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Assignment of the human gene for receptor-type protein tyrosine phosphatase IA-2 (PTPRN) to chromosome region 2q35→q36.1 and identification of an intragenic genetic marker

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Abstract. Using a mouse protein tyrosine phosphatase cDNA fragment as a probe, cosmid clones containing segments of the human IA-2 PTPase gene (PTPRN) were isolated. The gene was assigned to chromosome region 2q35→q36.1 by fluorescence in situ hybridization. In an intronic region of the IA-2 gene a polymorphic microsatellite sequence was found, which will be useful as a genetic marker for the 2q35→q36 region.

In recent years, protein tyrosine phosphatases (PTPases), which oppose the actions of protein tyrosine kinases (PTKs), have gained attention as regulators of important cellular processes, such as cell growth and differentiation (Walton and Dixon, 1993). The phosphotyrosine content of cellular proteins is determined by a balanced action of PTKs and PTPases; in some cases these enzymes have been shown to work in concert in signal transduction networks, which carry signals from the cell membrane to the nucleus (Brady-Kalnay and Tonks, 1994).

Until now, a substantial number of PTPase genes has been identified, which can be classified into two large subgroups: (1) the cytosolic and nuclear PTPases and (2) the receptor-type PTPases. As a rule, the cytosolic and nuclear PTPases have one tyrosine phosphatase domain, whereas the receptor-type PTPases generally contain two tandemly repeated cytoplasmic tyrosine phosphatase domains. Exceptions are, for example, PTP-SL (Hendriks et al., 1995) and IA-2 (Lan et al., 1994; Lu et al., 1994), which are transmembrane proteins with only a single tyrosine phosphatase domain.

We recently cloned a mouse PTPase cDNA fragment, mPTP38 (Hendriks et al., 1995), that was identical to sequences within mouse IA-2 cDNA (Lu et al., 1994). In this paper, we describe the use of mPTP38 as a probe to isolate mouse full-length cDNAs and human genomic cosmid clones for IA-2. The human IA-2 gene (PTPRN) was found to reside in chromosome region 2q35→q36.1.

Materials and methods

Isolation of IA-2 cDNA and genomic clones

The 368-bp PCR fragment mPTP38 (Hendriks et al., 1995), was labeled radioactively by random priming and used as a probe to screen a mouse brain cDNA phage library (Stratagene). Hybridization conditions were those as described by Hendriks et al. (1995). After hybridization, filters were washed two times at high stringency (0.1% SDS, 0.04 M sodium phosphate [pH 7.4], 1 mM EDTA) for 20 min at 65°C. Positively hybridizing phages were plaque-purified, and the inserts were rescued as pBlue-

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                    V   W   E   S   G   C   T   V   I   V   M   L   T   P   L   V   E   D   G   V
tctgtccctag  AT GGT GTG GGA GAG CGG CTG CAC CGT CAT CGT CAT GCA CAC CCC GCT GGT GGA GGA TGG TGT

 K   Q   C   D   R   Y   W   P   D   E   G   A   S   L   Y   H
CAA GCA GTG TGA CCG CTA CTG GCC AGT TGA GGG TGC CTC CCT CTA CCA C gtatataaggtcagcagggccccacagca

aggggacaatggatgtgggaagagtagatgatggcctttcgtaacgttttcttcaccaaatgagcactcaacagtcttttaaaaaggcttttat

tggccctgctcatgattcatacatatatgaatatatataaacacatatatgcatacccatatattcacatatatatacatgtgtgtgtcctgttt

acagaacatacatgtgtgtgtgtgtgtgtgtgtgtgtgtgtcctgtctagagtcaaaaagcgttcatagagattatgataaggcacagagatcga

gcccggggtgggcgaagaactccagcatgagattcgggtacc
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Fig. 1. Genomic sequence of part of the human PTP38/IA2 gene present in clone PTP38/7.16S (GenBank accession No. Z48226). Exon sequences that correspond to nucleotides 2,462 to 2,572 in the IA-2 cDNA sequence (Lan et al., 1994) are in capital letters; intronic regions are depicted in lower-case letters. Above the exonic sequence the deduced amino acid sequence is shown in single letter notation. The sequence amplified by primer set F388 and R388 is underlined. The microsatellite is doubly underlined.

Script SK plasmids (Stratagene), following the instructions of the manufacturer, and sequenced.

The same mouse probe was used to screen a human genomic library in cosmid vector Supercos 1, kindly provided by Dr Marten Hofker (University of Leiden, The Netherlands). Hybridization and washing conditions were as described above.

Characterization of a microsatellite at the human PTPRN locus

Sau3A subclones from cosmid PTP38/7 were screened for the presence of microsatellite sequences by hybridizing recombinants-containing filters for 2 h at 37 °C in 5 × SSPE, 0.3% SDS with a mixture of oligonucleotides that were 5′ end-labeled using [γ-^{32}P]ATP and T4 polynucleotide kinase (Life Technologies). The oligonucleotide mixture consisted of 100 ng of oligonucleotide $(GA)_7$, $(TG)_7$, $(CAC)_5$, and $(CTG)_5$, each. The insert of one positive clone (PTP38/7.16) was further shortened by *SstI* digestion and self-ligation, yielding clone PTP38/7.16S, and sequenced.

Polymerase chain reaction (PCR)

Based on sequence information in clone PTP38/7.16S, unique oligonucleotides flanking the IA-2 microsatellite were designed and tested in a PCR. PCR was performed in a 50-µl volume containing 40 ng of genomic DNA, 50 mM KCl, 20 mM Tris-HCl (pH 8.3), 1.0 mM $MgCl_2$, 0.01% gelatin, 0.1% Triton-X100, and 100 ng of oligonucleotides F388 (5′-GGCCCTGCTCATGATTC-3′ and R388 (5′-TCTATGAACGCTTTTTGACTC-3′). Oligonucleotide F388 was 5′ end-labeled with [γ-^{32}P]ATP and T4 polynucleotide kinase. After an initial denaturation step of 5 min at 95 °C, 1 U of *Taq* polymerase was added to the reaction mixture, and 32 cycles were performed; each cycle involved denaturation at 94 °C for 45 s, annealing at 43 °C for 30 s, and elongation at 72 °C for 40 s, with a final extension step of 5 min at 72 °C. PCR products were separated on 6% denaturating polyacrylamide gels, and bands were visualized by autoradiography at −80 °C.

Fluorescence in situ hybridization (FISH)

Nonradioactive in situ hybridization to normal human lymphocyte metaphase spreads was performed essentially according to De Leeuw et al. (1993). Briefly, cosmid DNA (1 µg) was labeled with biotin-14-dATP (Life Technologies) by nick-translation, purified, and ethanol precipitated together with a 50-fold excess of Cot-1 DNA (Life Technologies). Subsequently, 250 ng was dissolved in 12 µl of hybridization mixture consisting of 2 × SSC, 10% dextran sulfate, 1% Tween-20, and 50% formamide. The hybridization mixture was heat-denatured and then incubated overnight at 37 °C to heat-denatured chromosome spreads enclosed under a cover slip. Immunocytochemical detection of the hybridizing probes was achieved using fluorescein isothiocyanate (FITC)-conjugated avidin, followed by two amplification steps using rabbit-anti-FITC and mouse-anti-rabbit-FITC. A Zeiss epifluorescence microscope was used for visual examination of the chromosome slides. Digital images were obtained using a high-performance cooled CCD camera (Photometrics) coupled to a Macintosh IIci computer. The Oncor-Image F.I.S.H. software package (Oncor-Imaging) was used for analysis of the digital images.

Results and discussion

We recently isolated three novel mouse PTPase cDNA fragments (Hendriks et al., 1995) using degenerate primers based on conserved catalytic domains in the rapidly expanding gene family (Walton and Dixon, 1993). One of these fragments, mPTP38 (Z23060), corresponds to a 4-kb transcript that is exclusively expressed in mouse brain (Hendriks et al., 1995). To obtain full-length cDNA clones, a mouse brain cDNA library was screened using mPTP38 as a probe. In this way, a 3.6-kb cDNA clone (PTP38-32) was isolated and sequenced entirely. While this work was nearing completion, the sequence of a mouse cDNA clone designated mIA-2 was published (Lu et al., 1994) that appeared to be identical to our clone. The mouse IA-2 cDNA also exhibits 92% identity with the published human IA-2 cDNA (Lan et al., 1994) that was isolated from an insulinoma subtraction library. Very recently, the putative rat homolog of IA-2, called PTPLP, has been reported to show 98.4% homology in amino acid sequence with the intracellular part of the mIA-2 protein (Kambayashi et al., 1995).

Using cDNA fragment mPTP-38 as a probe, seven independent, but overlapping, human IA-2 genomic cosmid clones (PTP38/1 through PTP38/7), spanning approximately 50 kb, were isolated. Their identity as PTPRN gene-containing cosmids was confirmed by sequence analysis of an 0.8-kb subclone (PTP38/7.16S), originating from cosmid PTP38/7, that contains a region that is 100% identical to nucleotides 2,462 to 2,572 of the IA-2 cDNA (Lan et al., 1994) (Fig. 1). In the same subclone a microsatellite repeat of 14 CA dinucleotides was

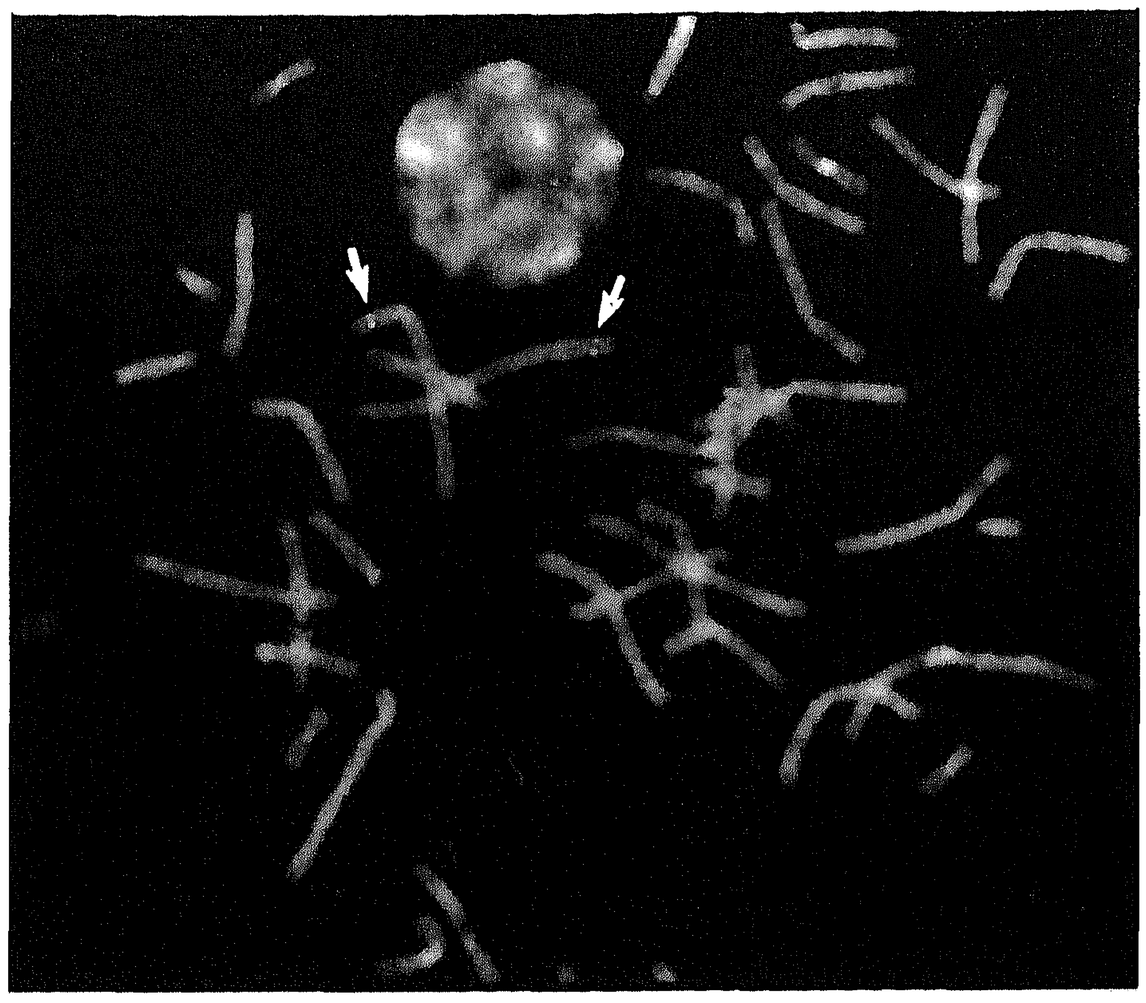

Fig. 2. Chromosomal localization of the human PTPRN gene by FISH. The arrows indicate the location of the hybridization signals of the biotinylated probe (cosmid PTP38/7) to human metaphase chromosome spreads.

identified. Oligonucleotides flanking this repeat were designed to amplify a segment of 163 bp and test the polymorphic value of this microsatellite, using DNA of 37 unrelated Caucasian individuals. Four alleles were observed, with frequencies of 0.054 (167 bp), 0.20 (165 bp), 0.73 (163 bp), and 0.04 (161 bp), yielding a heterozygosity value of 0.44. Thus, the polymorphic microsatellite repeat can serve as a genetic marker (D2S1753E) for the PTPRN locus.

The chromosomal localization of human PTPRN was determined by FISH using cosmid PTP38/7 as a probe. Analysis of 40 human metaphase spreads revealed single hybridization signals in the region 2q35→q36.1 in almost all spreads and usually on both homologs (Fig. 2). This chromosomal localization was confirmed by FISH of two of the other isolated PTP38/IA-2 cosmids, PTP38/4 and PTP38/6 (data not shown). We therefore were able to assign the human gene for the PTPase IA-2 to chromosome region 2q35→q36.1.

Expression of human IA-2 mRNA was observed in brain, pituitary and weakly in pancreatic tissue and enhanced expression of IA-2 mRNA was reported for pancreas-derived tumors, including insulinomas and a glucagonoma (Lan et al., 1994). This observation is suggestive of a potential oncogenic effect resulting from elevated levels of IA-2 protein. Interestingly, for the human, mouse, and rat protein no PTPase activity could be demonstrated (Lan et al., 1994; Lu et al., 1994; Kambayashi et al., 1995), and it has been proposed that IA-2 might influence the proper interaction of other PTPase molecules with their respective substrates. However, a causative role of IA-2 in insulinoma and glucagonoma tumorigenesis, as well as the proposed dephosphorylation inhibitory effect, remains to be demonstrated.

On theoretical grounds, PTPases are expected to have tumor suppressive potential. Therefore, we searched whether allelic loss of the 2q35→q36.1 region has been reported in tumors originating from tissues that normally express IA-2. Frequent allelic losses of chromosome 2q have been reported in non-small cell lung carcinoma, colorectal carcinoma, and neuroblastoma (Tsuchiya et al., 1992; Kohno et al., 1994; Shiseki et al., 1994), but involvement of PTPase IA-2 remains to be investigated. Although a potential tumor suppressor was suggested to be homozygously deleted at region 2q33 in a small cell lung carcinoma cell line (Kohno et al., 1994), based on our physical mapping data, the IA-2 locus appears to reside outside this region. The availability of a genetic marker at the PTPRN locus, as presented in this article, now enables loss of heterozygosity studies to evaluate whether IA-2 might be involved in these and/or other human tumors.

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