegitimate recombination between or transposition of repetitive elements (16–21), breakage at matrix attachment and/or topoisomerase II cleavage sites (22, 23), illegitimate actions of the V(D)J recombinase (24, 25), topoisomerase I ("DNA swivelase") (26–28), and B-cell switch recombinase (29, 30) enzymes, polymerase α pause sites (31) and Z-DNA (32–35).

Hprt mutations arising in somatic cells are unrelated to cancer or heritable genetic diseases. However, this reporter gene provides advantages over disease-related genes for studies of the mutation process itself. Hprt mutant cells are selectable, providing large numbers of mutational events arising in vivo or in vitro in human cells for analyses. Hprt mutational events do not alter cell proliferation characteristics, as do mutations in oncogenes or tumor suppressor genes, making it easy to assess their primary frequencies. The entire 57 kb of the hprt region has been sequenced permitting detailed molecular studies (36). Numerous linked DNA markers have been mapped allowing determination of sites of deletion endpoints (37, 38). Assays are available to measure and isolate in vivo and in vitro arising mutants from humans, and a worldwide data base has developed (39). Molecular mutational spectra for this gene are being determined in several laboratories.

We report here the molecular characterization of 24 independently arising intragenic deletions of the human hprt gene, isolated in our laboratory from a variety of in vivo and in vitro studies. These were obtained from a total of approximately 1600 mutant isolates. 509 of these were deletions or other large structural alterations in the DNA and 60 of these were deletions of only internal hprt exons (i.e. did not include exons 1 and 9). One class of intragenic deletion, i.e. deletion of exons 2 and 3 resulting from illegitimate action of V(D)J recombinase enzyme, has been studied in detail, reported elsewhere, and is not included here (24, 25). An additional sixty of the large scale events appeared to be other intragenic deletions. Molecular characterization of 24 mutations for which DNA was available is reported here. In these 24 intragenic deletions, we found a non-random distribution of breakpoints, an excess of direct repeats in breakpoint regions, the presence of inverted repeats near some breakpoints, the occurrence of breaks at topoisomerase II consensus cleavage sites, and some deletions with characteristics strikingly similar to those reported by others.

MATERIALS AND METHODS

Isolation of hprt Mutant T Cell Clones. Hprt T cell mutants were isolated from human cloning assays determining either in vivo or in vitro T cell mutant frequencies. For the in vivo studies, lymphocytes were isolated from peripheral blood and plated, either fresh or after cryopreservation in liquid nitrogen, at 1–10 cells/well in nonselective medium or at 2 x 10^4 cells/well in 10 μM 6-thioguanine selective medium. After 7–14 days of culture, growing colonies were determined microscopically and mutant frequencies determined. For in vitro studies, cells were exposed to ionizing radiations and then allowed a period of growth for mutation fixation and phenotypic expression before