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Chromosomal Assignment of the Gene for Protein Tyrosine Phosphatase HPTPδ

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Protein tyrosine phosphatase (PTP) negatively regulates the effect of protein tyrosine kinases and is implicated in the regulation of a variety of biological phenomena such as cell activation, differentiation and neoplastic transformation. To gain insight into the role of PTPs, we cloned the human receptor-type PTP gene and assigned the chromosome harboring the gene for HPTPδ by using DNAs from human-mouse hybrid cell lines and by fluorescence *in situ* hybridization. The results clearly demonstrated that HPTPδ gene maps to human chromosome 9p24.

Key words: Chromosomal mapping — FISH — HPTPδ — Tumor suppressor gene

Protein tyrosine phosphorylation is involved in a vast array of biological events including cell activation, growth, differentiation and neoplastic transformation.[1-4] This process is tightly regulated by two opposing enzymes, protein tyrosine kinases (PTKs) and protein tyrosine phosphatases (PTPs).[5,6] Given that PTKs act as transforming proteins if anomalously expressed, PTPs, the counter-regulator of PTKs, are hypothesized to be potential candidates for tumor suppressors. There have been several reports supporting this hypothesis.[7-10] For example, treatment of fibroblast-like cells with a PTP inhibitor, vanadate, resulted in an increase in the level of phosphotyrosine and induced transformation phenotypes.[7] One of the receptor-type PTP (RPTP) genes, HPTPγ, has been demonstrated to map to a chromosome 3p21, the deletion of which is often observed in renal cell carcinoma and lung carcinoma. Further, one HPTPγ allele was lost in renal and lung cancers, suggesting that HPTPγ is a candidate tumor suppressor gene.[8] However, Pallen's group recently demonstrated that overexpression of an RPTP, PTPα, resulted in activation of $pp60^{c\text{-}src}$ kinase with dephosphorylation at the Tyr527 negative regulatory site, and induced cell transformation and oncogenic capability.[11] These results raised the possibility that the action of PTPs is much more complex than simply acting as a negative regulator of cell transformation. To characterize further the role of PTPs in cell growth and transformation, we tried to isolate human RPTP genes and to determine the chromosomal localization of the isolated RPTP genes.

To identify human RPTP genes, two cDNA fragments (573-bp *Pvu*II and 945-bp *Pst*I fragments) encoding most of the PTP domains of mouse CD45 were used as probes. A human female brain (temporal lobe) cDNA library (Stratagene, #935205) was screened with the probes under low stringent conditions. A group of overlapping clones was obtained that turned out to code for HPTPδ gene.[12] The restriction map of the isolated clones is shown in Fig. 1.

To localize the chromosome harboring the HPTPδ gene, DNAs from 10 human-mouse hybrid cell lines provided by Dr. Masabumi Shibuya (The Institute of Medical Science, The University of Tokyo) were used. The pattern of retention of human chromosomes in these hybrid cell lines is shown in Fig. 2. Five μg of DNA from each hybrid and 2.5 μg of human and mouse DNAs were digested to completion with *Pst*I and subjected to electrophoresis on 0.8% agarose gel. The DNA was then transferred to Nytran nylon membrane (Schleicher & Shuell, Dassel, FRG) and hybridized to ^{32}P-labeled HPTPδ probe. The HPTPδ probe was a 424-bp fragment of clone 401 (see Fig. 1). Hybridization was done at 42°C overnight in 50% formamide, 5×Denhardt's solution, 0.02 *M* NaH_2PO_4, 0.2% sodium dodecyl sulfate, 5×SSC (750 m*M* NaCl, 75 m*M* sodium citrate) and 100 μg/ml sheared salmon sperm DNA. The hybridized membranes were washed under high stringent conditions and exposed to X-ray films with intensifying screens for 1 day at −70°C.

As illustrated in Fig. 3, the HPTPδ probe detected a 5.4-kb fragment in human genomic DNA and a 13.0-kb fragment in mouse genomic DNA by cross-hybridization. Having confirmed the specificity of the restriction

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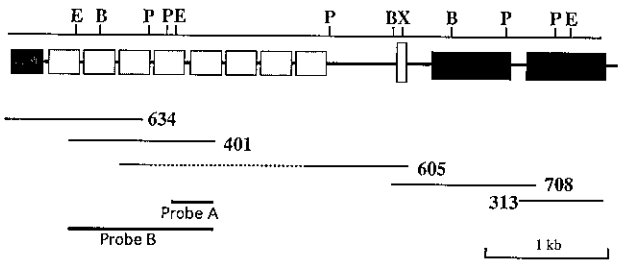

Fig. 1. Restriction map and schematic model of HPTPδ gene based on the report by Krueger *et al.*[12] Thin bars under the models indicate isolated clones. Probes A and B were used for genomic Southern blot analysis and FISH, respectively. B, *Bam*HI; E, *Eco*RI; H, *Hin*dIII; P, *Pst*I; X, *Xba*I.

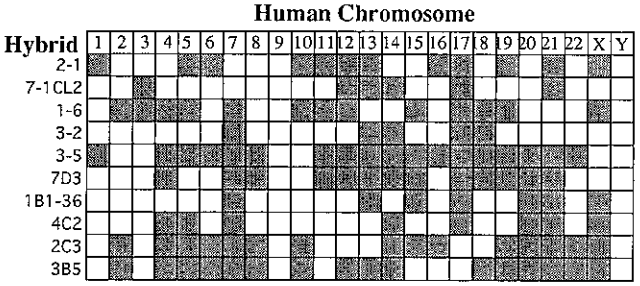

Fig. 2. The pattern of retention of human chromosomes in 10 human-mouse hybrid cell lines. A stippled box indicates that the hybrid retains the human chromosome indicated at the top.

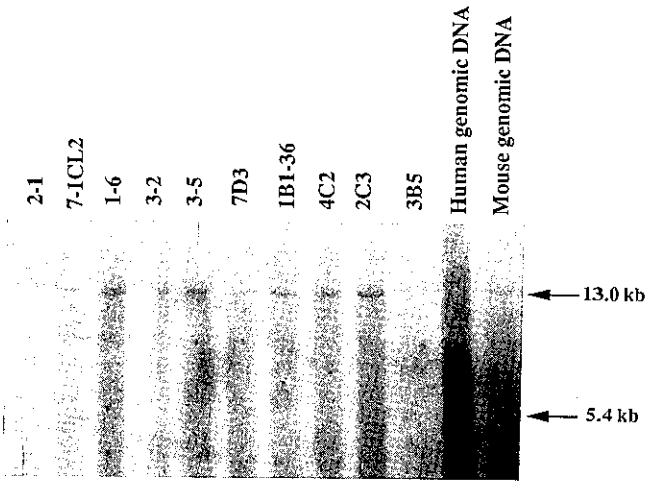

Fig. 3. Southern hybridization analysis of DNAs from human-mouse hybrid cell lines with HPTPδ probe. In control human and mouse DNAs, the HPTPδ probe detected a 5.4-kb and a 13.0-kb fragment, respectively. The same probe could not detect a 5.4-kb human-specific fragment in DNAs from any hybrids.

fragment length polymorphisms, DNAs from 10 human-mouse hybrids retaining various human chromosomes were analyzed. With the HPTPδ probe, a 5.4-kb human-specific fragment was not identified in any of the hybrid DNAs (Fig. 3). The result suggests that the HPTPδ gene locus resides on chromosome 9 or Y chromosome, neither of which retains any human chromosomes (Fig. 2). However, because the HPTPδ gene was cloned from a female brain cDNA library, the HPTPδ locus is considered to map to chromosome 9.

To characterize further the HPTPδ locus on chromosome 9, fluorescence *in situ* hybridization (FISH) was performed as previously reported.[13,14] Briefly, metaphase chromosomes were prepared by the thymidine synchronization/bromodeoxyuridine release technique. The slides were denatured in 70% formamide and 2×SCC at 75°C for 2 min, immersed in 70% ethanol at −20°C, and dehydrated. DNA probe for HPTPδ (clone 401 of 1.2 kb, see Fig. 1) was labeled with biotin, precipitated with salmon sperm DNA and *Escherichia coli* tRNA and dissolved in formamide. The slides were incubated with denatured DNA probe in a humidified box at 37°C for 16–18 h. After washing, the slides were incubated with avidin-FITC at 37°C for 40 min and then counterstained with propidium iodide.

A total of 82 metaphase cells were examined. Of these, 8 cells exhibited twin-spot signals on both homologs of chromosomes 9 at p24, as typically depicted in Fig. 4, and another 19 cells had twin-spot signals on one chromosome 9p24 or a single-spot on either one or both homologs. Such specific accumulation of the fluorescent signals could not be detected on any other chromosome. These results confirmed the somatic hybrid study and indicated that HPTPδ gene is localized to 9p24. We have recently cloned a murine counterpart of HPTPδ, named MPTPδ, and demonstrated that MPTPδ locus is tightly linked to coat color *brown* gene locus on mouse chromosome 4, a homologous region of human chromosome 9.[15]

There are several reports suggesting that chromosome 9 contains tumor suppressor genes for certain types of human cancers. For example, the introduction of chromosome 9 into a human endometrial carcinoma cell line completely suppressed the tumorigenicity of the cell line.[16] In addition, deletions or translocations in chromosome 9 have been observed in leukemias, melanoma, glioma and some colorectal carcinomas.[17–19] In this respect, it is also of note that the gene controlling the resistance to radiation-induced lymphomagenesis in the mouse (*Lyr*: lymphoma resistance) is located close to

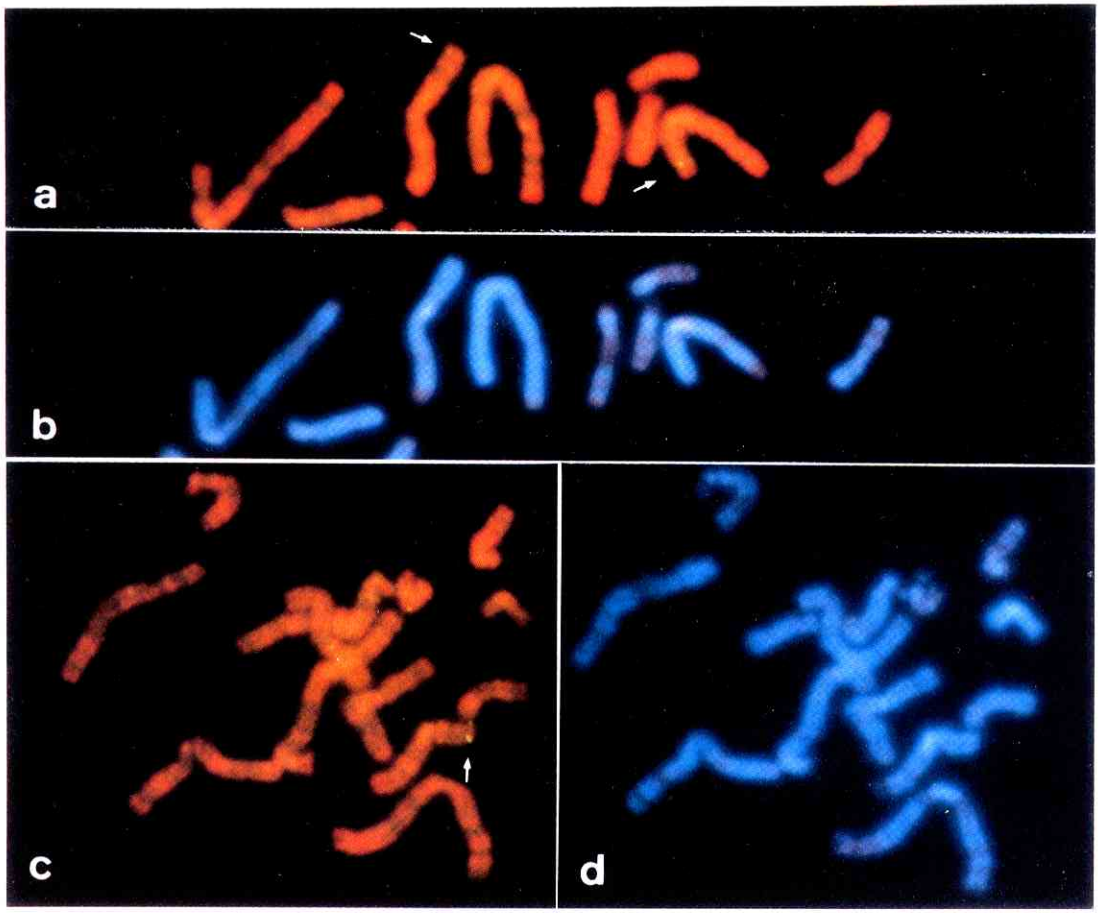

Fig. 4. FISH analysis on metaphase chromosomes with HPTPδ probe. (a) (c) R-banded chromosomes with double spot HPTPδ signals on 9p24 (arrows). Comparison of the fluorescence image with the G-banding of the same metaphases allowed localization of the hybridization signal to 9p24 (b, d).

the MPTPδ locus.[20] To establish firmly that HPTPδ or MPTPδ functions as a positive or negative regulator of oncogenesis, it would be necessary to examine how the introduction of HPTPδ and MPTPδ genes alters the biochemical machinery and tumorigenicity of the cancer cells.

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