Establishment of a Peripheral T-Cell Lymphoma Cell Line Showing Amplification of the c-myc Oncogene

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ABSTRACT

A new T-cell lymphoma cell line, designated T34, was established from freshly isolated lymph node tumor cells of a patient with non-Hodgkin's diffuse large cell lymphoma. The T34 cells, as well as the parental lymphoma cells, showed mature helper/inducer immunophenotypes in that they formed spontaneous sheep erythrocyte rosettes and reacted with OKT-3 and OKT-4 monoclonal antibodies. They were negative for OKT-6, OKT-8, terminal deoxynucleotidyl transferase, WT-1, and HLA-DR antigens. Molecular analysis revealed that the T34 cells contained 8- to 16-fold amplified c-myc DNA. The same genetic change was observed in parental lymphoma cells, indicating that the c-myc amplification had occurred in vivo. There was no gross rearrangement of the c-myc DNA. The c-myc gene of the T34 cell line was actively transcribed into normal-sized c-myc mRNA. Cytogenetic analysis showed that both the T34 and the parental lymphoma cells had a near-triploid karyotype with multiple structural chromosome changes. The terminal end of the long arm of chromosome No. 8, the chromosomal locus of the immunoglobulin heavy chain locus (9). Extending this observation, the possibility that c-myc is also involved in T-cell malignancies has been raised. Shima et al. observed that the joining sequence of the TCR-α was translocated to the 3'-end of the c-myc gene in a T-cell chronic lymphocytic leukemia cell line carrying a t(8;14)(q24;q11) chromosomal translocation (10). In this molecular recombination, the TCR-α may have played a role analogous to that of the immunoglobulin gene in Burkitt's lymphomas.

In this study, we established and characterized a lymphoma cell line having peripheral T-cell immunophenotypes. It was found that DNA from the cell line and from the parental uncultured lymphoma cells showed amplification of the c-myc oncogene. This report suggests that amplification of the c-myc oncogene, as well as molecular recombination with other genes caused by chromosomal translocations, is involved in the neoplastic process of T-cell malignancies.

INTRODUCTION

Application of a panel of monoclonal antibodies recognizing distinct T-cell-associated differentiation antigens to studies of T-cell malignancies has enabled the original subset and stage of differentiation of neoplastic T-cells to be determined (1). T-cell acute lymphoblastic leukemia and T-cell lymphoblastic leukemia cells display immunophenotypic characteristics consistent with various intrathymic stages of T-cell differentiation. In nearly all other types of T-cell malignancies, the neoplastic cells exhibit mature peripheral T-cell marker profiles (2, 3).

Chromosome abnormalities in these T-cell disorders have been described by a number of investigators. Abnormalities of chromosome No. 14 have been shown to be associated with the peripheral T-cell neoplasms and thought to involve the TCR-α chain gene (TCR-α) which has been mapped to the proximal band of the long arm of chromosome No. 14 (4–8). Recent studies suggest that cellular oncogenes are involved in tumorigenesis of hematolymphoid diseases. It is well established that, in Burkitt's lymphoma cells carrying a t(8;14)(q24;q32) chromosomal translocation, the c-myc oncogene is recombined with the immunoglobulin heavy chain locus (9). Extending this observation, the possibility that c-myc is also involved in T-cell disorders has been raised. Shima et al. observed that the joining sequence of the TCR-α was translocated to the 3'-end of the c-myc gene in a T-cell chronic lymphocytic leukemia cell line carrying a t(8;14)(q24;q11) chromosomal translocation (10). In this molecular recombination, the TCR-α may have played a role analogous to that of the immunoglobulin gene in Burkitt's lymphomas.

In this study, we established and characterized a lymphoma cell line having peripheral T-cell immunophenotypes. It was found that DNA from the cell line and from the parental uncultured lymphoma cells showed amplification of the c-myc oncogene. This report suggests that amplification of the c-myc oncogene, as well as molecular recombination with other genes caused by chromosomal translocations, is involved in the neoplastic process of T-cell malignancies.

MATERIALS AND METHODS

Case Report and Cell Culture. A 73-yr-old Japanese male was referred to our hospital from a dentist in August 1985, when he noticed an enlarged submandibular tumor. On examination, multiple lymph nodes could be palpated in the submandibular, cervical, and supraclavicular regions. A cytological study of an aspirated specimen of lymph node tissue detected atypical lymphoid cells. A lymphoma was suspected, and he was treated with a combination of cytotoxic drugs, as a result of which he showed a transient response. In December 1985, however, generalized lymphadenopathy and subcutaneous tumors appeared, and his illness rapidly progressed thereafter. Histological examination of biopsied lymph node tissue disclosed diffuse proliferation of lymphoma cells. The diagnosis was established as non-Hodgkin's malignant lymphoma, diffuse large cell type, according to the New Histological Formulation (11). The tumor cells were positive for spontaneous sheep erythrocyte rosette formation and expressed T-cell-specific antigens identified by OKT-3 and OKT-4 monoclonal antibodies. HTLV-I proviral DNA was not detected (12). The patient's serum was negative for anti-ATLA antibodies (13).

Cell suspensions of the biopsied lymph node tumor were cultured in RPMI 1640 medium supplemented with 20% fetal calf serum under standard conditions.

Cell Marker Analysis. Cells were examined by the rosette assay for sheep erythrocyte receptors (E-rosette) and for C3b- and Fcγ-receptors. Surface immunoglobulins were assayed by direct immunofluorescence.

The abbreviations used are: TCR, T-cell receptor; HTLV-I, human T-cell lymphotropic virus type I; ATLA, adult T-cell leukemia-associated antigen; E-rosette, erythrocyte rosette; TdT, terminal deoxynucleotidyl transferase; SSC, sodium dodecyl sulfate; FACS, standard saline citrate; poly(A)+ RNA, polyadenylated RNA; LCL, lymphoblastoid cell line; ATL, adult T-cell leukemia/lymphoma; DM, double minute; HSR, homogeneously stained region.
solution of 1 m NaCl, 5x Denhardt’s solution, 50 mM Tris-HCl (pH 7.4), 0.1% SDS, and heat-denatured salmon sperm DNA (100 µg/ml) at 65°C for 24 h. The filter was washed with 2x SSC at room temperature and then with 0.1x SSC-0.1% SDS at 65°C for 1 h and then exposed to X-ray film.

Northern Blot Analysis. Total cellular RNA was extracted from exponentially growing cells by the guanidinium-thiocyanate-cesium chloride method. Poly(A)* RNA was selected by oligo(dT)-cellulose column chromatography, electrophoresed on a 1.0% agarose gel containing 2.2 M formaldehyde, and transferred to a nylon membrane filter (BIOYNE; Pall Ultrafine Filtration, Glen Cove, NY). Hybridization using a nick-translated probe was performed in a mixture containing 50% fornamide, 5x Denhardt’s solution, 0.1% SDS, 50 mM phosphate buffer (pH 6.5), and heat-denatured salmon sperm DNA (250 µg/ml) at 42°C for 24 h. The filter was subsequently washed with 2x SSC at room temperature and then with 0.1x SSC-0.1% SDS at 65°C for 30 min.

DNA Probes. Specific probes for the first exon of the c-myc gene (a 0.6-kilobase KpnI/BamHI fragment of plasmid pMyc6514-1) and for the second exon (a 1.5-kilobase SstI fragment of plasmid pMyc6514-2) were kindly provided by Dr. Y. Taya (National Cancer Center, Chuo-ku, Tokyo) (18). Plasmid pHClβ1 which detects T-cell receptor β-chain gene rearrangements contained a 3.5-kilobase EcoRI/HindIII fragment representative of the Cβ1 constant region (19).

Karyotyping. Metaphase preparations obtained after exposure of cells to 0.05 µg/ml of Colcemid for 1 h were treated with 0.075 M KCl for 20 min and then fixed with methanol:acetic acid (3:1). Chromosomes were banded by the trypsin-Giemsa and quinacrine staining methods (20).

RESULTS

Establishment of the T34 Cell Line. After 2 wk of primary cultivation, the cells showed definite proliferation, and the established cell line was designated T34. The T34 cells grew in cultivation, the cells showed definite proliferation, and the proportion of OKT-4- and E-rosette-positive cells decreased, and a small percentage of the cells after long-term culture were positive for Tac antigens. These peculiar characteristics should correspond to absence of HTLV-1 genome, infection of which “activates” T-cells. Therefore, the T34 cell line had the characteristics of the parental T-cell lymphoma cells, both having mature helper/inducer immunophenotypes.

Southern Blot Analysis. DNAs extracted from T34 and parental lymph node tumor cells were studied by Southern blot analysis. The acute promyelocytic leukemia cell line HL-60, in which the c-myc gene had been amplified 16- to 32-fold (21), and an Epstein-Barr virus-transformed LCL were used as positive and negative amplification controls, respectively. Fig. 1C shows representative Southern analysis data for EcoRI-digested DNA extracted from these cells. The 12.5-kilobase EcoRI fragments of the c-myc gene of the parental lymphoma cells and T34 cells’ DNA, as well as that of HL-60 cells, were more intense than the corresponding band of LCL DNA. By quantitative comparison of c-myc bands of LCL and serially diluted T34 DNAs, the c-myc of the T34 cell line was found to be amplified 8- to 16-fold (Fig. 2).

Active Transcription of c-myc in the T34 Cell Line. RNA blot analysis was done with polyadenylated RNA extracted from LCL, an African Burkitt’s lymphoma cell line Daudi, Kobayashi cells (isolated from a Japanese patient with diffuse small noncleaved lymphoma (22)), and the T34 cell line. The Daudi and Kobayashi cells were previously found to have a t(8;14)(q24;q32) chromosomal translocation, and high levels of c-myc transcription in Daudi cells have been reported by others (9). Our results (Fig. 3) showed that c-myc transcripts in these cell lines were 2.3 kilobases long and that the level of c-myc transcripts in the T34 cell line seemed to be similar to or higher than those expressed in B-cell lymphoma cell lines carrying the t(8;14) chromosomal translocation. No aberrant c-myc mRNA was detected.

Cytogenetic Analysis. Metaphase cells obtained from the T34 cell line and from the parental lymph node tumor cells after short-term culture were karyotyped. All cells had a near-triploid karyotype and many structural abnormalities, including unidentified marker chromosomes (Fig. 4). The majority of the rearranged chromosomes were common to both the original lymphoma cells and the established T34 cells. Structural abnormalities of chromosome Nos. 1, 2, 3, 8, 11, 12, 13, 14, 15, 16, and 19 were found in all metaphase cells analyzed. The terminal end of the long arm of one or two No. 8 chromosomes was expanded. Double minute chromosomes were not detected by the conventional banding methods used.

DISCUSSION

Many human leukemia/lymphoma cell lines with T-cell properties have been established (23, 24). The differentiation stages of these cell lines have been studied by using monoclonal antibodies, and the most frequent antigen profile has been found to be that of intrathymic T-lymphocytes. In contrast, the Japanese ATL-derived cell lines, harboring the HTLV-1 genome, consistently express mature T-cell phenotypes (25, 26). Most of the ATL-derived cell lines show helper/inducer phenotypes and are strongly positive for activation markers detected by antibodies against HLA-DR and interleukin 2 receptors. Although the T34 cell line presented here also showed mature helper/inducer immunophenotypes, the cells failed to react with OKIa-1, and only a small percentage of the cells after long-term culture were positive for Tac antigens. These peculiar characteristics should correspond to absence of HTLV-1 genomes, infection of which “activates” T-cells. Therefore, the

Table 1

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<th>Markers</th>
<th>Lymph node cells, Dec. 1984 (%)</th>
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* Marker abbreviations: E, sheep erythrocyte rosette formation; EA, Fcγ receptors; EAC, C3b receptors; sIg, surface immunoglobulins.
† NT, not tested.
T34 cell line has a distinctive antigenic profile of mature peripheral T-cell lymphomas lacking HTLV-1 genomes.

Accumulating evidence suggests that the c-myc oncogene plays an important role in the pathogenesis of various tumors. Basically, two mechanisms for such a role have been proposed: (a) rearrangements of the c-myc DNA and (b) amplification of copies of the gene. Rearrangements of the c-myc have been recently reported in certain types of T-cell leukemia cell lines (10, 27), in addition to the Burkitt's tumor cells. Amplification of copies of c-myc DNA has been mainly observed in epithelial carcinoma cell lines (28–32). Our current studies of the T34 cell line demonstrated that the c-myc oncogene was amplified 8- to 16-fold and was actively transcribed. The original lymph node tumor cells also showed amplified c-myc, indicating that the c-myc amplification occurred in vivo, and this genetic change could be involved in the neoplastic process leading to this peripheral T-cell lymphoma. To our knowledge, this is the first report of a T-cell lymphoma cell line showing amplification of the c-myc oncogene.

Both the T34 cells and the parental lymph node tumor cells had near-triploid karyotypes with complex cytogenetic abnormalities, suggesting that they were derived from neoplastic cells selected through multiple karyotype evolutions (33). It is important to consider how the genetic change in the c-myc oncogene was involved in this oncogenic process. It has been demonstrated that c-myc rearrangement and recombination with the immunoglobulin gene play critical roles in the pathogenesis of Burkitt's lymphoma (34). However, studies of the N-myc gene in neuroblastomas have indicated that N-myc amplification influences tumor progression to advanced stages of the disease, and that tumor cells showing gene amplification had conferred on them a selective growth advantage (35). We therefore speculate that c-myc amplification in this T-cell lymphoma was a late development in the neoplastic process and exerted a function in the progression and proliferation of the transformed T-cells.

Several cytogenetic structures have been shown to be associated with DNA amplification, including extrachromosomal DM chromosomes (36), HSRs (37), and abnormally banded regions (38, 39). The latter two are chromosomal abnormalities. The failure to detect DMs in the T34 and parental lymph node tumor cells indicates that the amplified c-myc could be integrated into the chromosomes, and in this respect, the elongated long arm of chromosome No. 8 (8q+) identified by the banding

Fig. 1. Southern blot analysis of genomic DNAs extracted from an Epstein-Barr virus-transformed lymphoblastoid cell line (Lane 1), uncultured lymph node tumor cells (Lane 2), the established T34 cell line (Lane 3), and the acute promyelocytic leukemia cell line HL-60 (Lane 4). Five µg of DNA from each sample were electrophoresed after enzyme digestion. A and B show clonal rearrangements of the β-chain of the T-cell receptor gene (TCR-β).

DNAs were digested with EcoRI (A) and HindIII (B), and they were hybridized to a genomic probe representing the constant region. TCR-β germ line configurations which hybridized to this probe were 10.5 and 4.0 kilobases (kb) after EcoRI digestion and 7.8 and 3.4 kilobases after HindIII digestion. C demonstrates c-myc amplification. DNAs digested with EcoRI were hybridized with a probe corresponding to the first exon of the c-myc gene. A and C are autoradiographs produced using the same filter. Molecular sizes were determined using λ-phage DNA digested with HindIII as a marker.

Fig. 2. Estimation of the degree of c-myc amplification in T34 DNA. EcoRI-digested T34 DNA (5 µg) was serially diluted with a solution containing carrier salmon sperm DNA and was hybridized to a c-myc probe (Lane 1, 5 µg; Lane 2, 2.5 µg; Lane 3, 1.25 µg; Lane 4, 0.6 µg; Lane 5, 0.3 µg; Lane 6, 0.15 µg of T34 DNA; and Lane 7, salmon sperm DNA). Lane 8 contained 5 µg of DNA from the lymphoblastoid cell line used as a control. kb, kilobase.

Fig. 3. Expression of c-myc in the T34 cell line. Poly(A)* RNA (6 µg per lane) was hybridized with a probe corresponding to the second exon of the c-myc gene. RNAs were isolated from a lymphoblastoid cell line (Lane 1), Daudi cells (Lane 2), Kobayashi cells (Lane 3), and T34 cells (Lane 4). Size markers consisted of 28S and 18S ribosomal RNA.
c-myc AMPLIFICATION IN T-CELL LYMPHOMA

Fig. 4. G-banded karyotype of the T34 cell line. The chromosome number was 77 in this metaphase cell. Small arrows indicate rearranged chromosomes. Abnormalities of chromosomes No. 1 [der(1;7)(pter->1p12::7)], No. 2 [del(2)(p21p23)], No. 3 [2 × i(3p)], No. 11 [del(11)(q21q23)], No. 12 [der(12)(12;7) (p11;?)], No. 13 [der(13)(13;7) (p12–13;7), 2 × der(13)(13;7)(p12–13;7)], No. 14 [del(14)(q24)], No. 15 [der(15)(15;7) (p12–13;7), 2 × der(15)(15;7)(q24;7)], No. 16 [der(16)(16;7)(p11;7)], and No. 19 [der(19)(19;7)(p13;7), 2 × der(19)(19;7)(p13;7)] were seen in all metaphase cells analyzed. Derivative No. 8 chromosomes with additional materials at the terminal end of the long arm are indicated by arrow heads.

studies is of special interest. Since the terminal end of this chromosome (Band 8q24) is the locus for the single-copy c-myc gene (40), the 8q+ chromosome may reflect the site of c-myc amplification. Similar chromosome changes have been detected in HL-60 cells (39). Recently, Lacy et al. detected an expanded HSR-like region on Band 8q24 in an Epstein-Barr virus-infected BJAB Burkitt’s lymphoma cell line containing amplified c-myc (41). Of course, in situ chromosome hybridization studies will be necessary to confirm the site of amplification.

Expression of the c-myc oncogene has been found to correlate with the stage of cell differentiation. It is well known that the level of c-myc mRNA in HL-60 cells is reduced after induction of differentiation into mature macrophages and granulocytes (42). However, even though the T34 cell line presented here had mature peripheral T-cell immunophenotypes, the c-myc gene was actively transcribed at a level consistent with the degree of gene amplification. Thus, it seems that increased expression of the c-myc gene is possible in actively proliferating cells, irrespective of their stage of differentiation.

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REFERENCES

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