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A rare mRNA variant of the human lymphocyte-specific protein tyrosine kinase *LCK* gene with intron B retention and exon 7 skipping encodes a putative protein with altered SH3-dependent molecular interactions

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Abstract

A rare mRNA variant of the human lymphocyte-specific protein tyrosine kinase *LCK* gene that retains intron B and excludes exon 7 (B^+7^-) due to alternative splicing of the canonical LCK transcripts was identified and characterized. LCK B^+7^- mRNA is detected in all tested peripheral blood T lymphocytes total RNA samples but is apparently sequestered in the nucleus. The presence of intron B sequence does not disrupt the reading frame and results in the insertion of 58 aminoacids, containing a proline-rich region just upstream of p56lck SH3 domain. This putative isoform encodes an unstable 516 aminoacids protein (Lck^{B+7-}) which can be expressed in transfected COS-7 cells. Furthermore in Jurkat T cell extracts, a recombinant intron B plus SH3 p56lck domain fails to interact with some TCR-induced tyrosine phosphorylated polypeptides and known p56lck partners such as Sam68 and *c*-Cbl. The biological function of this rare messenger remains to be elucidated.

Keywords: LCK gene; Intron retention; Exon skipping; mRNA variant; Alternative splicing

Abbreviations: A, adenosine; AMV, avian myeloma virus; ATP, adenosine triphosphate; bp, base pair(s); C, cytidine; *c*-Cbl, cellular homolog of CAS-BR-M murine ecotropic retroviral transforming sequence; cdc2, cell division cycle 2; cDNA, DNA complementary to RNA; DMEM, dubelcco's modified Eagles medium; G, guanosine; Grb-2, growth factor receptor-bound protein 2; GST, glutathione-S-transferase; iB, intron B; IU, international unit(s); kb, kilobase(s); kDa, kilodalton(s); LCK, lymphocyte-specific protein tyrosine kinase; M, A/C; MAPK, mitogen-activated protein kinase; mRNA(s), messenger RNA(s); N, A/C/G/T; oligo(dT), oligodeoxythymidine-*n*; PBL, peripheral blood lymphocyte(s); PBMC, peripheral blood mononucleate cell(s); PBS, phosphate buffered saline; PCR, polymerase chain reaction; PI2K, phosphatidylinositol 3-kinase; PTK, protein tyrosine kinase; PVDF, polyvinyl difluoride; R, A/G; Ras-GAP, Ras guanosine triphosphatase activating protein; RT, reverse transcriptase; Sam68, src-associated mitotic cell protein; SCID, severe combined immunodeficiency; SDS-PAGE, sodium dodecylsulfate-polyacrylamide gel electrophoresis; SH*n*, src homology domain *type n*; SLP-76, SH2 domain-containing leukocyte protein 76-kDa; Src, rous sarcoma virus oncogene homolog; T, thymidine; TCR, T cell receptor; Tec, Tec protein tyrosine kinase; Th*n*, T helper *type n*; Wasp, Wilkott-Aldrich syndrome protein; Y, C/T.

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1. Introduction

The common pathway for TCR-activated signaling involves a protein tyrosine kinase (PTK) cascade including the Src family member p56lck (Palacios and Weiss, 2004). p56lck is predominantly expressed in T cells and mainly localized in microdomains. It plays a crucial role in early activation events, thymic differentiation, cell cycle progression, Th1/Th2 differentiation (Yamashita et al., 1998), apoptosis and homeostatic proliferation of naïve T cells. Like other Src family kinases, p56lck is organized in domains including a C-terminal kinase domain, Src homology domains (SH4, SH3 and SH2), and a N-terminal region. SH3 domain mediate protein interactions by binding proline-rich aminoacid sequences and is also important for intra- and intermolecular interactions that regulate the catalytic activity, the localization into membrane rafts (Patel et al., 2001), and the recruitment of downstream effectors such as *c*-Cbl, PI3K, Ras-GAP, SLP-76, Cdc2, MAPK, Sam68 (Togni et al., 2004). Markedly in T cells, decreased p56lck protein expression has been observed in a variety of cancers (Majolini et al., 1999) and in autoimmune diseases including type 1 diabetes (Nervi et al., 2000).

The human lymphocyte-specific protein tyrosine kinase *LCK* gene resides at chromosomal locus 1p35-p34.3 and is composed of a segment of approximately 14 kb containing 12 exons. This gene belongs to the large category of human genes that undergo alternative splicing and whose isoforms are involved in regulatory functions (Modrek et al., 2001). Expression of the LCK gene is under the control of two structurally distinct promoters separated by a genomic region of 35 kb, approximately, the so-called distal and proximal promoters (Takadera et al., 1989). The promoter alternate usage produces two major mRNAs in human, designated type I when transcribed from the proximal promoter and type II from the distal promoter. Type I and II mRNAs differ only in their non coding 5′ end. In each class, several mRNAs are transcribed through the use of alternative transcription initiation sites or by alternative splicing (Rouer and Benarous, 1992). Interestingly, an alternative splicing produces two type II mRNAs. The mRNA IIA is the most abundant in mature T cells whereas the minor mRNA IIB, lacking exon 1′ encoding for the N-terminal domain of Lck, is devoid of the coding sequence for the interaction motif with CD4 and CD8 coreceptors (Huse et al., 1998). Another alternative splicing produces an exon 7-less mRNA encoding for an unstable Lck protein isoform. The sole expression of this defective exon7-less transcript was correlated with a signaling deficit downstream of CD3/TCR complex in a young immunodeficient patient (Goldman et al., 1998). However, further analysis evidenced the presence of both transcripts in all tested human cells expressing the *LCK* gene (Nervi et al., 2000; Rouer et al., 1999). It encodes a truncated p56lck protein (LckΔ7) lacking the ATP binding site and with the characteristics of a p56lck cell-signaling regulator (Germani et al., 2003).

During an attempt to elucidate the molecular basis of p56lck deficiency in type 1 diabetes (Nervi et al., 2000), we have identified a rare human LCK transcript retaining intron B (iB) and lacking exon 7. It contains an open reading frame encoding for 516 aminoacids (Lck^{B+7-}) with the insertion of 58 mostly hydrophobic proline-rich aminoacids just upstream of the SH3 domain and lacking the ATP binding site. GST pull down experiment with recombinant p56lck intron B+SH3 domain evidenced loss of interaction with known partners of p56lck including Sam68 and *c*-Cbl. Lck^{B+7-} mRNAs were detected in all tested total RNA samples prepared from various T lymphocyte subsets regardless of the diabetic phenotypes of the individuals. However, the physiological relevance of this transcript isoform is unclear since it appears predominantly sequestered in the nucleus of T lymphocytes.

2. Materials and methods

2.1. Cells

Peripheral blood mononuclear cells from healthy volunteers or type 1 diabetic patients were purified by Ficoll-Hypaque (Nervi et al., 2000). The human leukemia Jurkat (clone H6.2) (Nunes et al., 1993) and the Lck-deficient Jurkat mutant (JCam1.6) (Penninger et al., 1993) T cell lines were cultured in RPMI 1640 supplemented with 10% heat-inactivated fetal bovine serum, 2 mM glutamine, 100 IU/ml penicillin and 100 μg/ml streptomycin. The COS-7 cells were grown in DMEM supplemented as described above.

2.2. LCK mRNA cloning and sequencing

Total RNA was extracted from 10^7 resting PBMC with Trizol reagent (Life Technologies) and converted into cDNA using oligo(dT) primers (Promega) and Moloney murine leukemia virus reverse transcriptase (Invitrogen). For cloning, an aliquot of the cDNA (1/25) was used as template and the complete *LCK* gene coding sequence was amplified by PCR in a 50 μl-mixture including 1 μl high fidelity *Pfu* Taq polymerase (Promega) and 50 μM of two primers matching its 5′ and 3′ ends, respectively (sense 1F: 5′-ATGGGCTGTGGCTGCAGCTCACACCC-3′, reverse 1530R: 5′-TCAAGGCTGAGGCTGGTACTGGCCC-3′). Cycling conditions included an initial denaturation at 94 °C for 2 min followed by 30 cycles at 95 °C for 1 min, 65 °C for 2 min, 74 °C for 4 min, and a final extension step at 74 °C for 5 min. The PCR products were separated by gel electrophoresis and purified using a Nucleospin kit (Macherey–Nagel) and subcloned into the pUC18 shuttle vector. To determine the presence of intron B sequence within *bona fide* LCK mRNAs, full length cDNA were also synthesized from total RNA according to the GeneRacer™ protocol (Invitrogen Life Technologies) including dephosphorylation, decapping and reverse-transcription with AMV reverse