SHORT COMMUNICATION

Structure and Chromosomal Localization of the Human PD-1 Gene (PDCD1)

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A cDNA encoding mouse PD-1, a member of the immunoglobulin superfamily was previously isolated from apoptosis-induced cells by subtractive hybridization. To determine the structure and chromosomal location of the human PD-1 gene, we screened a human T cell cDNA library by mouse PD-1 probe and isolated a cDNA coding for the human PD-1 protein. The deduced amino acid sequence of human PD-1 was 60% identical to the mouse counterpart, and a putative tyrosine kinase-association motif was well conserved. The human PD-1 gene was mapped to 2q37.3 by chromosomal *in situ* hybridization.

The phenomenon called programmed cell death (PCD) is essential for generation of the complex structural and functional organization of various organs and systems in the living organism. Most of the cells undergoing PCD require transcriptional activation of genes that are essential for cell death (4). To understand the molecular mechanism of PCD, it is important to isolate genes that are newly activated upon cell death. We have assumed that many, if not all, cells killed by PCD express similar genes. We thus set out to isolate genes commonly activated upon PCD by subtractive hybridization using two different mouse cell lines that show PCD; IL-3-dependent hematopoietic progenitor cell LyD9 and T-cell hybridoma 2B4.11. As the first of such genes, we have isolated cDNA encoding PD-1, which is a cell surface membrane protein of the immunoglobulin superfamily (2). PD-1 is strongly induced in thymus when an anti-CD3 antibody is injected into the mouse and a large number of thymocytes are killed. Although the function of the PD-1 protein is unknown, strong correlation of its expression upon programmed cell death suggests that this protein may be involved at some stage of PCD. We report here isolation, characterization, and chromosomal mapping of the human homolog of the mouse PD-1 gene.

We prepared a cDNA library from mRNA of a human NK-like cell line YTC3, screened 1.2×10^6 colonies with a mouse PD-1 cDNA, and isolated 23 positive clones. The longest clone, h#9, contained an insert of about 2.1 kb, in good agreement with the size (2.3 kb) of the polyadenylated transcript detected by Northern blot hybridization (see below). The nucleotide sequence of clone h#9 was determined, and the deduced amino acid sequence was aligned with the previously determined mouse PD-1 sequence (Fig. 1A). The longest open reading frame encodes a putative human PD-1 protein of 288 amino acids. The sequence contains two hydrophobic regions, one at the N terminus and the other in the middle of the protein, which presumably serve as a signal peptide and a transmembrane segment, respectively. A computer search of the protein and nucleic acid databases (GenBank) revealed no significant identity to any known protein or nucleotide sequences except for the mouse PD-1. The human PD-1 protein sequence is 60% identical to the mouse PD-1 protein sequence.

The residues characteristic of the immunoglobulin superfamily (7) are conserved between the mouse and the human PD-1, including two cysteine residues (Cys^{54} and Cys^{123}) with 68-residue interval and four amino acids (Arg^{94}, Phe^{95}, Asp^{117}, and Gly^{119}). Both mouse and human PD-1 proteins have a variant form of the consensus sequence (two YXXL with a spacer) that is conserved in the cytoplasmic domains of the CD3 chains (δ, ϵ, γ, ζ), and the β and γ chains of the high affinity FcεR (1). Recent comparison of the consensus motif indicates that the sequence between a pair of YXXL can be quite variable (Fig. 1B). Among the proteins with this motif, the human PD-1 has the longest spacer (21 residues), followed by the mouse PD-1 (19 residues). The human FcγRIIA with 15-residue spacer was shown to be able to transduce signal as chimeric molecules, in which the extracellular domain of the human CD4 or CD16 antigen was joined to the transmembrane and intracellular domains of the human FcγRIIA (3). It remains to be tested whether the human and mouse PD-1 proteins are capable of transducing any signal.

Northern blot analysis using human PD-1 cDNA as probe detected the 2.3-kb transcript in two human T-cell lines, YTC3 and JM cells (Fig. 2A). A higher expression level was seen in YTC3 cells, whereas JM cells

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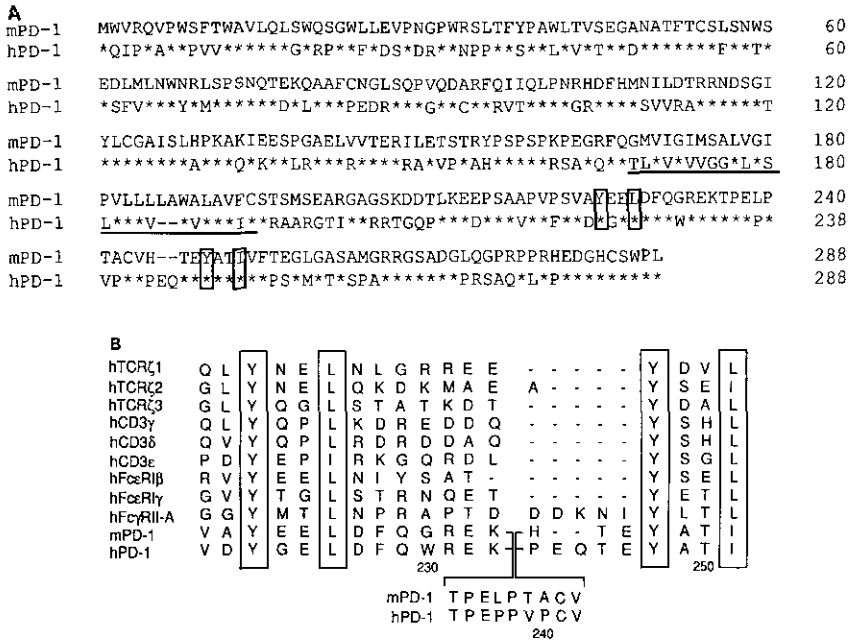

FIG. 1. Structure and alignment of human PD-1 protein. Five micrograms of poly(A)$^+$ RNA extracted from YTC3 cells was used for cDNA library construction by using the TimeSaver cDNA synthesis kit (Pharmacia) and was cloned into the λgt10 as described (5). Duplicate sets of phage library filters were hybridized using the mouse PD-1 cDNA probe (2). The filters were washed in 1× SSC and 0.1% SDS at 60°C for 10 min. cDNA inserts isolated from the human cDNA library were subcloned and sequenced by the dideoxynucleotide chain termination method using a modified T7 DNA polymerase (United States Biochemical) and [α-^{32}P]dCTP (3000 Ci/mmol, Amersham). Nucleotide sequences of both strands of DNA were determined. Nucleotide sequence is deposited in GenBank (Accession No. L27440). **(A)** Amino acid sequences of the human and mouse PD-1. Asterisks represent identical amino acid residues. YXXL motifs are boxed. Transmembrane region is underlined. **(B)** Comparison of the YXXL motifs of the mouse and human PD-1 with other human YXXL motif-containing proteins.

gave only a weak signal. Although we did not observe any signals in MOLT-4F and CCRF-CEM, very faint signals were detected in Jurkat, HPB-ALL, and CESS cells after a prolonged autoradiograph exposure (data not shown).

Southern blot analysis using the human PD-1 cDNA probe detected a single positive PD-1 gene band (23-kb *Eco*RI, 5.6-kb *Bam*HI, and 23-kb *Hin*dIII bands). Under a lower stringency condition, however, another additional band appeared around 3 kb in *Bam*HI digests,

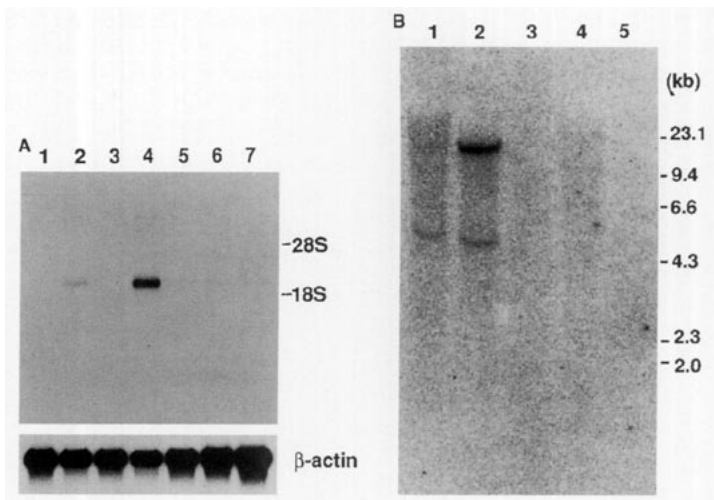

FIG. 2. Expression and cross-hybridization of the PD-1 gene. Isolation of genomic DNA from various animal cells, restriction enzyme digestion, Southern hybridization, extraction of total cellular RNA, purification of poly(A)$^+$ RNA, and Northern hybridization were carried out by standard methods (5). **(A)** Three micrograms of poly(A)$^+$ RNA from the human T- or B-lymphoid cell lines indicated was used. Filters were hybridized with either human PD-1 cDNA or β-actin probe. Lane **1,** MOLT-4F (T); lane **2,** JM (T); lane **3,** CCRF-CEM (T); lane **4,** YTC3 (T); lane **5,** Jurkat (T); lane **6,** HPB-ALL (B); lane **7,** CESS (B). **(B)** DNA of various animals were digested with *Bam*HI. The filter contained 10 μg of genomic DNA except for the *Drosophila* (1 μg). Filter was hybridized with mouse PD-1 probe under a low-stringency condition. Origins of DNA are lane **1,** human; lane **2,** mouse; lane **3,** rabbit; lane **4,** *Xenopus;* lane **5,** *Drosophila.*

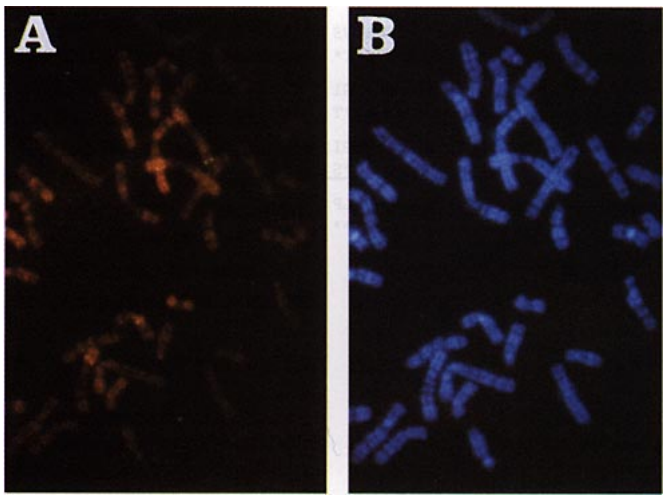

FIG. 3. Chromosomal localization of the human PD-1 gene by fluorescence *in situ* hybridization. **(A)** Hybridization signals at 2q37.3. **(B)** R-bands in the same metaphase plate. Chromosomal *in situ* hybridization was carried out using phage DNA containing the PD-1 gene labeled with biotin–11-dUTP by nick-translation. Chromosome spreading and hybridization were carried out as described previously (5). A genomic DNA library of *Sau*3A partial digests of esophageal cancer cell line DNA was constructed using the Lambda DASH II vector provided by Dr. Y. Nishiyama (Kyoto University). Human PD-1 DNA clones were isolated from this library by hybridization with the human PD-1 cDNA probe.

but not in the other lanes (data not shown). Although DNA sequences that hybridized to the mouse PD-1 cDNA cross-hybridized to the human DNA, we could not detect any significant hybridization signals in the *Drosophila, Xenopus,* and rabbit DNA (Fig. 2B).

To determine the chromosomal location of the human PD-1 gene, a phage library containing human genomic DNA was screened with a human PD-1 cDNA probe. Of 1×10^6 recombinant phages, two positive plaques were isolated and characterized by restriction mapping and Southern blot hybridization analysis. Although the two clones overlapped each other in an about 14-kb region, one of the clones was found to have a 2-kb deletion. Subsequently a 200-bp fragment encoding the region from Gly^{47} to Arg^{114} was amplified by PCR, subcloned, sequenced to confirm the identity of the phage clone, and used for chromosomal mapping.

Of the 30 metaphases examined, 28 (93%) had a specific hybridization signal with complete twin spots on both homologs at the telomeric region of the long arm of chromosome 2, and the remaining 3 metaphases (10%) exhibited incomplete signal and/or double spots on either or both homologs of chromosome 2. By high-resolution R-banding analysis, the PD-1 gene was assigned to the band 2q37.3 (Figs. 3A and 3B). There are no obvious human genetic disorders mapped to 2q37.3 that show any symptom related to immune disorders.

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