SHORT COMMUNICATION

Chromosomal Localization of Four Human VH1-like Protein-Tyrosine Phosphatases

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Four human protein-tyrosine phosphatase (PTPase) genes of the VH1-like subclass were cloned by low-stringency screening of a genomic library. These genes were localized to their respective chromosomes by G-banding and fluorescence *in situ* hybridization. The genes were localized to unique regions of different chromosomes: CL100, a stress-induced PTPase, to 5q35; PAC-1, a mitogen-induced nuclear PTPase, to 2q11; hVH-3 to 10q25; and hVH-4 to 10q11. © 1994 Academic Press, Inc.

A critical step in the regulation of several cellular processes is phosphorylation and dephosphorylation of protein tyrosyl residues (1, 11, 22). The balance of tyrosine phosphorylation is controlled by the dynamic action of protein-tyrosine kinases (PTKs) and protein-tyrosine phosphatases (PTPases) (5, 12, 20). The processes regulated by PTPases comprise cell growth and proliferation, including cell cycle regulation, signal transduction, and oncogenic transformation (2, 20).

Over 40 unique PTPases have been cloned and characterized. A common catalytic motif is found in all PTPases, HCxxxxxR (2). The invariant cysteine residue has been shown to form a phosphorylated intermediate in the catalytic dephosphorylation of substrate (8). These enzymes belong to one of two general classes, the receptor-like PTPases or the intracellular PTPases (2). A subclass of intracellular PTPases has recently been described, following the cloning of a novel PTPase from Vaccinia virus (VH1) (7). This enzyme was unique in that it would hydrolyze proteins containing phosphoserine/threonine and tyrosine. The VH1 family of phosphatases had amino acid identity to the cell cycle regulator protein cdc25, which was subsequently shown to be a "dual specificity" phosphatase (4, 6, 17). Other members of this subclass have been recently discovered and include a PTPase from Baculovirus (BVH1) (10) and yeast (YVH1) (9). Several mammalian VH1-like PTPases have also been cloned, and these include 3CH134 from mouse 3T3 cells (3), CL100, which is the human homolog of 3CH134 (15), and PAC-1 from human T cells (21). All of these mammalian VH1-like PTPases appear to be immediate early response gene products.

To identify additional members of the VH1 PTPase subclass, we screened a human genomic DNA library (leukocyte DNA, Clontech, Palo Alto, CA) with a 523-bp fragment of CL100. The partial cDNA fragment of CL100 was cloned by PCR from a human placental cDNA library (Stratagene, La Jolla, CA) using a pair of specific oligonucleotides that corresponded to the full-length human cDNA sequence at nucleotide positions 756–776 and complementary to positions 1259–1279. The PCR conditions (94°C, 1 min; 55°C, 1 min; 72°C, 1 min) were carried out for 35 rounds of amplification. Approximately 1 million independent recombinants of a human genomic library were screened. Prehybridization was performed in 30% formamide/5× SSPE/5×

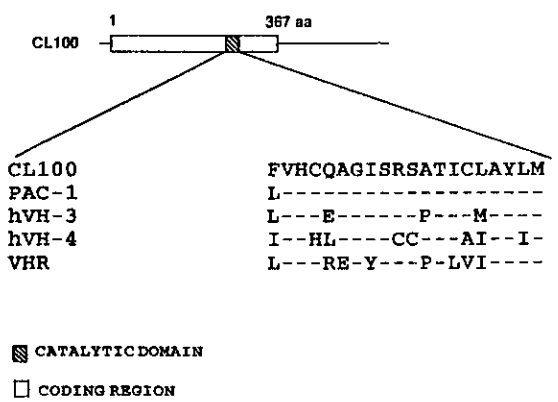

FIG. 1. Schematic representation of human VH1-like PTPase and active site region. Amino acid sequences were derived from cloned genomic DNA fragments. VHR, a previously cloned member of this subclass of PTPases and a dual specificity phosphatase, is included for comparison (13). Active site region amino acids are shown compared to CL100, with conserved residues indicated by a dashed line.

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TABLE 1

Chromosomal Localization of VH1-like Phosphatases by FISH

Clone	Chromosome localization	Total cells examined	No. cells w/label on both homologs[a]	% double dots to band region[b]
CL100	5q35	20	20	100
hVH-3	10q25	12	12	100
hvH-4	10q11	20	19	100
PAC-1	2q11	9	8	100

[a] In all cases 100% of cells had localization of spots to both chromatids of at least one homolog of the indicated chromosome. In 59/61 cells examined by FISH/GTG-banding, localization of "double dots" was to both homologs.

[b] In all cases the only double fluorescence signals observed were localized to the representative band region indicated.

Denhardt's/0.1% SDS/100 μg/ml denatured salmon sperm DNA. Hybridization was carried out in the same solution with the addition of the PCR-amplified probe (2×10^6 cpm/ml) labeled with a Multiprime labeling kit (Amersham, Arlington, IL). Filters were washed successively in 1× SSC/0.1% SDS at 42°C for 15 min, 50°C for 30 min, and 60°C for 30 min.

Several PTPase clones were identified by this low-stringency hybridization and subsequent restriction mapping and sequencing (Fig. 1). These phosphatases showed the greatest degree of structural identity to the VH1-like phosphatases and demonstrated the greatest amount of amino acid identity within the previously identified active site sequence of the PTPases. This region also included an expanded segment of amino acid identity at the carboxy terminal portion of the active site, AY(L/I)M (Fig. 1). During our investigations, one of these clones was published: PAC-1, a mitogen-induced nuclear PTPase (21).

Genes mapped to regions of chromosomal disruptions have indicated the role of kinases (19) and PTPases (14) in carcinogenesis. To identify the chromosomal location of each VH1-like phosphatase and to investigate whether these genes reside at the location of recurring chromosome alterations in a malignancy, we performed

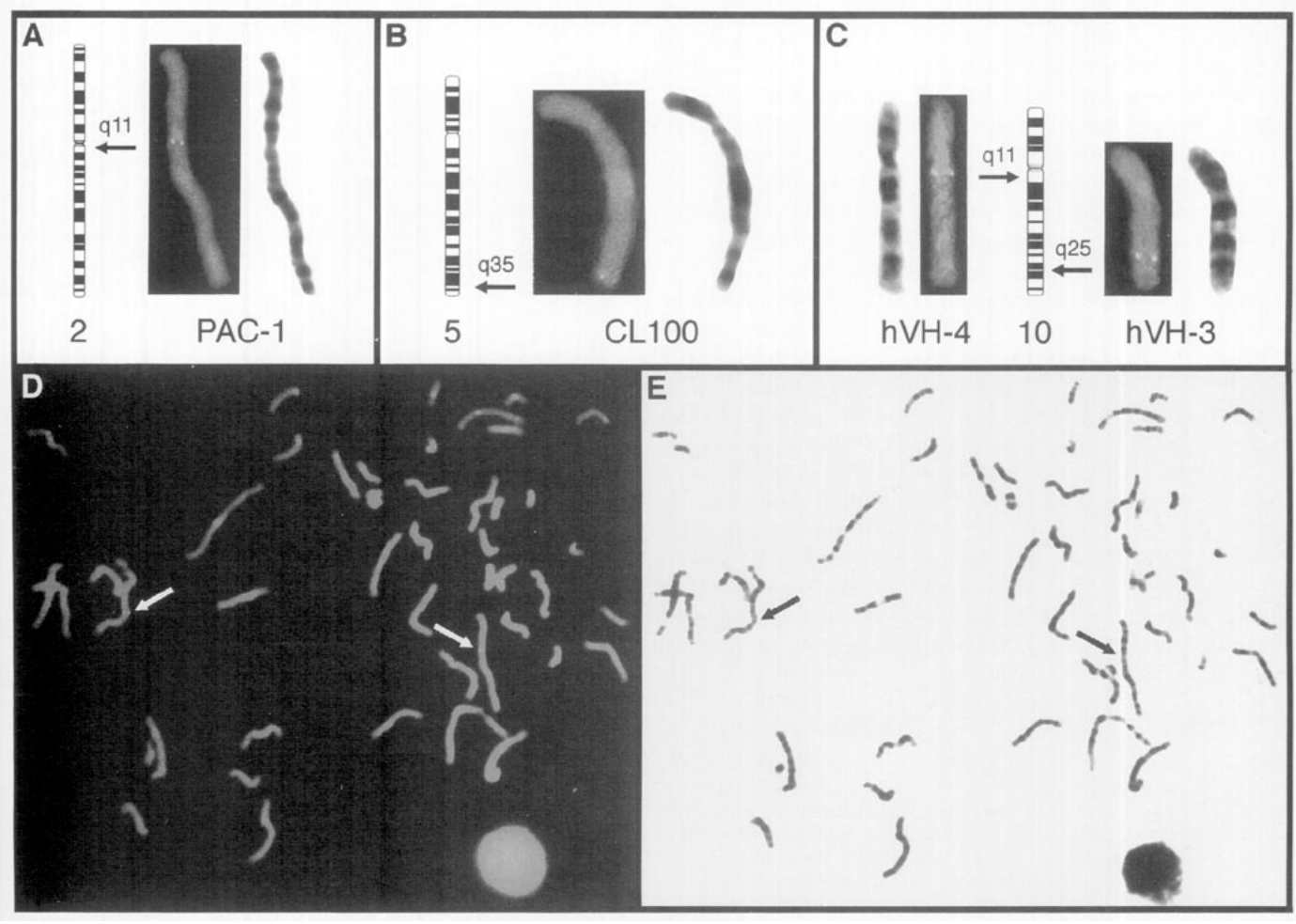

FIG. 2. Illustration of FISH localizing all four VH1-like phosphatases. (**A**) Chromosome 2 idiogram (left), labeled chromosome 2 (center), and the identical G-banded chromosome 2 (right) localizing the PAC-1 gene to 2q11. (**B**) Chromosome 5 idiogram (left), labeled chromosome 5 (center), and the identical G-banded chromosome 5 (right) localizing the CL100 gene to 5q35. (**C**) Chromosome 10 idiogram (center) flanked by the FISH-labeled and G-banded chromosomes localizing hVH-3 and hVH-4 genes to 10q25 and 10q11, respectively. (**D**) Fluorescence *in situ* hybridization pattern of biotin-labeled PAC-1 DNA on human metaphase chromosomes. Arrows point to the chromosomes hybridizing with the probe. (**E**) The same metaphase chromosome spread stained with a G-banding technique using trypsin–Giesma. The arrows indicate the 2q11 chromosome segment on the two homologous chromosomes as the site of probe localization.

fluorescence *in situ* hybridization (FISH) for each VH1-like gene. Probes utilized for FISH analysis were cloned λ phage with genomic DNA inserts ≥15 Kb. One microgram of each clone was labeled with biotin and hybridized to human metaphase chromosomes as previously described (16). An average of 15 metaphase cells per VH1-like clone were examined, with individual information on each clone presented in Table 1. In all cases only chromosomes in which both chromatids displayed a signal were included for analysis, making the background hybridization essentially zero. Further, the same cells hybridized for FISH had all been previously G-banded (using trypsin–Giemsa) and photographed to allow direct comparisons of the results and for making band assignments. The results demonstrated that these four VH1-like phosphatases each can be localized to unique regions of three different chromosomes (PAC-1 to 2q11; CL100 to 5q35; hVH-4 to 10q11; and hVH-3 to 10q25); see Fig. 2.

To determine whether the VH1-like phosphatases mapped to chromosomal regions frequently altered in a human malignancy, we compared the chromosomal localization to the information within the Catalog of Chromosome Aberrations in Cancer (18). Three of the four genes mapped within regions frequently involved in recurring chromosome alterations in cancer. PAC-1 (2q11) coincides with a band region frequently involved in translocations in myeloproliferative disorders and in several adenocarcinomas (e.g., kidney, thyroid, and breast cancer). The localization of CL100 (5q35) is coincident with a band region that is very frequently lost in cases of refractory anemia and is also a region frequently lost or translocated in various other adult leukemias and solid tumors. Finally, the localization of hVH-4 to 10q11 is a region associated with multiple endocrine neoplasia syndromes, and this region is also involved infrequently in various adult leukemias. We recognize that mapping a gene to a chromosomal region involved in recurring chromosome alterations does not directly imply a role for that sequence in the etiology of the disorder. However, it remains tempting to speculate that VH1-like phosphatases may play a role in altered growth regulation and, by analogy to other growth regulatory sequences, could be the target of chromosome rearrangements in human malignancies.

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