Deregulation of c-myc by Translocation of the α-Locus of the T-Cell Receptor in T-Cell Leukemias

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Two human T-cell leukemias carrying a t(8;14)(q24;q11) chromosome translocation were studied for rearrangements and expression of the c-myc oncogene. For one leukemia, rearrangement was detected in a region immediately distal (α') to the c-myc locus; no rearrangements of c-myc were observed in the second case (DeF). However, studies with hybrids between human and mouse leukemic T-cell lines indicated that in the leukemic cells of DeF, the breakpoint is chromosome 14 occurred between genes for the variable (Vα) and the constant (Cα) regions for the α chain of the T-cell receptor. The Cα locus had translocated to a region more than 38 kilobases 3' to the involved c-myc oncogene. Since human c-myc transcripts were expressed only in hybrids carrying the 8q+ chromosome but not in hybrids containing the normal chromosome 8, it is concluded that the translocation of the Cα locus 3' to the c-myc oncogene can result in its transcriptional deregulation.

Some human T-cell malignancies carry specific chromosome rearrangements and inversions, that involve chromosome region 14q112.1 (2), the location of the locus for the α-chain of the T-cell receptor (2). One of the most common chromosome alterations in acute lymphocytic leukemia of the T-cell type is a t(11;14)(p13;q11) chromosome translocation (3). We have shown previously that the chromosome break at band 14q11 in these tumors directly involves the locus for the T-cell receptor between the genes for the variable (Vα) and for the constant (Cα) regions of the α-chain of the T-cell receptor and that the Vα genes are proximal and the Cα gene is distal to the 14q11 chromosome breakpoints (4). Thus the orientation of the α-locus of the T-cell receptor relative to the centromere is the opposite of that of the human immunoglobulin heavy-chain locus on chromosome 14.1

Recently a translocation between chromosomes 8 and 14, with breakpoints at 8q23 (the locus of the α-chain oncogene) (6) and 14q11, the locus for the α-chain of the T-cell receptor (2), has been described in several T-cell neoplasms (7-9). These findings suggest that the locus for the α-chain may be involved in c-myc deregulation in some T-cell malignancies, similarly to the role of the human immunoglobulin loci in c-myc and bel-2 deregulation in Burkitt lymphoma (9) and in other B-cell malignancies (10, 11). We have examined two cases of T-cell leukemias with a chromosome translocation t(8;14) involving band 14q11 and the distal long arm of chromosome 8. Figure 1A

Table 1. Human genes in DeF-BW5147 cell hybrids.

<table>
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<th>mat</th>
<th>myc</th>
<th>IgH</th>
<th>Cα</th>
<th>Vα</th>
<th>NP</th>
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<th>Human chromosome†</th>
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<td>DeF</td>
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<td>8q+ 14 14q-</td>
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<td>+</td>
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|*|Percentages of metaphases containing the relevant human chromosome. A minimum of 18 metaphases of each hybrid were examined. +, major; ±, 1 to 10%; +, 10 to 30%; ++, 30 to 50%; +++, >50%; ND, not done. For human c-myc translocation, + and + were determined on the basis of analysis of the α' in Fig. 4.

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Fig. 1. (A) Representative karyotype of leukemic cells from patient DEF with t(8;14) translocation and pericentric inversion of chromosome 9 as the only abnormalities; the latter was shown to be a constitutional variant in normal T cells during clinical remission. The breakpoint on chromosome 14 is at band q11, and the segment translocated to chromosome 8 reflects loss, and possible rearrangement, of chromosome 14 material. (B) Representative karyotype of T-cell leukemia cell line SK/W-3 with t(8;14)(p24;q11). In addition to two copies of the 8q+ chromosome and a 14q−, resulting from the translocation, other abnormalities (arrows) include t(3q;4q), t(9p;11p), t(12q−, and absence of the other chromosome 14. These results are consistent with the previous cytogenetic analysis of SK/W-3 cells (9).

The c-myb gene. We then used three probes (pCA1.75, pPA1.3SB, and pPA0.25) specific for the flanking region 3' to the c-myb oncogene (Fig. 2A) to determine whether the rearrangements occurred within 38 kb 3' to the c-myb oncogene. We did not detect rearrangements within this region of DNA in DEF cells.

On the other hand, we detected rearrangements distal (3') to the c-myb gene in the SK/W-3 cells (Fig. 2B). The breakpoint in these cells was between the first Eco R1I and the first Hind III site 3' to the c-myb oncogene (Fig. 2A). Since the orientation of the λ and κ immunoglobulin loci (J3, J4) and of the locus for the α-chain of the T-cell receptor is the same (8), the present result is consistent with the "variant" t(8;14,22) and the c(2,8) chromosome translocations in Burkitt lymphomas, where the breakpoints are 3' to the involved c-myb oncogene (J5, J8). Thus, appears to be heterogeneity in breakpoints on chromosome 8 in T-cell leukemias with a t(8;14) and in Burkitt lymphoma (16).

To determine whether the breakpoint in DEF leukemic cells, as predicted on the basis of the orientation of the α-chain locus of the T-cell receptor (4), is 3' to the c-myb oncogene and whether the breakpoint in T-cell leukemias carrying the t(8;14) chromosome translocation involves the locus for the α-chain directly, we fused DEF leukemic cells with BW5147 mouse T-cell leukemia cells that are deficient in hypoxanthine phosphoribosyltransferase (4). The hybrids were assayed for the presence of human chromosomes, for the c-myc oncogene, which is located at band 8q11 (17), the c-myb oncogene, for the Vα and Cα regions, and for the expression of nucleotide phosphorylase (NDP), the human constitutive isozyme that has been localized to a region of chromosome 14 proximal to the Vα genes at 14q11 (4). Hybrids 563 BR2 and 563 BSA-BC10 (that have retained the 8q+ chromosome) contained the human c-myc oncogene and the Cα gene, but had lost the Vα gene and NDP expression (Table 1 and Fig. 3). On the contrary, hybrids 563 BD3, 563 AA1, and 563 BD2 (that have retained the 14q− chromosome) contained Vα genes and expressed human NDP, but had lost the human c-myc and the Cα gene. Since hybrid cells carrying the 8q+ chromosome in the absence of both the normal chromosome 8 and the 14q− chromosome contain the germine c-myc, while hybrid cells with only the 14q− chromosome have lost c-myc, the chromosome 8 breakpoint in the DEF leukemic cells must be 3' (distal) to the involved c-myb.

This is similar to the variant chromosome translocations in Burkitt lymphomas (J3, J4). We have previously shown that the 5' end of the c-myb oncogene is more proximal than its 3' end at band q24 of chromosome 8 (8). In addition, since hybrid cells containing the 8q+ chromosome in the absence of the other relevant human chromosomes also retained all three DNA segments (pCA1.75, pPA1.3SB, and pPA0.25) 3' to the c-myb oncogene (data not shown), we conclude that the chromosome breakpoint in DEF cells is more than 38 kb 3' to the involved c-myb oncogene. Finally, these results indicate that the chromosome breakpoint on chromosome 14 is between the Vα and the Vα genes.

We have previously shown that transloca-

Fig. 2. Rearrangement distal to the c-myb locus in human T-cell leukemia cells. (A) Restriction map of the region 3' to the c-myb locus on chromosome 8. The restriction map was obtained by chromosome walking with overlapping genomic clones of chromosome 8 of human PA 682 cells (19). (B) Digestion with Eco R1I, Kpn I, and Hind III and hybridization with pCA1.75 DNA showed a germline and a rearranged band in SK/W-3 cells (lanes 1). Hybridization with pCA1.75 DNA showed a germline band and a rearranged band in SK/W-3 cells (lanes 1). Hind III and hybridization with pCA1.75 DNA showed a germline and a rearranged band in SK/W-3 cells (lanes 1). BL2 is a Burkitt lymphoma with a t(8;14) and 123 kb rearrangement (3') to the involved c-myb oncogene (lanes 3). We have cloned the breakpoint of BL2 cells and found it to be approximately 10 kb 3' to the involved c-myb oncogene. The band representing the rearranged c-myb locus is more intense than the germline band because SK/W-3 cells contain two copies of 8q+ (Fig. 2B). Abbreviations: E, Eco R1I; H, Hind III; K, Kpn I; X, Xba I; and S, Sst I.

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tion of the c-myc locus to the heavy-chain locus on chromosome 14 (6), or the translocation of either the Λ or the κ locus to the c-myc oncogene on chromosome 8 (13, 14), results in the transcriptional deregulation of the c-myc oncogene involved in the translocation (9, 13, 14). In Burkitt lymphoma, the c-myc oncogene involved in the translocation fails to respond to the normal transcriptional control and is expressed constitutively at elevated levels (9, 13, 14) while the c-myc oncogene on the normal chromosome 8 in Burkitt lymphoma is transcriptionally silent (9, 13, 14). Therefore, we have examined the expression of the c-myc oncogene involved in the (8;14) chromosome translocation versus that of the c-myc oncogene on the normal chromosome 8 in somatic cell hybrids between BWS147 mouse leukemia and DeF human leukemic cells.

We have previously shown that the transcripts of the mouse and the human c-myc oncogene can be distinguished by S^35 nucleoside protection experiments (9). Hybrid 563 BAS-BC10 (containing the 8q^+ chromosome) expressed human c-myc transcripts while hybrids 563 BCS and 563 BD3 (containing the normal 8) did not (Fig. 4, lanes 6, 7, and 8). The lower c-myc expression in hybrid 563 BAS-BC10 compared to that of the parental DeF cells is due to the presence of the 8q^+ chromosome in only 16 of 28 metaphases examined in the hybrid and to the fact that the parental mouse BWS147 cell is near tetraploid. Thus, the involved human c-myc gene is diluted at least four- to fivefold in the hybrid. Hybrid 563 AC3, which contains the 8q^+ chromosome in 10% of its cells but has lost the normal chromosome 8, also expressed human c-myc transcripts (Table 1). The results indicate that the translocation of the 8p locus of the T-cell receptor to a region 8q of the c-myc oncogene results in its transcriptional deregulation in a T-cell background. This observation closely parallels previous findings concerning c-myc deregulation in Burkitt lymphomas (9, 13, 14). Thus the locus for the α-chain of the T-cell receptor seems to contain genetic elements capable of activating gene transcription in vivo over considerable chromosomal distances.

REFERENCES AND NOTES

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17. J. P. Crotwell et al., EMBO J. 4, 2245 (1985).
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