Chromosomal translocation in T-cell leukemia line HUT 78 results in a MYC fusion transcript

(human T-cell leukemia virus type 1/Sézary syndrome/gene fusion/oncogene activation/genetics of human leukemia)

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ABSTRACT Primary cultures and established cell lines derived from human T-cell leukemias were analyzed for genomic rearrangements in the region 3' of the MYC locus. A T-cell leukemia line, HUT 78, whose 3' MYC region is rearranged, carries a chromosome t(2;8) juxtaposition; i.e., a locus derived from chromosome region 2q34 is attached to the 3' end of one MYC allele. The t(2;8) rearrangement in the HUT 78 cell lines results in expression of a fused transcript encompassing the MYC gene and a locus designated TCL4 (T-cell leukemia / lymphoma 4), which normally resides on chromosome 2. The steady-state level of MYC-TCL4 fusion transcripts in HUT 78 cells is significantly higher than the MYC RNA level found in several other B- and T-cell lines. The production of fused MYC-TCL4 transcripts in a leukemic cell line raises the possibility that other B- and T-cell leukemias may express MYC fusion transcripts as an integral step in their pathogenesis.

A majority of human hematopoietic malignancies carry non-random chromosomal alterations (1). These include translocations, inversions, extra copies, and deletions. A large body of evidence indicates that these rearrangements are directly involved in the pathogenesis of human leukemias and lymphomas (2). In Burkitt lymphomas, chromosomal translocations have been shown to involve the MYC gene on chromosome 8 and one of the immunoglobulin gene loci located on chromosome 14, 2, or 22 (3-5). In the so-called variant translocations, which carry a t(2;8) or t(8;22) translocation, the MYC gene remains on chromosome 8 and either the κ or λ light-chain locus, respectively, translocates into the 3' flanking region of the MYC gene (4, 5).

In a number of T-cell leukemias, there is a t(8;14)(q24;q11) translocation in which the gene coding for the T-cell receptor α chain becomes relocated to a position 3' of the MYC gene on chromosome 8 (6, 7). These t(8;14) translocations in T-cell leukemias are analogous to the variant translocations in Burkitt lymphoma, as they occur 3' of the MYC gene, and as a consequence of the translocation, the MYC gene does not respond to its normal regulatory mechanisms and is transcribed inappropriately (8-10).

In this study we investigated an unusual MYC rearrangement in a human leukemic T-cell line, HUT 78, derived from an individual with Sézary syndrome whose serum contained antibodies to human T-cell leukemia virus type I (HTLV-I) (11).

MATERIALS AND METHODS

Molecular Cloning. High molecular weight DNA was partially digested with the restriction enzyme Sau3A1 and fragments ranging from 15 to 23 kilobases (kb) in length were selected by fractionation over a 10-40% sucrose gradient. The appropriate fractions were ligated into the λ phage EMBL3A. Conditions for hybridization and washing of the nitrocellulose filter replicas of bacteriophage plaques have been described (12).

Molecular Probes. pRyc7.4 is a MYC cDNA probe that includes a segment of exon 2 and 806 nucleotides of exon 3 (13). The 3' MYC probe, pCA1.7S, contains a 1.7-kb SstI insert in pUC19 (14). Probe p78Hc0.5 is a 0.5-kb HindIII insert. The probe p78SE0.5 contains a 0.5-kb SalI-EcoRI insert in pUC19. Probe p78H1.5 contains a 1.5-kb HindIII fragment in pUC19. Probe p78RSH0.7 contains a 0.7-kb SalI-HindIII insert in pUC19. The location of the insert of each probe is indicated on the restriction maps presented in Fig. 2.

RNA isolation and Northern blot analysis. Total cellular RNA was extracted by the guanidinium isothiocyanate method described by Davis et al. (15). RNA samples were denatured at 68°C with 50% (vol/vol) formamide/2.2 M formaldehyde and electrophoresed in 1% agarose gels containing 2.2 M formaldehyde, essentially as described by Yancopoulos and Alt (16). RNA was transferred and hybridized as described (17).

Cells, isolation, propagation, and characterization of most parental cells and somatic cell hybrids used in this study have been described (18-20). The presence of specific human chromosomes or regions of chromosomes has been confirmed by DNA hybridization using probes for genes assigned to specific human chromosome regions. Fig. 3 depicts diagrammatically the chromosomes or partial chromosomes retained in most hybrids used in this study for chromosome localization of the locus, termed TCLA, that is juxtaposed 3' to the MYC locus in HUT 78. A hybrid retaining an 8q+ chromosome (8pter-8q24:27p12-2pter) from a Burkitt lymphoma with a t(2;8)(p12;q24) translocation (4) and hybrids PB5 and CSK-12, retaining partial chromosomes 2 (defined by presence or absence of chromosome 2-linked probes), have allowed a regional localization of the TCLA locus.

Southern blot analysis. DNAs from human peripheral blood lymphocytes or human cell lines, mouse cell lines, and rodent-human hybrid cell lines were prepared by cell lysis, proteinase K digestion, phenol extraction, and ethanol precipitation. Cellular DNAs were digested with an excess of appropriate restriction enzymes, sized in 0.6% agarose gels, transferred to nitrocellulose filters, and hybridized under conditions recommended by the manufacturer (17).

Chromosomal in situ Hybridization. Metaphase chromosome preparations were obtained by culturing peripheral blood lymphocytes from a normal male subject (46,XY) for 72 hr in RPMI-1640 medium supplemented with 15% fetal bovine serum.

Abbreviation: HTLV-I, human T-cell leukemia virus type I.
In situ hybridization for the mapping of the chromosome 2-derived sequences was performed by a modified protocol (19, 21). Slides were aged 10–14 days at 4°C and treated with RNase A (Sigma) for 1 hr at 37°C. Chromosomal DNA was denatured at 70°C for 2 min in 70% formamide/2 X SSC (1 X SSC is 0.15 M NaCl/0.015 M sodium citrate, pH 7.0). Probe DNA was denatured in hybridization mixture (25% formamide/2 X SSC/10% dextran sulfate). The final concentration of probe DNA placed on each slide was 0.07 µg/ml. Hybridization was carried out at 37°C for 18 hr. Slides were then rinsed at 39°C, dehydrated in an ethanol series, air-dried, dipped in nuclear track emulsion (Kodak NTB-2), fan-dried, and stored in light-tight boxes at 4°C. At different time intervals, slides were developed and fixed at 15°C, air-dried, and then stained with a modified Wright-Giemsa staining protocol (19). Each slide was dipped for 10–16 sec in a pH 9.2 borate buffer (50 mM Na2B4O7/2.5 mM Na2HPO4) at 35°C and then stained for 2–3 min in a staining dish containing 3 parts pH 9.2 buffer and 1 part Wright-Giemsa stain (Baker). Grains situated on nonoverlapping chromosome regions were counted and scored.

RESULTS

MYC Rearrangement in HUT 78 Leukemic Cells. The human leukemic T-cell line HUT 78, derived from a patient with Sézary syndrome (11), has been reported to have a complex karyotype (22). Our analysis of the HUT 78 karyotype indicates that the cells have a near-triploid chromosome number, 65–70, with multiple rearrangements including at least 15 marker chromosomes, largely unidentifiable. One large marker is a 2q+ with the breakpoint apparently distal to band q33. There is also a possible 8q-, apparently deleted at band q24. The extra material on the 2q+, however, is too large to represent a simple t(2;8) translocation.

Southern blot analysis using the pRyc7.4 probe detects a germ-line fragment of 11.6 kb and a rearranged fragment of 13.1 kb in HindIII-cleaved HUT 78 DNA (Fig. 1 a). HindIII-cut HUT 78 DNA exhibits a germ-line fragment of 11.6 kb and a rearranged fragment of 6.2 kb when analyzed with a genomic probe, pCA1.7S, located 1 kb 3’ of the MYC probe pRyc7.4. That the HindIII germ-line pattern is the same for both the probes, whereas the rearranged fragments have different sizes, allows one to narrow the location of the 3’ MYC rearrangement in HUT 78 to within the 1-kb DNA segment that separates these two probes. Also, the ability to detect rearranged HUT 78 DNA fragments by Southern blot analysis using the probes pRyc7.4 and pCA1.7S with additional restriction enzymes (Fig. 1) excludes restriction fragment length polymorphisms as an explanation of the new HindIII fragment.

We did not find any evidence for the involvement of HTLV-I in the 3’ MYC rearrangement, as a probe containing the entire HTLV-I genome, pMT-2 (23), did not hybridize to HUT 78 DNA on Southern filters (data not shown).

To analyze the 3’ rearrangement in the HUT 78 cell line, a full genomic phage library was prepared from the HUT 78 cellular DNA. The library was screened with the pRyc7.4 and pCA1.7S probes. Two classes of clones were obtained and classified as the rearranged clones representing the genomic rearrangements detected by the pRyc7.4 probe (class A) and the pCA1.7S probe (class B). Unexpectedly, no clones representing the germ-line MYC locus were obtained among the 25 phase clones analyzed in detail. The restriction maps of representative overlapping phage clones of each group are shown in Fig. 2.

The localization of the HUT 78 3’ rearrangement was further defined by sequencing the 3’ MYC region of the HUT 78 class A phage clones and comparing this DNA sequence to the germ-line MYC sequence. Starting 251 nucleotides downstream from the translation termination codon within the 3’ untranslated region of the MYC gene, a DNA segment of the class A phage clones has the nucleotide sequence 5’-ACAATGT-TTCTCTGTAATAATTTGACTTAATACAAT-3’, whereas the germ-line MYC sequence counterpart is 5’-ACA-ATGTTCCTCTGTAATAATTTGACATTAATACAAT-3’ (24). A comparison of these two sequences indicates that the divergence of the HUT 78 sequence from the germ-line counterpart occurs 271 bases downstream of the MYC translation termination codon. Thus the HUT 78 MYC rearrangement occurs 3’ of the MYC translation termination codon and at a point 5’ of the two normal MYC hexanucleotide polyadenylation signal sequences (24).

Chromosome Mapping of the DNA Segment Recombined with MYC. To determine the chromosomal origin of the DNA

![Fig. 1. Hybridization of DNA from the HUT 78 cell line to the MYC probe pRyc7.4 (a) and the 3’ MYC probe pCA1.7S (b). DNAs (10 µg) isolated from MOLT-4, a T-cell leukemia cell line used as a germ-line control (lanes A), and HUT 78 (lanes B) were cut with the indicated restriction enzymes.](image-url)
Fig. 2. Restriction maps of regions surrounding the breakpoint in HUT 78. (a) The germ-line TCL4 locus on chromosome 2, covering the unarranged translocation region. (b) The rearranged MYC locus representing the genomic rearrangements detected by Southern blotting using the MYC probe pRyc7.4. (c) The germ-line MYC locus on chromosome 8. Exons of MYC are indicated by boxes 1, 2, and 3. (d) The rearranged locus representing the genomic rearrangements detected by Southern blotting using the pCA1.7S 3' MYC probe. Black bar, chromosome 2 DNA regions; white bar, chromosome 8 DNA regions. Probes are shown as hatched boxes. E, EcoRI; B, BamHI; H, HindIII; X, Xba I.

segment juxtaposed 3' of the MYC gene, repeat-free probes were generated from the non-MYC portion of the two classes of clones. Probes generated from the class A phage clones are referred to as p78Hc0.5 and p78SE0.5. A probe from a class B phage clone is referred to as p78RSH0.7. The location of each of these probes is indicated in Fig. 2. The probe p78SE0.5 was in turn used to isolate the complementary germ-line DNA locus from a B-cell DNA library that is unarranged for this region (Fig. 2). From this unarranged locus the probe p78H1.5 was subcloned (Fig. 2). The normal chromosomal location of the probes p78Hc0.5, p78SE0.5, p78H1.5, and p78RSH0.7 was determined by correlating the presence of these sequences with the presence of specific chromosomes in a panel of somatic cell hybrid DNAs. Parental and hybrid DNAs were digested with an appropriate restriction enzyme, fractionated in agarose gels, transferred to nitrocellulose, and hybridized to the respective subcloned probes to detect the presence of the cognate restriction

Fig. 3. Presence of the TCL4 gene in a panel of 18 rodent-human somatic cell hybrids. □, Hybrid named in the left column contains the chromosomes indicated in the upper row; ⬤, presence of the long arm (or a part of the long arm, indicated by a smaller fraction of stippling) of the chromosome shown above the column; □, presence of the short arm (or partial short arm) of the chromosome listed above the column; □, absence of the chromosome listed above the column. The column for chromosome 2 is boldly outlined and stippled to highlight correlation of presence of this chromosome (or region of this chromosome) with the presence of the various TCL4-region probes. The pattern of retention of the TCL4 probes in the panel is shown in the column at right, where the presence of the probe in the hybrid is indicated by a stippled box with a plus sign and absence of the probe is indicated by an open box enclosing a minus sign.
fragments. The results of this analysis, summarized in Fig. 3, demonstrate that the presence in the hybrids of the subcloned probes, collectively referred to as the TCL4 locus, correlates with a region on chromosome 2. Results in Fig. 3 also establish a regional localization of the TCL4 locus on chromosome 2, since hybrids retaining only 2p (CSK-12 and J14-2) are negative for the TCL4 probes, whereas hybrid PB5 is positive; hybrid PB5 does not retain the HOX4 locus or the COL3A1 gene, both of which have been mapped to the distal long arm of chromosome 2 (19, 25). Thus, we conclude from analysis of somatic cell hybrids that the TCL4 locus is on the long arm of chromosome 2, centromeric to the HOX4 and COL3A1 loci.

To determine more precisely the location of the TCL4 locus on chromosome 2, chromosomal in situ hybridization (19, 21) was performed with the [3H]-labeled p78H1.5 plasmid. After in situ hybridization, a total of 603 chromosomally localized grains were counted over 211 metaphases; 15% of the grains were localized over the long arm of chromosome 2, with 77% of the 2q grains observed over 2q31→2qter. Fig. 4 depicts the grain distribution over all chromosomes and shows the major peak of hybridization at chromosome 2q34.

Expression of a Fusion Transcript in HUT 78. The influence of the t(2;8) rearrangement of MYC expression in the HUT 78 cell line was examined by Northern blot analysis. The steady-state level of MYC transcripts in HUT 78 (Fig. 5a, lane 3) is significantly elevated relative to the human B-cell line GM1500 6TG and the T-cell lines SUP-T1 and Jurkat. This is in agreement with previous RNA dot blot experiments, which showed a high MYC transcript content for HUT 78 as compared to several other T-cell lines (22).

HUT 78 (Fig. 5a, lane 3) shows novel MYC transcripts of 3.8 kb and 6.8 kb. The third MYC transcript detected in HUT 78 appears to be slightly smaller than the normal 2.4-kb MYC transcript observed in the other cell lines. The expression of the novel-size transcripts in HUT-78 suggests the possibility of chimeric transcripts composed of MYC sequences attached to chromosome 2-derived sequences. A TCL4 probe, p78Hc0.5, hybridizes as does the MYC probe to the 3.8-kb and 6.8-kb transcripts (Fig. 5b, lane 3) in HUT 78 RNA. Thus the TCL4 probe appears to detect two chimeric messages in HUT 78 cells. Clearly, more studies on the TCL4 locus and the MYC-TCL4 fusion transcripts are needed to ascertain their genetic structure and relevance to tumor pathogenesis.

**DISCUSSION**

This study shows that the human leukemic T-cell line HUT 78 has a structurally abnormal MYC locus as a result of a rearrangement beginning 271 nucleotides 3' of the MYC translation termination codon. We have defined the rearrangement by molecular cloning of the region 3' of MYC in HUT 78 and shown it to be due to a t(2;8)(q23;q24) chromosome translocation. At the cytogenetic level, a possible 8q−, deleted at 8q24, and a large 2q+ marker were observed with the breakpoint apparently distal to band q33. The extra material on the 2q+, however, is too large to represent a simple t(2;8) translocation.

A consistent consequence of chromosomal translocations involving the MYC locus in B- and T-cell malignancies is the alteration of MYC regulation. MYC activation has been attributed to diverse genetic changes affecting MYC expression: interruption of trans-acting control regions within exon 1 and flanking regions (29–31), changes in posttranscriptional stability (32), an escape from translational suppression (33), and sequence mutations (30, 34). Our laboratory has proposed that MYC association with cis-acting positive regulatory elements in the immunoglobulin heavy-chain locus or a T-cell antigen-receptor locus as the consequence of a chro-
mosomal translocation event leads to transcriptional MYC deregulation and malignant transformation (2, 8, 9).

We have shown in this report that a chromosomal translocation involving the MYC gene can lead to the creation of fusion transcripts with another locus. The TCLA locus is normally located on chromosome 2 but is juxtaposed 3' to the MYC gene in HUT 78. The steady-state level of MYC–TCLA chimeric transcripts is higher than the MYC RNA level found in the other cell lines shown in Fig. 5.

In HUT 78, three abnormal MYC transcripts are detected. Two of the transcripts are larger than the normal 2.4-kb MYC RNA and hybridize with both the MYC and the TCLA probes. Since the 3' MYC rearrangement occurs 5' to the two MYC polyadenylation signal sequences, it is not unexpected that the two larger MYC transcripts in HUT 78 are chimeric transcripts between the MYC locus and the TCLA locus. The third transcript appears abnormal: its electrophoretic mobility indicates that it is slightly smaller than a normal MYC transcript. All three of the transcripts are expressed at a higher level than the MYC transcripts of the other cell lines shown in Fig. 5a. By Northern analysis we do not detect a normal MYC RNA in HUT 78. It is plausible that all three MYC transcripts are from the rearranged MYC locus on chromosome 8 and not from the MYC gene on the normal chromosome(s) 8 present in the karyotype. This interpretation is consistent with the previous findings that the translocated MYC gene is expressed and the normal MYC gene on the uninvolved chromosome 8 is not expressed in Burkitt lymphoma cells and in somatic cell hybrids with mouse plasmacytoma cells (10).

The high steady-state level of the MYC–TCLA fusion transcripts in HUT 78 suggests that the MYC gene is involved in transforming the involved T cells. This suggestion is strengthened by analogy to Burkitt lymphoma, where the translocated MYC gene is expressed inappropriately and frequently at an elevated level (10). The higher steady-state level of the HUT 78 MYC–TCLA transcripts relative to the MYC RNA level found in other B- and T-cell lines (Fig. 5) may be due to an increase in the posttranscriptional stability of the fusion transcripts. Stability of the fused MYC RNA could be a consequence of the fact that the HUT 78 rearrangement occurs within a 140-base-pair region that is primarily responsible for the short half-life of normal MYC message (35); additionally, stability of the fused MYC RNA could be affected by some features of the attached TCLA sequences. Alternatively, the TCLA locus juxtaposed to the MYC gene may carry cis-acting regulatory sequences that influence the steady-state level of the MYC–TCLA transcripts.

Although the present finding involves a single case with multiple abnormalities in the genome, the finding that the leukemia-derived T-cell line HUT 78 produces a MYC–TCLA fusion transcript suggests an additional mechanism for activation of the MYC oncogene in the molecular pathogenesis of leukemia. Recently, a specific chromosome 2 abnormality involving a region near TCLA, (2;13)(q13;q14), was described in several cases of rhabdomyosarcoma (36). Whether the TCLA gene is directly involved in this nonrandom translocation should be investigated.

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