A New Member of the Proprotein Convertase Gene Family (LPC) Is Located at a Chromosome Translocation Breakpoint in Lymphomas

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Abstract

A new member of the proprotein convertase gene family (LPC) has been identified at a chromosome translocation breakpoint occurring in a high grade lymphoma. The translocation t(11;14)(q23;q32) has been molecularly cloned and shown to be the result of a fusion between an intron in the 3' untranslated region of LPC with a sequence close to the switch region Sγ4 of the IGH locus. The LPC gene encodes a protein of 785 amino acids with substantial homology to furin and the other members of the proprotein convertase family and represents a novel target for chromosome translocation and subsequent deregulation.

Introduction

Abnormalities at chromosome band 11q23 have been recorded in previous studies of lymphoma karyotypes. In a recent survey of 43 cases of non-Hodgkin’s lymphomas, 3 of 21 examples that lacked the translocation t(11;14) have been shown to contain 11q23 abnormalities. The RCK gene, known to be the target of the t(11;14) translocation in the RCK-8 cell line (9), has not yet been reported to be involved in other primary lymphomas. The RCK gene, therefore, lies at an undetermined distance in the 3' direction. The open reading frame (Fig. 2) was found to encode a new member of the proprotein convertase family; we have, therefore, named this gene LPC (lymphoma proprotein convertase). The methionine codon is preceded by an in-frame stop codon at base 36. The transcription starts at a single site and is preceded by an in-frame stop codon at base 36. The transcription pattern of LPC was investigated by Northern analysis of various tissues and cell lines. The expression pattern of LPC was investigated by Northern analysis, and a 3.5-kb transcript was observed at a low level in a wide variety of human tissues (Fig. 3). A similar expression of a 3.5-kb transcript was found in mouse tissues with a minor mRNA transcript.

Materials and Methods

Exon Amplification. Cos6 DNA was double digested with BamHI and BglII and after purification, cloned into the pSPI3 vector. Exon trapping was performed (11), and clone etp12 was isolated. DNA of clone etp12 was used to screen a cDNA library derived from the Jurkat cell line. This library was constructed using random primers and inserts were ligated into the vector BSSV (gift from J. Dunne, Imperial Cancer Research Fund).

DNA Sequencing. One μg of plasmid DNA was Taq cycle sequenced using the Applied Biosystems PRISM Ready Reaction Dye-Deoxy terminator sequencing kit, and the products were extracted with phenol/chloroform. Samples were run on an ABI 373A DNA sequencer, and the results were analyzed using DNAstar software. Oligonucleotide primers were synthesized at appropriate positions to allow full-sequence analysis on both strands.

Southern Analysis. Genomic DNA was prepared from tumor samples, and Southern analysis was performed as described previously (12).

Northern Analysis. Multiple tissue Northern blots were obtained from Clontech. The blots had 2 μg of polyadenylated RNA in each lane and were hybridized to the etp12 probe at 42°C in a solution containing formamide and washed at 50°C for 4 min in 0.1× SSC (1× SSC is 150 mM NaCl, 15 mM Na₃Citrate), 0.1% SDS. The blots were autoradiographed for 2–3 days.

Results and Discussion

An example of the t(11;14(q23;q32) translocation has been identified previously in a case of MLCLS.2 Molecular cloning demonstrated that this translocation was an example of a fusion between the IGH locus on chromosome 14 and an unknown sequence on chromosome 11 (12). It was further demonstrated that this fragment, which did not appear to contain coding sequence, mapped centromeric to HRX and, therefore, did not lie in the vicinity of RCK, which lies telomeric to HRX (13). The layout of genomic sequence at the breakpoint is shown in Fig. 1A. Using as source DNA a chromosome 11 cosmid (cos6) encompassing this breakpoint region, exon trapping identified a short region of potential coding sequence (etpl2). A cDNA library prepared from the Jurkat cell line was screened with this short probe, and several cDNA clones were identified. Sequence analysis of these overlapping clones determined a large open reading frame (Fig. 1, B and C). The open reading frame (Fig. 2) was found to encode a new member of the proprotein convertase family; we have, therefore, named this gene LPC (lymphoma proprotein convertase). The methionine codon is preceded by an in-frame stop codon at base 36. The genomic layout of 4 of the 3' exons of LPC (exons a–d) were determined by both Southern hybridizations to cosmid and genomic human DNA and by sequence analysis and are indicated in Fig. 1, A and B. Exons a, b, and c lie on cosmid cos6, whereas exon d is on a 7.8-kb genomic BamHI fragment that is not present on cos6 and, therefore, lies at an undetermined distance in the 3' direction. The precise position of the breakpoint has been determined previously on cos6 (12); therefore, it can be concluded that this t(11;14(q23;q32) translocation did not affect the coding region of LPC and took place in an intron within the 3' tail of this gene.

The expression pattern of LPC was investigated by Northern analysis, and a 3.5-kb transcript was observed at a low level in a wide variety of human tissues (Fig. 3). A similar expression of a 3.5-kb transcript was found in mouse tissues with a minor mRNA transcript.
The cloning and identification of LPC resulted from our observation of a t(11;14)(q23;32) translocation in a single example of MLCLS. This disease is being increasingly recognized as a discrete entity (14, 15). To investigate whether this event may have occurred in other examples of MLCLS, a series of 5 lymphomas with a similar histopathological classification was investigated by Southern analysis using probe A indicated in Fig. 1A. The results are shown in Fig. 4, and it is clear that tumor L2 has abnormal bands on digestion with both EcoRI and BamHI, strongly suggesting that rearrangement to this region of 11q23 has taken place. It can, therefore, be concluded that the LPC gene may be target for chromosome translocation in a proportion of MLCLS tumors. The full size of the intron in which these breakpoints occurred remains to be determined; therefore, other breakpoints could exist outside the region examined thus far. The full extent of LPC involvement in translocations in lymphomas remains to be determined. The cytogenetic evidence suggests that 11q23 abnormalities are a low but consistent proportion of the genetic events in lymphomas, and alterations to LPC may account for a proportion of those.

LPC is the seventh member of the proprotein convertase gene family to be identified [the others are FUR, PACE4, PC1/3, PC2, PC4, and PACE4 (16)] and is the first to be directly implicated in oncogenesis. The six established mammalian processing enzymes have in common significant similarities to yeast kexin and bacterial subtilisins. FUR is the founder member of this family and was isolated as an expressed region upstream to the FES oncogene on chromosome 15q25 (17). The PACE4 gene also maps to chromosome 15q25, and both FUR and PACE4 are widely expressed. PC5/6, located on chromosome 9, has a similar ubiquitous expression. Both PC1/3 (at 5q15–21) and PC2 (at 20p11) are restricted in their expression to neuro-endocrine tissue, whereas PC4 (on human chromosome 21) is expressed only in testicular germ cells. By several criteria, LPC seems to be a typical member of this family of processing enzymes. The LPC protein can be divided into several domains by analogy with other members of the family. As indicated in Fig. 2, there is a leader sequence, a pro domain, a catalytic domain, and a P or middle domain. As with the other members, the catalytic and middle domains of LPC are highly conserved. In particular, the catalytic domain of LPC contains the active sites Asp187, His228, and Ser306, and also the Asn329 residue important for oxyanion stabilization (Fig. 2). The catalytic and middle domains of LPC have been compared to those of the other six family members and were found to be most similar to those of PACE4 (49.6 and 35.3%, respectively). In general, LPC is slightly less related to the other members than they are to each other. However, the overall structure and sequence homology indicates that LPC can be regarded as full member of this family. The divergence between the COOH region of LPC and the other family members is also a consistent feature of this group of proteins.

Most chromosomal translocations have been found to target genes with the characteristics of transcription factors (18). The results presented here are the first example of a translocation breakpoint within a protease gene. It may be speculated that this event deregulates LPC expression, either by the loss of elements in the 3′ tail of the mRNA, or by the juxtaposition of the Eμ enhancer. An interesting parallel has recently been reported in HIV lymphomas (19). Clonal integration of the HIV genome within the 3′-untranslated tail of the FUR gene has been found in samples from AIDS patients with T-cell lymphomas. Thus, it can be speculated that in these tumors the presence of the HIV genome disrupts FUR gene expression in a manner analogous to chromosomal translocation. Currently, the FES gene, which lies within 1000 bp of the FUR gene, cannot be excluded as another possible target for viral integration.

A large range of biologically active molecules including growth factors and peptide hormones are produced by cleavage of precursor translation products at specific sites. In vivo and in vitro experiments implicate this family of proteases in cleavage at particular basic residues (20). It is likely, therefore, that deregulation of LPC expression in a chromosomal translocation could...
Fig. 2. The composite sequence of LPC mRNA and its predicted protein product. The catalytic domain is underlined and the middle domain is indicated with a dotted line. The residues of the catalytic triad (Asp187, His228, and Ser230) are double underlined as is the residue of the oxyanion hole (Asn329). Arrowhead, position of the intron that contains the breakpoint for the t(11;14)(q23;q32) translocation.
have a profound effect on the processing of biologically important molecules. The in vivo substrates of LPC are unknown, but abnormal or ectopic production of growth factors could play an important role in lymphomagenesis.

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References


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