An Apparent Interlocus Gene Conversion-like Event at a Putative Tumor Suppressor Gene Locus on Human Chromosome 6q27 in a Burkitt’s Lymphoma Cell Line

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Abstract

A region of minimal deletion in B-cell non-Hodgkin’s lymphoma (B-NHL) has recently been defined between D6S186 and D6S227 spanning 5-9 Mb at 6q26-q27, predicting the presence of at least one tumor suppressor gene (TSG) at this locus. During the construction of a deletion map in the B-NHL tumor panel, we report the identification of a Burkitt’s lymphoma cell line, BL74, having an apparent homozygous deletion at the D6S347 locus, internal to the critical region. Since this case may facilitate the localization of the target TSG, a detailed structural molecular characterization and search for candidate genes were undertaken at this locus. While BL74 underwent a loss of heterozygosity at 6q26-q27, D6S347 was also likely subjected to a somatic interlocus gene conversion-like event between two homologous but distinct loci, resulting in the homozygous replacement of a 1860- to 2067-bp segment of one locus with the corresponding segment copied from the other locus. Two genes at this locus were identified, but their lack of expression in B-cell lineages tentatively excludes them as candidate TSGs. Another still unidentified gene at this locus may be disrupted by the gene conversion-like event, which would represent a novel mechanism of TSG inactivation.

Key words: gene conversion; chromosome 6q27; tumor suppressor locus; Burkitt’s lymphoma

1. Introduction

Recurrent deletions at specific chromosomal loci in a given tumor type are the hallmark of the presence of tumor suppressor genes (TSGs). Although deletion coupled with a point mutation in the remaining allele have been the classic means to achieve TSG inactivation, other types of DNA lesions have been described including homozygous deletions, rearrangements, chromosomal translocations, and epigenetic mechanisms like hypermethylation. While the mapping of a consensually deleted region in a series of tumors has been useful in the localization of the corresponding TSG, often the genomic region to be cloned and surveyed for candidate genes can be quite large, and the effort required to accomplish the endeavor daunting. Positional cloning strategies to identify TSGs can be vastly facilitated by the detection of smaller or more local DNA upheavals such as homozygous deletions as is the case with RB1, WT1, BRCA2, MADH4 (DPC4, SMAD4), and PTEN or chromosomal translocations as is the case with NF1 and EXT1. Hemizygous deletions of chromosome 6q27 have been described at high frequency in multiple tumor types including B-cell non-Hodgkin’s lymphoma (B-NHL) as well as ovarian, breast, salivary gland, and melanoma cancers. Regions of minimal deletion including or within 6q27 have been well defined in several tumor systems. Most of these critical regions have been estimated to be over 1 Mb, and thus are not immediately amenable to positional cloning strategies. Efforts to approach this problem have relied on the evaluation of known candidate genes, consideration of the partial overlap of consensus regions amongst the different tumor studies, and restriction of the critical region by identifying new cases with small deletions. An homozygous deletion at distal 6q has been described in only one
melanoma cell line, but since this deletion spans at least 49 cM, it is of little utility to restrict the critical region. Recently, we have identified, mapped, and cloned a region of minimal molecular deletion in B-NHL at 6q26-q27 (RMD-1) between D6S186 and D6S227 covering a large genomic region (5-9 Mb). In the process of assaying loss of heterozygosity (LOH) in the B-NHL panel, we encountered a case, with a previously documented LOH restricted to 6q26-qter, having an apparent homozygous deletion internal to RMD-1.

2. Materials and Methods

2.1. DNA samples

The B-NHL panel used in this study has been previously reported. In particular, BL74 is a Burkitt’s lymphoma (BL) cell line that is matched with a “normal” lymphoblastoid cell line (LCL), IARC290B, from the same individual. Both cell lines were grown in Iscove’s modified Dulbecco medium (Life Technologies, Gaithersburg, MD) with 10% fetal calf serum in 5% CO2 at 37°C. The somatic cell hybrid DNA panel included A9 (Neo-6), which retains chromosome 6 as its only human material, and previously characterized hybrid cell lines that also permitted submapping of chromosome 6 loci. Aqueous genomic DNA was prepared by cell lysis, proteinase K digestion, “salting out” extraction, and ethanol precipitation as described. To obtain high-molecular-weight human genomic DNA, LCL cells (IARC632) were embedded in agarose plugs at 2.5 x 10⁷ cells/ml and treated with proteinase K followed by phenylmethylsulfonyl fluoride. Cosmid, phage, and plasmid DNA were purified by standard alkaline lysis extraction procedures.

2.2. DNA probes

The probe p4J4vs-34F is a 0.7-kb repeat-free EcoRI fragment derived from cosmid 4J4vs-34 (D6S347 at 6q27) and subcloned into the pBluescript KS+ vector (Stratagene, La Jolla, CA). Other probes used for Southern and/or Northern analysis included a human TCP10 cDNA (pHu7-9; kind gift of Lee Silver), a human MAX cDNA, and human I.M.A.G.E. cDNA clones 415697 and 129630 (Genome Systems, St. Louis, MO).

2.3. Southern blot analysis

DNA was digested with the appropriate restriction endonuclease and electrophoresed in a 0.9% agarose gel. Gels were treated and transferred to Duralon-UV filters (Stratagene, La Jolla, CA) by standard techniques. Probe p4J4vs-34F was labeled with [α-32P]dCTP by the random priming technique. After overnight hybridization, filters were washed at 60°C in 0.2 x SSC (standard saline citrate) and 0.1% SDS (sodium dodecyl sulfate) and then autoradiographed using intensifying screens.

2.4. Genomic cloning and restriction mapping

Genomic libraries of BL74 and IARC290B were constructed by partial Sau3AI digestion of high-molecular-weight aqueous genomic DNA and ligation of gel-purified 9- to 22-kb fractions into LambdaGem-11/BamHI vector (Promega, Madison, WI). The recombinant phage DNA was in vitro packaged using Gigapack II extract (Stratagene, La Jolla, CA). Library screening was performed by plaque hybridization using the p4J4vs-34F probe. Restriction mapping of the purified phages was accomplished by a partial digestion strategy. Briefly, phage insert with flanking T7 and SP6 promoter sites was completely excised from the vector with Sfi I, and subjected to complete and partial digestions with a series of restriction enzymes (EcoRI, HindIII, Nde I, Sac I, Xba I, and Mlu I). Following electrophoresis through an agarose gel and transfer to membrane, the blot was sequentially hybridized to T7 and SP6 oligonucleotides which were previously kinased with [γ-32P]ATP. Other cosmids from this locus were mapped in an analogous manner. Filters were stripped between probes. Hybridization was carried out in 5 x SSPE, 5 x Denhardt’s solution, and 0.5% SDS at 37°C. Final washing was performed in 5 x SSPE and 0.1% SDS at 50°C.

2.5. DNA sequencing

Phage fragments were subcloned into pBluescript KS+ prior to sequencing. Sequence reactions were performed by dye terminator cycle sequencing using both vector- as well as insert-derived primers and analyzed by automated fluorescence detection on an Applied Biosystems Model 373A DNA sequencer (Perkin-Elmer, Foster City, CA). Sequence alignments were accomplished using the Pileup program (default settings) of the Wisconsin Sequence Analysis Package (version 7.3) (Genetics Computer Group [GCG], Madison, WI). Inverted and direct repeats were identified using the StemLoop (using default parameters of Krawinkel et al., 1986) and Repeat programs, respectively. All sequences underwent sequence similarity searches of GenBank using BLASTN and BLASTX against the nr and dbEST databases via the National Center for Biotechnology Information (NCBI) server.

2.6. Pulsed-field gel electrophoresis

Plugs were prepared for digestion by extensive washing in TE pH 8.0 (10 mM Tris-Cl pH 8.0, 1 mM EDTA pH 8.0) followed by equilibration in 1 x of the appropriate restriction endonuclease buffer on ice. The plugs were single- and double-digested in situ with a series of rare-cutting restriction endonucleases: Nru I, Not I, Mbu I, BssHII, Sac II, and Sfi I. Following the digestions, plugs were equilibrated in 0.5 x standard Tris-borate-EDTA (TBE) buffer and were size-fractionated on 1% pulsed-field certified agarose gels (Bio-Rad) in 0.5 x TBE.
at 14°C using a contour-clamped homogenous field apparatus (CHEF Mapper system, Bio-Rad) with linear ramping and pulse times of 60-110 sec with a 120° angle between electrodes for 24 hr at 6 V/cm. Gels were stained in ethidium bromide (0.5 μg/ml), photographed, UV irradiated at 60 mJ, and blotted to nylon membranes. After hybridization, sizes of bands were estimated by comparison of their mobilities with size standards (Saccharomyces cerevisiae chromosome, low range, and lambda ladder PFG markers, New England Biolabs, Beverly, MA).

2.7. RNA extraction and northern analysis
RNA from cell lines (IARC174 and IARC290B, LCLs; BL53 and BL74, BLs; 697, common acute lymphoblastic leukemia cell line; BJAB, B-cell lymphoma cell line; Ly-1, diffuse large-cell lymphoma cell line; and U266, multiple myeloma cell line) and tissues (spleen, tonsil, and testis) was extracted by the guanidine isothiocyanate method. A 20-μg aliquot of total RNA was electrophoresed through a 1% agarose-2.2 M formaldehyde gel and transferred to a Duralon-UV membrane. Probes were 32P-labeled by random priming and expressed with 0.5 mg of sheared total human placental DNA (Sigma, St. Louis, MO) when necessary. Hybridization of the same filters to a human MAX cDNA probe was used as a control for the amount of RNA loaded in each lane and for the sensitivity of the filter. After overnight hybridization, filters were washed at 60-64°C in 0.1-0.2 x SSC and 0.1% SDS.

2.8. Exon trapping
The input genomic phage was both partially digested with Sau3A1 and completely digested with BamHI, BglII, BamHI/BglII, and PstI. Exon trapping was carried out according to methods previously described.

3. Results

3.1. Detection of a DNA alteration at D6S347 in IARC290B/BL74 by Southern blot analysis
BL74 is a previously characterized BL cell line, that despite having two cytogenetically intact chromosomes 6, has a terminal LOH event spanning 6q26-qter (including 15 distinct loci tested) distal from marker CEPH715b9R. An apparently functional telomere is present. During the construction of a 6q deletion map in a panel of B-NHL cases, a polymorphic marker at D6S347, which was subsequently mapped internal to B-NHL RMD-1 by mapping of a 6q26-q27 yeast artificial chromosome contig, was used to evaluate for LOH. A portion of the LOH results on the tumor panel for this marker is shown in Fig. 1A. The probe for this locus, pJ4vs-34F, detects a two-allele restriction fragment length polymorphism (RFLP) with restriction enzyme Sac I (Genome Database [GDB] accession ID: 162448).

In addition to the two alleles at 5.8 kb and 1.2 kb, the probe detects a constant fragment at 2.3 kb. Although case IARC290B/BL74 is noninformative for the Sac I RFLP and thus cannot be assessed by LOH analysis, the invariant band at 2.3 kb was homozygously absent in the tumor-derived cell line versus its corresponding normal LCL. The 2.3-kb constant fragment showed no variation in 54 normal chromosomes surveyed (data not shown) and was present in the remainder of the B-NHL tumor panel (Fig. 1A and data not shown).

Of 71 B-NHLs studied with 30 loci at 6q26-q27, this cell line was the only case demonstrating a potential homozygous deletion. Since the discovery of even one cell line or xenograft with a small homozygous deletion can be extraordinarily useful for the localization and identification of the target TSG, the D6S347 region was examined in more detail and scanned for nearby candidate genes. To determine if the Sac I allelic and nonallelic systems were part of the same locus or were separate loci, probe pJ4vs-34F was completely sequenced. No Sac I sites were identified in the probe sequence or found experimentally (data not shown), suggesting that the allelic and nonallelic systems are distinct, but homologous loci. Both loci mapped to chromosome 6 by somatic cell hybrid mapping (Fig. 1B).

Since these hybrids contain only one copy of an intact or partial chromosome 6, the presence of both bands (2.3 kb and 1.2 kb) in single hybrids provides support that the two bands represent two distinct loci, rather than a single polymorphic system. With respect to Sac I in IARC290B, we have termed the locus representing the 2.3-kb invariant fragment as “affected”, since it is not present in BL74. Similarly, the 1.2-kb Sac I band represents the “unaffected” locus since it appears unchanged in BL74. Furthermore, PFG analysis of this probe demonstrated that both loci coexist on approximately 140-kb MluI and 450-kb BssHI genomic fragments (data not shown). The panel of restriction enzymes used to analyze this LCL/BL pair was expanded to a total of four (Fig. 1C). Like Sac I, an apparent homozygous deletion of one locus was observed with an additional enzymatic Xba I. Curiously, two other restriction enzymes, HindIII and EcoRI, displayed the germline configuration of bands, detecting no homozygous deletion, in BL74 upon Southern analysis.

3.2. Molecular cloning of “unaffected,” “affected,” and “hybrid” loci in IARC290B/BL74
To further understand the nature of the DNA alterations in IARC290B and BL74, genomic libraries were constructed from these cell lines and screened with the pJ4vs-34F probe. Nearly two dozen phage clones from each library were retrieved and mapped by restriction analysis (Fig. 2). As predicted from the Southern blot
Figure 1. Southern hybridization analysis of p4J4v-34F (D6S347). (A) LOH screening on partial B-NHL panel DNAs digested with SacI. A two-allele RFLP is observed with allelic bands at 5.8 kb and 1.2 kb. Case T-NC89 is heterozygous at the RFLP and does not demonstrate any allelic loss. Both case BL64 which is homozygous for the upper allele and the remaining cases which are homozygous for the lower allele are noninformative with respect to the LOH analysis. Case BL74 demonstrates complete, homogenous absence of an invariant band at 2.3 kb. (B) Same probe mapped onto a chromosome 6 somatic cell hybrid panel digested with SacI (see Koi et al., 1989; Boyle et al., 1992 for description of hybrids). Both the allelic and nonallelic SacI bands map to the same 6q26-q27 hybrid subregion. (C) Multienzyme analysis of same probe on IARC290B (normal, N) and BL74 (tumor, T) DNA pairs. While an invariant 6.5-kb band (no polymorphisms detected with XbaI in 54 normal chromosomes tested; data not shown) seems to be homozygously deleted in BL74 after XbaI digestion, no alterations are detected when the same DNAs are digested with HindIII and EcoRI.

analysis, two species of clones, representing the “unaffected” and “affected” loci were recovered from the IARC290B library. Identical maps were obtained from p4J4v-34F clones isolated from a human placental genomic library (data not shown). Unexpectedly, two species of clones were also retrieved from the BL74 library. One species was identical to the unaffected locus of IARC290B. The second species appeared to be a hybrid or composite between the unaffected and affected loci, possibly resulting from some type of recombination between the two loci. An internal portion of the map of this hybrid locus (between the second EcoRI and single NdeI sites) appears identical to an inner segment of the unaffected locus and is flanked by the map of the affected locus. This observation suggests that the unaffected locus may be the sequence donor to the recipient affected locus, resulting in the hybrid locus (thick curved arrow in Fig. 2). Part of the left flanking region (between the first XbaI and second EcoRI sites) has an apparently identical map for both the unaffected and affected loci and thus, is indeterminate with respect to its possible origin at this point.

The restriction map of the clones is consistent with the Southern blot observations (Figs. 1, 2). For example, the apparent homozygous deletion of the 2.3-kb SacI affected band in BL74 can be explained by the “creation” or gaining of a new SacI site at the hybrid locus generating a 1.2-kb fragment identical in size with the fragment detected at the unaltered unaffected locus. Unlike SacI, the EcoRI fragment detected by the probe at the hybrid locus is coincidental in size to the normal affected locus. Thus, when cutting BL74 genomic DNA with EcoRI, a germline configuration of bands is observed with respect to size.
3.3. Nucleotide sequence analysis

To increase the resolution of the locus comparison in both IARC290B and BL74, the region suspected of including and flanking the postulated recombination product of the hybrid locus was subjected to nucleotide sequencing together with the analogous segments of the unaffected and affected loci. Assuming the recombination event introduced a contiguous sequence tract from the unaffected locus into the affected locus, the analysis of the nucleotide sequence divergence between these loci should define the extent of interaction. The extent of the internal region of the hybrid locus that is identical to the unaffected locus covers at least 1860 bp. Identical sequences at the recombinational boundaries (162 bp for the left side and 45 bp for the right side) between the unaffected and affected loci would not allow the identification of precise "breakpoints". Thus, the unaffected portion of the hybrid locus could extend up to 2067 bp (1860 bp + 162 bp + 45 bp). Flanking the recombination boundaries on both sides, the sequence of the hybrid locus is identical to the sequence of the affected locus of IARC290B. Most of the left flanking region of the hybrid locus (between the first XbaI and second EcoRI sites) which had an apparently identical restriction map for both the unaffected and affected loci could now be distinguished by sequence analysis as being identical to the unaffected locus (see Fig. 3A). Remarkably, between these XbaI and EcoRI sites (0.98 kb), there is a 97% sequence identity between the unaffected and affected loci, with preservation of the XbaI, SacI, BglII, and EcoRI restriction sites. This high level of identity between the two loci explains why the probe used can easily detect both loci at high stringency by Southern blot analysis. Overall, for the region completely sequenced (essentially all four loci in Fig. 2), there is an approximately 80% sequence similarity between the two loci. Of the 1860 bp of the unaffected-derived portion of the hybrid locus, 1323 bp are identical between the two loci, while all of the remaining positions are diagnostic for the unaffected locus. Despite the replacement of sequences at the affected locus with presumed sequences of the unaffected locus, unique sequences of the normal affected locus can nevertheless be considered homozgyously deleted in BL74. The sequence of the unaffected locus of BL74 remains completely unaltered compared with the unaffected locus of IARC290B.

Figure 2. Molecular cloning and mapping of the unaffected (left, gray shaded rectangle), affected (upper right, hatched rectangle), and hybrid (lower right, mixed gray and hatched rectangles) loci from IARC290B (normal, N) and BL74 (tumor, T) genomic DNA. The unaffected locus is the same in both N and T, while the second locus of the tumor has a 1.86- to 2.07-kb region (determined by sequence analysis; boldface outlined gray rectangle with white borders) apparently derived from the unaffected locus (thick curved arrow). The left and right white-boxed regions contain sequences identical at all loci and represent the boundaries of the postulated recombination event in BL74. The placement of the probe, pJ4vs-34F, used in Fig. 1, determined by sequence analysis, is shown as a rectangle with the name of the probe inside it. Note that the probe detects both 1.2-kb and 2.3-kb SacI fragments at the normal unaffected and normal affected loci, respectively, but, in the tumor cell line, detects only 1.2-kb SacI fragments at both loci (SacI fragments represented by thin horizontal lines with sizes underneath each locus). B, BglII; E, EcoRI; H, HincIII; N, NdeI; S, SacI; X, XbaI. The leftmost EcoRI site at the unaffected locus is beyond the map shown, but is located 12 kb from the shown EcoRI site. A number of potential recombination motifs, determined by sequence analysis, are indicated above the map: χ, chi-like sequence; numbers in boxes, site and quality score of palindromes (inverted repeats) in bond units (relative ability of a sequence to form stem-loop structures); in some cases p represents the probability of coincidence between a point of presumed recombination and the palindrome as calculated according to Krawinkel et al., 1986;33 pairs of small arrows, sites of direct repeats (only those larger than the theoretically expected longest direct repeat to occur by chance (> 11 bp in this case) are shown); SSR, simple sequence repeat. Extended MluI mapping and PFGE analysis would reverse the shown orientation of the unaffected locus with respect to the affected locus (see Fig. 4). In addition, the PFGE analysis suggests that the two loci are separated by at most 140 kb of each other (slanted broken lines) (see Fig. 4).
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Figure 3. Sequence comparison of the unaffected (U), affected (A), and hybrid (H) loci from IARC290B (normal) and BL74 (tumor) at and adjacent to the left "recombination junction" (left white-boxed regions in Fig. 2) (panel (A)) and within the core of the recombination event (panel (B)). (A) Left "recombination junction" region is shown. Blank spaces underneath Normal-U sequence represent sequence identity among the loci. Only sequence differences are shown. To facilitate maximum alignment, dots represent gaps upon GCG Pile-Up alignment. Uppercase sequences derive from the normal unaffected locus, whereas lowercase sequences derive from the normal affected locus. There is a 162-bp region of identity (italics, between arrows) among all loci, left of which the tumor hybrid locus resembles the normal affected locus (diagnostic at three positions shown). Note the preservation of the first Sae I site in the tumor hybrid locus (see Fig. 2). Restriction sites are noted and underlined. A χ-like sequence is shown at all loci within the recombinational breakpoint sequences (below asterisks). A 13-bp imperfect inverted repeat separated by a potential 26-bp stem is denoted below the carets. (B) Sequence alignment within core of the recombination event and "creation" of an Xba I site (second Xba I site in Fig. 2) at the tumor hybrid locus. By gaining this Xba I site at this locus, the probe p4J4vs-34F recognizes only 1.4-kb Xba I fragments in the tumor, while losing the capability to detect the larger 6.5-kb fragment (see Figs. 1, 2). All sequences at the tumor hybrid locus resemble the unaffected locus here. A perfect 13-bp direct repeat is overlined at all loci except the normal affected locus. As seen throughout all sequences, the unaffected loci of IARC290B and BL74 are identical.

3.4. Long range transcriptional map at D6S347

Mapping of the unaffected, affected, and hybrid loci was extended by incorporating overlapping cosmid clones of the unaffected locus30 and through the use of PFGE of normal human genomic DNA (Fig. 4). The presence of both unaffected and affected loci on a single 140-kb genomic fragment together with a Mlu I restriction map of the clones, suggested that the two loci were in relative proximity and oriented in opposite directions with respect to their homologous regions. Several approaches were used to identify candidate genes at this locus. First, a series of probes encompassing approximately 20 kb of the affected locus and 20 kb of the unaffected locus (Fig. 4) were tested on Northern blots of a total RNA panel consisting of B-cell containing tissues and B-cell lines at different stages of differentiation including BL74. No transcripts could be identified in this way. Second, the TCP10 gene, which has been mapped adjacent to D6S347 on a yeast artificial chromosome contig of 6q27,25 was tested for cross-hybridization on this long range map. The TCP10 cDNA cross-hybridized to a fragment of the unaffected locus over 20 kb from the region believed to have been the sequence donor for the hybrid locus. The expression pattern of this gene has been reported in testis.31 Our Northern results on total RNA confirmed expression in adult testis, but not in a B-cell-derived total RNA panel (data not shown). Finally, a phage clone covering the normal affected locus was subjected to "internal" exon trapping. Upon BLAST analysis, one of two unique recovered trapped exons (IET-2D1, 182 bp) was essentially identical to part (rl sequence) of a 2.1-kb cDNA clone (I.M.A.G.E. 415697; GenBank accession no.: W78931) derived from a normalized and subtracted fetal liver/spleen library32 and previously mapped within an interval (D6S264-D6S1697) at 6q27.54 Backmapping
of this exon demonstrated hybridization to a genomic fragment about 5 kb away from the epicenter of the presumed recombination event at the BL74 hybrid locus (Fig. 4). A 3'-derived cDNA probe (515 bp) (I.M.A.G.E. 129630; GenBank accession no.: R16618 + R16560) assembled from a UniGene (NCBI) cluster of overlapping ESTs (Hs. 22033), including I.M.A.G.E. 415697, also hybridized to a fragment of the unaffected locus. It has not been determined if the two cross-hybridizing genomic fragments are from the same, related, or duplicated transcription units, and if the gene is disrupted by the insertion of new sequences at the hybrid locus in BL74. Total RNA Northern analysis of this cDNA revealed no expression in B-cell lineages. Expression was also not observed by reverse transcription-polymerase chain reaction (data not shown). PCR analysis of a panel of cDNA libraries showed expression only in an adult liver cDNA library (data not shown). Thus, the two genes (TCP10 and Hs. 22033) identified at this locus were excluded as viable candidates based on their RNA expression patterns.

4. Discussion

We report herein a locus, D6S347 (6q27), mapped internal to the B-NHL critical region (B-NHL RMD-1), exhibiting a homozygous loss of a genomic fragment in BL74 and have provided a detailed structural characterization of this locus and search for nearby candidate genes. Molecular cloning, mapping, and sequencing of D6S347 of both normal (IARC290B) and tumor (BL74) DNA revealed that the normal locus consists of two distinct, but physically close homologous loci (termed unaffected and affected) (Figs. 2–4). In the tumor DNA, a 1860- to 2067-bp internal segment of the affected locus is absent and replaced in toto by a copy of the corresponding homologous portion of the unaffected locus. Since BL74 has been reported to exhibit an LOH spanning a large, contiguous interval of 6q26-qter including the D6S347 locus,25 this region has been reduced to homozygosity, and hence, the absence and replacement of the 1860- to 2067-bp affected locus manifests as a homozygous event.

The properties of the DNA alteration described in BL74 at D6S347 are consistent with gene conversion. Gene conversion describes a nonreciprocal exchange of homologous sequences between a pair of nonallelic DNA sequences (interlocus) or allelic sequences (interallelic).55 At the hybrid locus in BL74, a 1860- to 2067-bp portion of the unaffected locus appears to act as the sequence donor which is unidirectionally transferred to a corresponding homologous stretch of another distinct locus, the normal affected locus, acting as recipient or acceptor. Flanking sequences on both sides of this 1860- to 2067-bp tract are not exchanged. The sequence of the donor remains unaltered after this recombination event. The converted tract extends through several diagnostic restriction enzyme sites, allowing detection by Southern blot analysis (see Figs. 1, 2). The fidelity of the gene con-
version is complete, in that all sequence positions of the hybrid locus that diverge from the normal affected locus are identical to the corresponding homologous positions of the unaffected locus. Furthermore, the unaffected and affected loci share a high level of homology which has been described as an important variable in promoting efficient gene conversions in other systems, presumably by stabilizing heteroduplex intermediates.\textsuperscript{56,57} Formally, gene conversion can only be proved when all products of the recombination can be simultaneously recovered and directly analyzed as the case following a single meiosis in some fungi (i.e., tetrad/octad analysis). However, in higher eukaryotes in which one cannot isolate, in general, all recombination products after chromosomal segregation in mitosis or meiosis, the term gene conversion has been liberally applied to describe any potential recombination, in somatic or germ cells, in which an internal segment of a sequence is replaced by the corresponding copy of a homologous sequence. In this case, the evidence for gene conversion, like that presented here, is circumstantial and based on sequence comparisons whereby the suspected converted locus can be compared with its most likely donor sequence.

Mechanistic molecular models of the interlocus gene conversion-like event, involving single-strand exchanges or recombination and mismatch repair acting on heteroduplexes, have been previously described.\textsuperscript{40,58–60} A number of sequence elements have been reported to loci suspected of undergoing gene conversion, which may be involved in initiating or mediating recombination by serving as sites for the initiation of locus alignment,\textsuperscript{39,44} strand breakage and invasion,\textsuperscript{49} and the recognition of recombination enzymes.\textsuperscript{33} \(\chi\)-sequences have been defined in prokaryotes as activators of recombination,\textsuperscript{61} and they have also been speculated to be involved in eukaryotic recombination events.\textsuperscript{41–44,46,47,50} They have been found at high frequency adjacent or within gene conversion tracts.\textsuperscript{41,42,47} Similarly, palindromic (inverted), direct, and simple sequence repeats have been described at these sites, some of which are unlikely to be situated at these loci by chance.\textsuperscript{33,39,40,44,45,51} The presence of all these classes of motifs flanking or within the 1860- to 2067-bp gene conversion tract in BL74 (Figs. 2, 3) suggests that they may also have a role in this recombinatorial process.

Two alternative mechanisms \textit{in lieu} of gene conversion may also underlie the generation of the hybrid locus in BL74, though they would be less likely to occur. First, an unequal double crossover event occurring between the unaffected and affected loci could result in a reciprocal exchange of sequences over 1860-2067 bp, such that, unlike gene conversion, both participants in the exchange would be modified. However, one may expect that such a double crossover would be improbable over such a small genomic distance. Second, sequential single point mutations may have accumulated independently at the affected locus. However, in this case, each mutation would be subject to selection. In contrast, in gene conversions, some mutations would be silent "passengers" and simply co-converted with the selected mutation. In addition, a single recombinational event such as gene conversion that transfers a complete uninterrupted tract of sequence would accomplish the same result as multiple point mutations or a double crossover in a single event, providing a more simple and economical explanation. Thus, we consider the creation of the hybrid locus most probably due to a gene conversion-like event.

Gene conversions have been used to explain the generation of pathogenetic alleles of some human genes such as \textit{CYP21B} in 21-hydroxylase deficiency,\textsuperscript{42} \textit{VWF} in von Willebrand disease,\textsuperscript{47} \textit{SMN}\textsuperscript{T} in spinal muscular atrophy,\textsuperscript{62} and \textit{PKD1} in autosomal dominant polycystic kidney disease.\textsuperscript{52} The deleterious alterations such as point mutations that are introduced into these functional genes are thought to be stored in the corresponding internal portion of their pseudogenes or homologous genes that act as sequence donors in gene conversion. In all of the above examples, only the end products of ancestral gene conversion events, occurring in meiosis during an evolutionary time frame can be analyzed. In contrast, in some cases, gene conversion-mediated mutations have been shown to occur \textit{de novo} in the germline, so that they are present in an affected case but not in either parent.\textsuperscript{45,60,63} Similar \textit{de novo} germline gene conversions have been described at the \(H2-K\) gene of murine MHC (interlocus)\textsuperscript{64} and human \textit{HLA-DPB1} (interallelic).\textsuperscript{65} Somatic or mitotic gene conversions have also been described at the human \textit{COL17A1} gene\textsuperscript{61} and at the immunoglobulin locus in avians,\textsuperscript{57} hybridomas,\textsuperscript{66} and a multiple myeloma patient.\textsuperscript{67} These somatic gene conversions occurred in \textit{vivo} and/or \textit{in vitro}. Somatic gene conversion has also been proposed as a mechanism of LOH limited to a small genomic extent.\textsuperscript{68} For this category of LOH, gene conversion is interallelic.

The fact that we are unable to isolate the hybrid locus from IARC290B DNA indicates that the gene conversion did not occur in the germline of the patient but occurred somatically. One cannot distinguish if this \textit{de novo} gene conversion event in BL74 was established \textit{in vivo} or \textit{in vitro} during tissue culture, since the primary tumor was not available for analysis. In any case, the gene conversion event may confer upon the cell a growth advantage by inactivating a TSG at that locus, analogous to the generation of pathogenetic alleles of some human genes. In contrast, \textit{de novo} gene conversion-mediated mutations have been shown to occur \textit{de novo} in the germline of the patient but occurred somatically. One cannot distinguish if this \textit{de novo} gene conversion event in BL74 was established \textit{in vivo} or \textit{in vitro} during tissue culture, since the primary tumor was not available for analysis. In any case, the gene conversion event may confer upon the cell a growth advantage by inactivating a TSG at that locus, analogous to the generation of a tumorigenic lesion.\textsuperscript{69} In both scenarios, BL74 would be clonal with respect to the gene conversion event, consistent with the observations by Southern blot analysis (Fig. 1A, C). Distinguishing between these possibil-
Figure 5. One proposed model of IARC290B/BL74 genetic lesions at 6q26-qter including D6S347. The two chromosome 6 homologues are distinguished by boldface and regular vertical lines. In the assumed normal germline state, both homologues are present, each possessing an affected locus (hatched box, A or a; uppercase and lowercase represent the different alleles) and unaffected locus (gray box, U or u). The actual proximal to distal order of the unaffected and affected loci is not known. A more proximal locus, CEPHy715b9R (black box, C or c), is also shown. The first lesion is thought to be the interlocus gene conversion-like exchange between the affected (recipient) (in this case at the boldface homologue) and unaffected (donor) loci to generate a unique mosaic or hybrid locus (mixed gray and hatched box, H). The source of the donor unaffected locus could reside on the same chromatid (in cis) (curved, closed arrow) in which local chromatin folding of approximately 100 kb brings the two loci together in an inverted orientation such that their corresponding homologous regions can properly align, sister chromatid (not shown), or nonsister chromatid (curved, open arrow). Interlocus gene conversion between sister or nonsister chromatids would result from unequal pairing of chromatids. LOH extending through D6S347 reduces this locus to homozygosity as the second step. LOH most likely proceeds by a mitotic recombination mechanism (represented by the crisscross) between CEPHy715b9R (C) and the centromere since heterozygosity is observed at loci proximal to CEPHy715b9R and homozygosity at all loci including and distal to CEPHy715b9R. The presence of two cytogenetically intact chromosomes 6 in BL74 by both conventional karyotyping and fluorescence in situ hybridization (data not shown) is consistent with this model. Both lesions are thought to have occurred somatically since they are both absent in IARC290B.
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