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IDENTIFICATION OF HOMEOBOX GENES EXPRESSED IN HUMAN T-LYMPHOCYTES

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Homeobox genes encode transcription factors involved in the process of normal development and differentiation of cells and organs. Recently, some homeobox genes are found implicated in hemato-lymphoid neoplasms. To assess the role of homeobox genes in normal development and neoplastic transformation of T-cells, we have surveyed for homeobox-containing gene expression in a human T-cell leukemia line with a polymerase chain reaction. Ten members of homeobox genes were isolated, including one novel sequence. The novel sequence was most related to mouse *Cdx*. One of the others, HOXB7, is expressed in most malignant T-cell lines and CD4-positive peripheral blood T-cells. Together with the recent observations, these results suggest that the protein products of these genes may participate in normal functions and/or transformation of T-cells.
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Immune response to foreign antigens requires coordinated expression of many genes, such as cytokines, their receptors, and adhesion molecules. The intrinsic regulation of these genes requires transcription factors governing the expression of other genes. Among potential candidates of such transcriptional regulators are the homeobox-containing proteins. These proteins, initially discovered during investigation of developmental mutants of *Drosophila melanogaster*, are DNA-binding proteins which share a common DNA-binding peptide motif. Homologues of the homeobox genes have been demonstrated in human as well (1)(2). Recent studies demonstrated that some homeobox-containing genes are expressed in human lymphocytes (3)(4)(5). In the mouse, genetic study using gene targeting demonstrated that the product of a divergent homeobox-containing gene, Oct-2, is involved in B-cell maturation (6). Meanwhile, studies of non-random chromosomal breaks found in some human lymphoid neoplasms indicate that homeobox-containing genes, such as Pbx-1 and HOX11, are involved in malignant transformation of lymphocytes (7)(8)(9). Bearing these in mind, we investigated the expression of homeobox genes in human T-lymphocytes.

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Materials and Methods

Cells. Leukemic T-cell lines were cultured in RPMI 1640 medium supplemented with 10% fetal calf serum, L-glutamine, and penicillin-streptomycin. Peripheral blood mononuclear cells were obtained from normal healthy donors with a protocol involving density-dependent separation using Lymphoprep (Nycomed). CD4-positive or CD8-positive T-Cells were enriched to over 99% purity by fluorescence-activated cell sorting using FACStar (Becton-Dickinson).

RNA purification. Total cellular RNA was prepared from the above sources by using acid-guanidium-phenol-chloroform method.

Cloning of Jurkat cell homeobox sequences. Poly(A)+ RNA from Jurkat human T-cell leukemia line was reverse-transcribed into single-stranded cDNA using MMTV-RT (Gibco-BRL) and oligo(dT)$_{12-18}$ (Pharmacia). Homeobox sequences were amplified in a polymerase chain reaction using a pair of degenerate primers, 5E and 3F. The 5E primer was 5'-AAAGGATCCTGCAGA(AG)(CT)TXGA(AG)AA(AG)GA(AG)TT-3', spanning the nucleotide positions 43-59 of the homeobox flanked with BamHI and PstI sites ('X' denotes inosine). The sequence of the 3F primer was 5'-ACAAGCTTGAATTCATXC(GT)-XC(GT)(AG)TT(CT)TG(AG)AACCA-3', complementary to the nucleotide positions 142-162 of the homeobox with HindIII and EcoRI sites. The oligomers were synthesized on a DNA synthesizer (381A DNA synthesizer; Applied Biosystems). The polymerase chain reaction was performed with AmpliTaq DNA polymerase (Perkin-Elmer/Cetus) according to the manufacturer's recommendations with some modifications. The thermal condition of the reaction was as follows: 1 min at 94°C (denaturation), 1 min at 40°C (annealing), 2 min at 72°C (extension) for 30 cycles, on a DNA thermal cycler (Perkin-Elmer/Cetus). The resultant product was purified and cloned into BlueScript KS(-). Sequence determination of cloned inserts was performed using Sequenase Ver. 2.0 kit (United States Biochemical). The sequences were compared with published human homeobox sequences (10).

RT-PCR amplification and detection of HOXB7 message. RT-PCR amplification of HOXB7 message was done as follows: 2 μg of total cellular RNA was treated with 1 unit of RNase-free DNase (RQ1; Promega) at 37°C for 60 min, in a 50μl reverse transcriptase buffer of 50mM Tris-HCl (pH 8.3), 75mM KCl, 3mM $MgCl_2$, 1mM DTT, 0.5U/μl RNase inhibitor (HPRI; Takara), and 1mM dNTP; the mixture was heated to 90°C for five minutes after the addition of 2μg oligo(dT)$_{12-18}$, then chilled on ice for 5 min; RNA was reverse-transcribed into cDNA in the presence of 8U/ml of MMTV-RT; one fifth of the RT reaction product was used in the polymerase chain reaction using HOXB7-specific oligonucleotide primers. Condition of the PCR was as follows: 30 sec at 94°C, 1 min at 55°C, 1 min at 72°C for 30 cycles. The sequences of the specific oligomers were 5'-AGTAACTTCCG-GATCTACCC-3' (corresponding to nucleotides 463-482 of Ref. 11) and 5'-TCTGCTT-CAGCCCTGTCTTG-3' (complementary to nucleotides 715-734). The RT-PCR products were run on 3% agarose gels in TAE buffer, Southern-blotted to Hybond-N (Amersham) membranes, and visualized by hybridizing at high-stringency conditions to the radio-labelled HOXB7 internal probe. The internal probe was obtained during cloning of the Jurkat homeobox sequences.

Results

Detection of homeobox sequences in a T-cell line.

In order to study the expression of homeobox genes in human T-lymphocytes, poly(A)+ RNA from Jurkat T-leukemia cells was reverse-transcribed and PCR-amplified with two degenerate primers. The degenerate oligomers were chosen from sequences corresponding to highly conserved amino acid stretches in the classical *Antennapedia*-class (*Antp*-class) homeodomain. A DNA fragment of expected size was obtained and subcloned. Sequence analysis of 41 independent clones revealed the existence of ten different homeobox genes with the following frequency; HOXB7 (25 clones), HOXC4 (4 clones), HOXA7 (3 clones), HOXB3 (2 clones), HOXB4 (2 clones), HOXA1 (1 clone),

HOXA4 (1 clone), HOXA5 (1 clone), HOXA6 (1 clone), and one previously undescribed clone, designated JRX (1 clone). The two degenerate primers could theoretically allow the amplification not only of most *Antp*-class homeobox sequences but also of some divergent homeobox sequences, however, nine of the ten isolated sequences were found among the *Antp*-class homeobox genes and only one (JRX; Fig. 1) belonged to the divergent classes of homeobox genes. The nine *Antp*-class homeobox genes were not a random representation because five were from HOXA locus, three from HOXB locus, only one was from HOXC locus, and no sequences from HOXD locus was identified.

Expression of HoxB7 in normal and malignant T-cells.

Because of the highly skewed representation of HOXB7 in the above RT-PCR survey, it was important to demonstrate the expression of this gene in various T-cells. An RT-PCR assay was established for this purpose. Gene-specific oligonucleotide primers were synthesized for HOXB7, based on published sequence (11). The primers were chosen from sites flanking an intron so that mRNA-derived products could be analysed separately from products derived from unspliced RNA and genomic DNA. The products were analyzed by hybridizing to radiolabelled HOXB7 probe. RNA samples from malignant human T-cell lines representing various stages of differentiation were analyzed using this method. HOXB7 expression was detected in twelve out of the fifteen T-cell lines examined (Fig. 2a). There was no apparent correlation between HOXB7 expression and the differentiation stages of the cell lines. Next the expression of HOXB7 among normal T-lymphocytes was analyzed (Fig. 2b). HOXB7 message was detectable in RNA samples from normal peripheral blood mononuclear cells. After cell separation by fluorescence activated cell

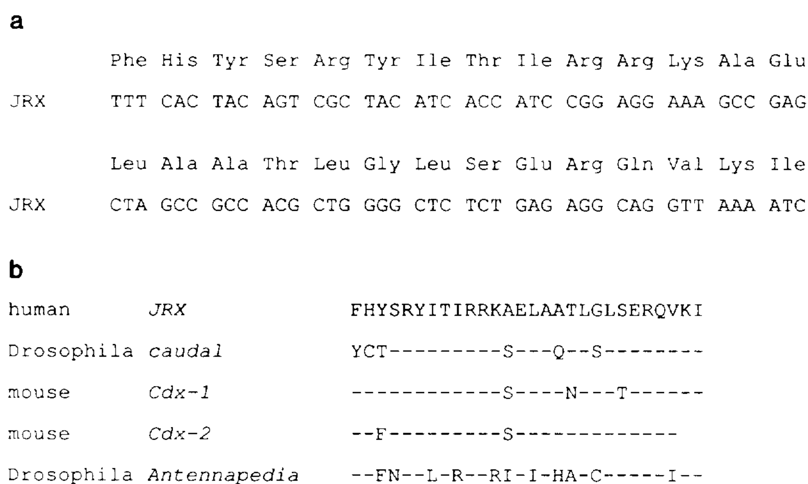

Figure 1. Sequence of the JRX homeodomain. a) Nucleic acid and predicted amino acid sequences of the JRX homeobox region internal to sequences used for PCR amplification are shown. **b)** Comparison of the JRX homeobox amino acid sequence. The homologous region from the published sequences of Drosophila *caudal* (16), mouse *Cdx* (15), *Cdx-2* (13), and Drosophila *Antennapaedia* (17) are included for comparison.

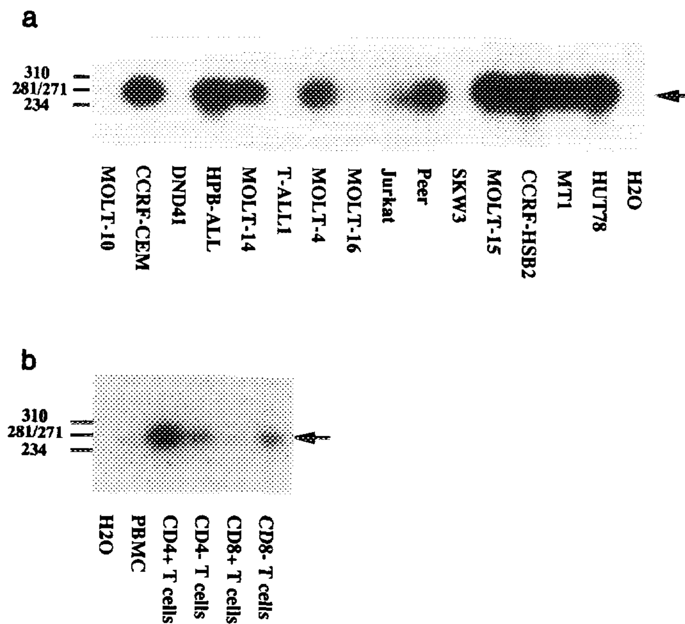

Figure 2. RT-PCR analysis of HOXB7 expression in T-cell lines (a) and normal peripheral blood T-cells (b). Two micrograms of total cellular RNA was reverse-transcribed into single-strand cDNA, one-fifth of which was used for the HOXB7-specific polymerase chain reaction (see Materials and Methods). The expected size of amplified products was 272 base pairs, indicated by an arrow. A very faint expression is detected in MOLT-10 cell line. The experiments were done at least twice, with the same results. The integrity of cDNA samples was checked with PCR amplification of β-actin and β2-microglobulin sequences (data not shown). The amplified products were visualized by hybridizing the blotted membranes with ^{32}P-labelled internal probe. H2O: RT-PCR done without reverse transcriptase. PBMC: peripheral blood mononuclear cells.

sorting, the message was reproducibly more abundant in CD4-positive helper T-cells than in CD8-positive cytotoxic T-cells.

Discussion

Homeobox-containing genes are potent regulators of gene expression during embryogenesis and differentiation in various organisms. Recent reports indicate that some of the homeobox genes are expressed in various hematopoietic cells and play some roles in normal function and/or malignant transformation of these cells. In this communication we studied the expression of homeobox genes in human T-cells. In order to survey the expression of multiple members of the homeobox gene family, cDNA sample obtained from Jurkat T-cell line was subjected to a polymerase chain reaction using degenerate oligonucleotide primers. The primers correspond to conserved amino acid sequences in the homeodomain. This approach has been taken by us and other authors (12)(13)(14). Our results reported here demonstrate the feasibility of this approach in human T-cells. In this study, ten different homeobox gene products were detected. Other groups have studied the

expression of multiple homeobox genes in lymphoid cells using gene-specific RT-PCR and RNase protection (5). Our result is consistent with published data, such that there is an absence of expression of HOXD cluster genes in T-cells. Although our approach lacks quantitative information about homeobox gene expression, one advantage of our approach is that it is possible to obtain sequence information about unrecognized homeobox genes through the use of degenerate primers during PCR-amplification. Indeed one novel sequence, JRX, was identified in our analysis. By sequence comparison with other homeobox genes, the JRX gene is most homologous to mouse Cdx-2 (13) in its amino acid sequence (Fig. 1b). Whether this sequence represents a human homologue of the reported mouse Cdx-1 (15), Cdx-2 (13), or other undescribed genes awaits cloning of the entire sequence.

Because of the demonstrated DNA-binding and gene-regulating capacities of homeoproteins, it is possible that the protein product of these genes could contribute to some aspects of differentiation and/or function of normal T-lymphocytes. In order to elucidate this possibility, we investigated the expression of one homeobox gene, HOXB7, in various T-cells. For this purpose, we have developed a sensitive RT-PCR assay for this gene. Using this method, it was possible to study HOXB7 expression in T-cell lines and normal T-cell subsets. Although it was reported that HOXB7 message was expressed in a number of malignant T-cell lines and normal T-cells (3)(4), the identity of the T-cell subsets which contain these messages has never been studied. In this communication it was demonstrated that HOXB7 expression was widespread among malignant T-cell lines (twelve out of fifteen). Among the normal T-cells, the message was detected preferentially in CD4-positive helper T-cells than in CD8-positive cytotoxic T-cells. Also, we have observed that the expression of HOXB7 increased after T-cell activation with lectin (data not shown), similar to the observation made by another group (3). It is tempting to speculate that the expression of HOXB7 in T-cells may be related to phenotype of these cells and that increases in HOXB7 expression after T-cell activation may result in changes in the phenotype.

Whether the protein products of homeobox-containing genes identified in this communication plays a significant role in malignant transformation of T-lymphocytes, such that has been proposed for HOX11 gene product in T-ALL cases carrying t(10;14), needs further investigation.

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