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Chromosomal localization of the protein tyrosine phosphatase G1 gene and characterization of the aberrant transcripts in human colon cancer cells

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Abstract

We have recently described the isolation of the human PTPG1 gene which encodes a member of intracellular protein tyrosine phosphatases that may be candidates for tumor suppressor genes. In order to investigate the abnormality of the PTPG1 transcript in various human cancer cell lines, we have analyzed the consensus catalytic region of PTPG1 cDNA, using the reverse transcriptionpolymerase chain reaction. In a colorectal carcinoma cell line, DLD-1, we found three aberrant transcripts. Sequencing analysis revealed that one had a missense point mutation and the remainders contained 77 bp and 173 bp deletions, respectively. These alterations might directly affect their phosphatase activities. Our findings provide the first evidence for the aberrant transcripts of the protein tyrosine phosphatase in human cancer cells, and suggest that the aberration of PTPG1 gene might be involved in the tumororigenesis. Moreover, the human PTPG1 gene is localized on chromosome 7q11.23, a region with frequent abnormalities implicated in some human cancers.

Key words: Protein tyrosine phosphatase; Point mutation; Alternative splicing; Human chromosome 7

1. Introduction

Protein tyrosine phosphatases (PTP) and protein tyrosine kinases (PTK) are involved in the regulation of tyrosine phosphorylation-mediated signaling. Such signaling is critical for the regulation of cell proliferation, differentiation and neoplastic transformation [1,2]. Tyrosine phosphorylated proteins can be specifically dephosphorylated through the action of PTP's [3–5], which are therefore likely to have as important a role as PTKs in the control of cellular growth and differentiation. Furthermore, given that hyperphosphorylation of protein tyrosine residues can cause cell transformation, it is plausible that lack of dephosphorylation resulting from loss of a PTP function may also wreak an oncogenic effect. Indeed, various lines of evidence have linked PTPs to cell cycle regulation, cell proliferation, and malignant transformation [6–9]. These studies suggest that PTPs may be candidates fr tumor suppressor genes.

We have previously isolated a human PTPG1 cDNA encoding a 88 kDa protein tyrosine phosphatase from an adult colon tissue cDNA library [10] and the other group have recently reported isolation of the virtually identical gene from HeLa cell [11]. PTPG1 belongs to a subfamily of intracellular PTPs and is characterized by following features: a long non-enzymatic domain located at the C-terminus which includes PEST sequences encoding proteins of short half-life in eukaryotes [12]; and some potential phosphorylation sites for cdc2 kinase, casein kinase II and double-stranded DNA-dependent kinase. The existence of the latter suggests that PTPG1 is regulated through phosphorylation in the cell cycle or by some mitogenic stimulation [10].

Cytogenetic studies of cancer cells have revealed an association between the accumulation of several genetic changes and the tumorigenic process [13]. In view of the potential importance of PTPs in tumorigenesis, we have used RT-PCR to investigate whether the PTPG1 gene could be altered in a tumor-associated manner. Here we report the presence of aberrant transcripts of PTPG1 in colon carcinoma cells and the characterization of its structure. In addition, we report that the chromosomal locus of the PTPG1 gene was mapped to a region of human chromosome 7q11.23.

2. Materials and methods

2.1. Cells and cultures

All of the cell lines used for these studies were obtained from the Japanese Cancer Research Resources Bank (Tokyo). The cells were maintained in RPMI1640 containing 10% FCS, with the exception of

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Abbreviations: PTP, protein tyrosine phosphatase; PTK, protein tyrosine kinase, RT-PCR, reverse transcription-polymerase chain reaction; cDNA, complementary DNA.

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those noted. Colo320DM, WiDr, BM314, HeLa, CV-1 and NIH3T3 were maintained in Dulbecco's modified MEM containing 10% FCS, and SW948 and SW1474 were in L15 containing 10% FCS. The distinct clones of DLD-1 were isolated by selecting colonies growing in soft agar, as described previously [14].

2.2. Isolation of RNA and Northern blot analysis

Total RNA was prepared from human cell lines by the procedure of Chomczynsky and Sacci [15]. About 10 μg of total RNAs were electrophoresed in 1.0% agarose formaldehyde gels and blotted onto nitrocellulose filters. The filters were hybridized overnight at 42°C with a ^{32}P-labelled full-length cDNA probe, as previously described [10].

2.3. Polymerase chain reaction, subcloning and sequencing of PTP domain

Total RNA from various human cell lines were converted to double-stranded cDNA with random hexamers and Moloney murine leukemia virus reverse transcriptase. These cDNAs were used as templates for in vitro amplification with *Taq* DNA polymerase and PTPG1 specific oligonucleotides (5′-AATGGGGAGGACAACTTCG-3′ as a sense primer, 5′-TTGGGCAATAGCTCTATGAAC-3′ as an anti-sense primer) and β-actin specific oligonucleotides (5′-CTGTCTGGCGGCACCACCAT-3′ as a sense primer, 5′-GCAACTAGTCATAGTCCGC-3′ as an anti-sense primer). The PCR was carried out under the following conditions: denatured at 94°C for 1 min, annealed and extended at 60°C for 1 min; 30 cycles for PTPG1; 20 cycles for β-actin. Fragments of approximately 800 bp, which cover the whole catalytic domain of PTPG1, were isolated from a low-melting-point agarose gel, subcloned into pBlueScript SK$^-$ plasmid (Stratagene) and sequenced. The nucleotide sequence was determined in both directions by the chain termination method with a Sequenase kit using 7-deaza-2′-deoxy-CTP (USB). In DLD-1, the PCR reactions were independently performed twice and sequenced at least three clones from each reaction for any DNA fragment to ensure against reverse trandscription or *Taq* polymerase artifacts.

2 4. Southern blot analysis

Genomic DNAs from various cell lines and tissues were extracted by proteinase K digestion, phenol–chloroform extraction and ethanol precipitation. Ten μg of genomic DNA was digested with the restriction endonucleases *Bcl*I, *Bsa*HI, *Hin*fI, *Pst*I and *Bam*HI. The blot was hybridized to the ^{32}P-labelled 0.6 kb cDNA fragment from 5′ side clone, λG1-C2 [10], or 3.0 kb of full-length PTPG1 cDNA and subjected to autoradiography for two days.

2.5. Expression of the PTPG1 protein and the antibody preparation

GST-PTPG1 constructs were generated in order to express the PTPG1 in *E. coli*. The N-terminal 1.5 kbp *Eco*RI fragment (corresponding to amino acids 1–493), or the C-terminal 1.4 kbp *Eco*RI fragment (corresponding to amino acids 515–780) was fused in frame to the glutathione-*S*-transferase gene in the pGEX vector. The fusion proteins were purified by affinity chromatography on a glutathione column [16]. Each of the purified fusion proteins (400 μg per rabbit) was injected subcutaneously with complete Freund adjuvant into two female New Zealand rabbits. Booster immunizations were given at 2-week intervals with 200 μg of the same preparation in incomplete Freund adjuvant. After the third boost, the IgG fraction was prepared from antisera using Wattman DE52 gel ion-exchange chromatography.

2.6. Immunoblotting and immunoprecipitation

The cells were washed with ice-cold phosphate-buffered saline and immediately lysed in lysis buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1% NP40, 0.1% sodium deoxycholate, 4 mM EDTA, 10 mM NaF, 10 mM $Na_4P_2O_7$, 2 mM Na_3VO_4, 1 mM PMSF, 1 μg/ml leupeptin, 10 μg/ml soybean trypsine inhibitor, 10 μg/ml tosyl phenylalanine chloromethyl ketone, 1 μg/ml aprotinin) at 4°C for 30 min. After removal of cell debris by centrifugation (12,000 × *g*, 30 min), lysates were immediately boiled for 5 min in sample buffer. For immunoblotting, protein (150 μg) from each lysate was fractionated on 10% polyacrylamide gels, transferred to a PVDF filter (Immobilon-P, Millipore), and then probed with the appropriate antibody. After three washes with TBST (20 mM Tris, pH 7.5, 150 mM NaCl, 1% Tween 20), PTPG1 was visualized by incubation of the immunoblot with alkaline phosphatase-conjugated anti-rabbit antibody (Sigma) followed by 5-bromo-4-chloro-3-indoyl-phosphate and Nitroblue tetrazolium treatment.

For immunoprecipitation, K562 cells were labeled for 4 h in methionine-free RPMI1640 containing 1 mCi of [^{35}S]methionine (100 μCi · ml^{-1}, 1200 Ci · mmol^{-1}; NEN). The labeled cells were lysed at 4°C in 1 ml of lysis buffer. The lysates were centrifuged at 12,000 × *g* for 30 min at 4°C, and aliquots of the supernatants were used for immunoprecipitation 8 μg of the antibody was added to the labeled cell lysates and incubated for 1 h at 4°C. The immune complexes adsorbed to protein G-Sepharose were washed extensively with lysis buffer, and then analyzed by 10% SDS-polyacrylamide gel electrophoresis (SDS-PAGE) followed by fluorography.

2.7 Chromosomal mapping

Normal female metaphase chromosomes were obtained by thymidine synchronization bromo-deoxyuridine release. The probe, pG1, containing a 3.0 kb fragment of the full-length human PTPG1 cDNA, was labeled by nick translation. Fluorescence in situ hybridization was performed as described by Pinkel et al. [17]. The fluorescent signals of hybrid probes were amplified with avidin-fluorescein isothiocyanate (Boehringer, Mannheim, Germany) and biotynylated anti-avidineD (Vector, Burlingame, CA) according to the same protocols [17].

3. Results

3.1. Expression of aberrant PTPG1 transcripts in cancer cells

We performed reverse transcription-polymerase chain reaction (RT-PCR) to investigate whether abnormal transcripts of PTPG1 gene can be found in human cancer cells. The sequence of the 3.0 kb cDNA encoded by the human PTPG1 gene had already been determined [10]. A pair of oligonucleotide primers that amplified the region encompassing the phosphatase domain coding sequence, corresponding to nucleotides 86–895, were synthesized. Fig. 1 shows the primers and the RT-PCR products generated from various human cancer cell lines. Using the primers, we detected a single band in all human cancer cell lines except for a colon cancer line, DLD-1. Interestingly, we found three products from DLD-1 mRNA, one of the expected size (approximately 810 bp, named DC1), a second, less abundant fragment, of about 730 bp (named DC2) and a third, equally abundant fragment, of about 630 bp (named DC3) (Fig. 1a). Thus, DLD-1 cells appear to contain three forms of the PTPG1 transcript, suggesting that the PTPG1 gene of DLD-1 may encode some abnormal phosphatase domains.

Since a previous report demonstrated that DLD1 cells were heterogenous and could be divided into two clones, A and D [14], we tested whether these aberrations occurred in both clones after subcloning of DLD-1 cells. The aberrant transcripts shown in Fig. 1 were subsequently detected in both cloned lines (data not shown); however, no significant abnormalities could be found by Northern blot analysis using total RNA (data not shown).

3.2. Nucleotide sequences of the aberrant transcripts in DLD-1 cells

To characterize the primary structure of the aberrant

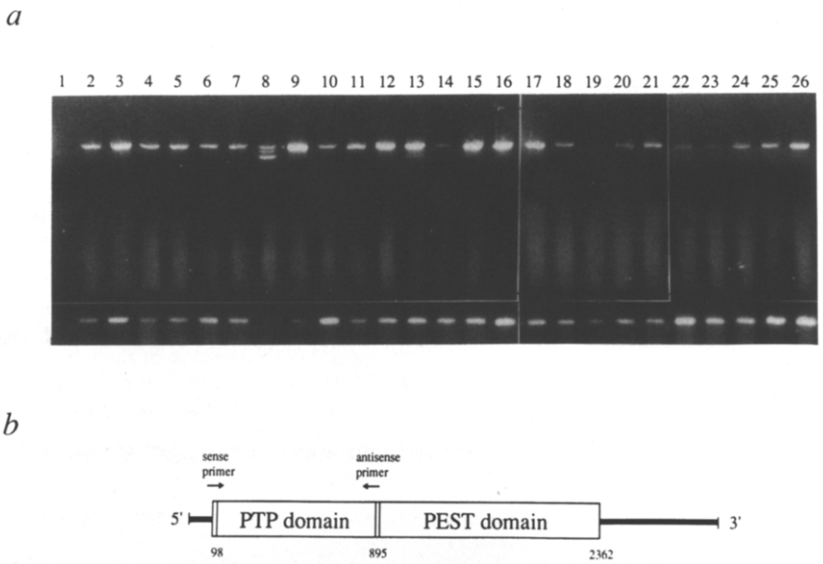

Fig. 1. (a) Expression of PTPG1 mRNA in various cell lines and aberrant PTPG1 transcripts in the phosphatase domain from DLD-1. One μg of total RNA extracted from cells was used for RT-PCR. β-Actin mRNA was used as an internal control. The sizes of PCR products are indicated on the right. Analyzed cell lines are as follows: (1) negative control; (2) Colo38; (3) Colo201; (4) Colo320DM; (5) Colo320HSR; (6) SW948; (7) SW1474; (8) DLD-1; (9) WiDr; (10) CHCY1; (11) BM314; (12) KATOIII; (13) MKN1; (14) MKN28; (15) MKN74; (16) NUGC3; (17) HUH7; (18) CHC4; (19) CHC32; (20) HLE; (21) Chang; (22) Molt3; (23) Molt4; (24) HL60; (25) Daudi; (26) K562. (b) Positions of the primers used are shown above a schematic diagram of the predicted protein structure of normal PTPG1. Numbers refer to nucleotide positions of domain bounderies.

transcripts in DLD-1 cells, each of the PCR fragments from DLD-1 was subcloned and sequenced. Sequencing analysis revealed that the DC1 transcript contained a single-nucleotide change at positions 201 (A-to-G) (Fig. 2a). This point-mutation resulted in a single amino acid substitution, or a missense mutation at codon 61 (lysine-to-arginine). DC2 and DC3 transcripts did not contain the point-mutations, but had deletions of 77 bp and 173 bp, respectively, corresponding to nucleotides 228–304 and 228–400 of the authentic PTPG1 gene transcript (Fig. 2b). Each of the deletions resulted in a frame shift, and a termination codon (TAA or TGA) was found at

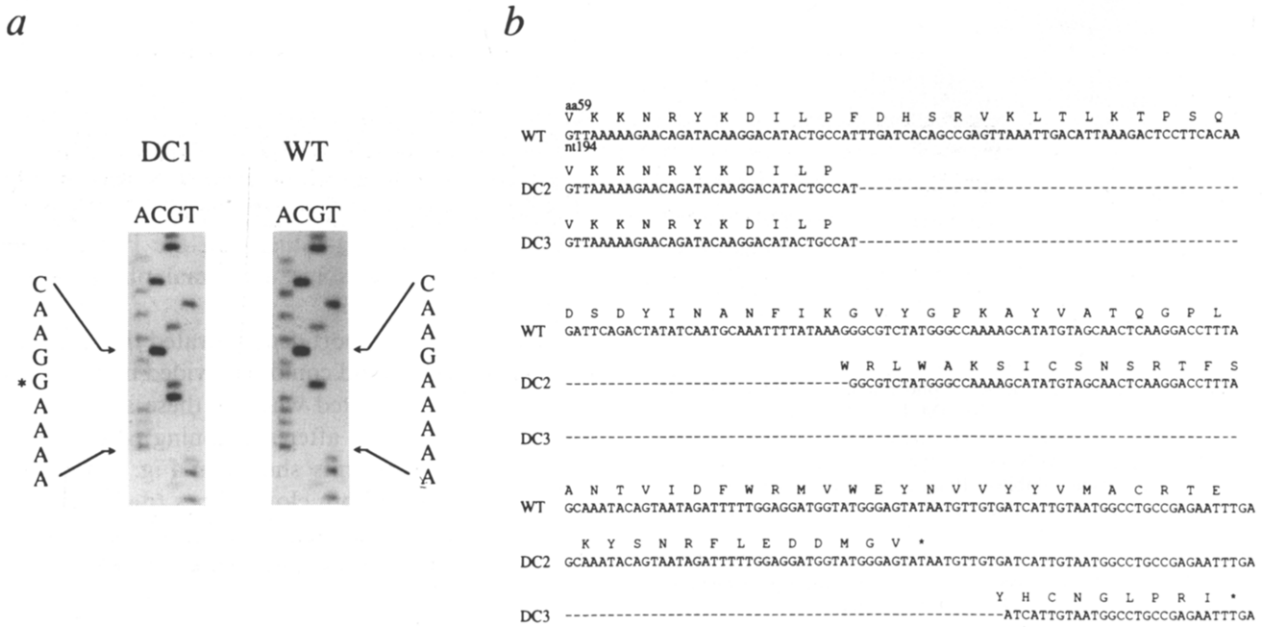

Fig. 2. Abnormalities of PTPG1 transcripts encompassing the phosphatase domain in DLD1 cells. (a) Sequencing analysis of the DC1 fragment from DLD1 and wild-type (WT) PTPG1 cDNA. The asterisk marks the A-to-G substitution in the DC1 sequence. Sequence from DC1 RT-PCR product show a single-nucleotide change at position 201. (b) Comparison of the nucleotide (nt) and predicted amino acid (aa) sequences containing a portion of the phosphatase domain in PTPG1 of wild-type, DC2 and DC3. Sequences from DC2 and DC3 show 77 bp and 173 bp deletions, respectively.

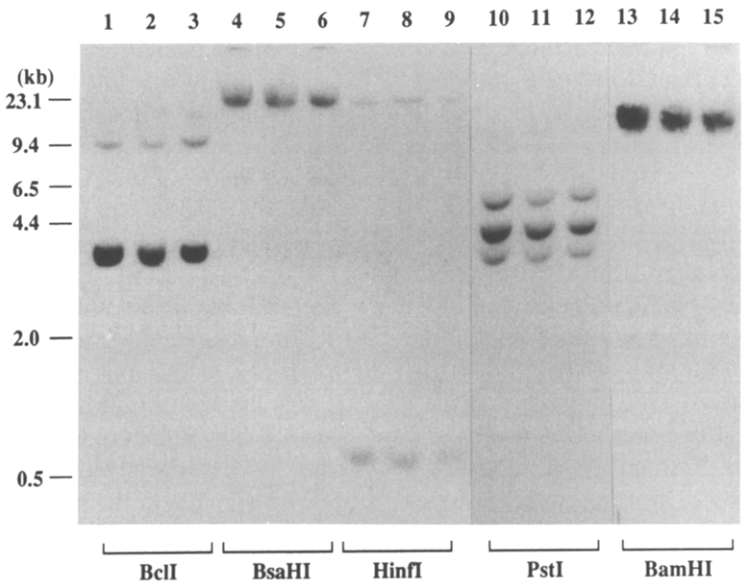

Fig. 3. Southern blot analysis of the PTPG1 gene. Ten μg of genomic DNA from normal colon tissues (lanes 1, 4, 7, 10 and 13). COLO320DM (lanes 2, 5, 8, 11 and 14) and DLD-1 (lane 3, 6, 9, 12 and 15) was hybridized with a 0.6 kb fragment from λG1-C2. DNA was digested with *Bcl*I (lanes 1–3), *Bsa*HI (lanes 4–6), *Hin*fI (lanes 7–9), *Pst*I (lanes 10–12) and *Bam*HI (lanes 13–15). Numbers indicate the size of DNA markers in kb.

a position 391 (for DC2) or 427 (for DC3) of the authentic cDNA. Therefore, the smaller PTPG1 transcripts, DC2 and DC3, may encode the truncated PTPG1 proteins with additional 29 and 9 amino acids following amino acid no. 69, respectively (Fig. 2b).

3.3. *Southern blot analysis of the PTPG1 gene*

Southern blot analysis of genomic DNA was performed to confirm whether aberrant PTPG1 transcripts in DLD-1 cells are ever produced by rearrangement of PTPG1 gene. The 0.6 kb fragment of PTPG1 cDNA (see section 2), which covered the deleted regions, was used as a probe. The sizes of the resulting bands were the same among the DNAs from DLD-1, COLO320DM and a normal colon tissue when digested with some restriction enzymes whose sites were located in the deleted regions or beside them (Fig. 3). No rearrangement or amplification of PTPG1 gene was detected when the full-length of

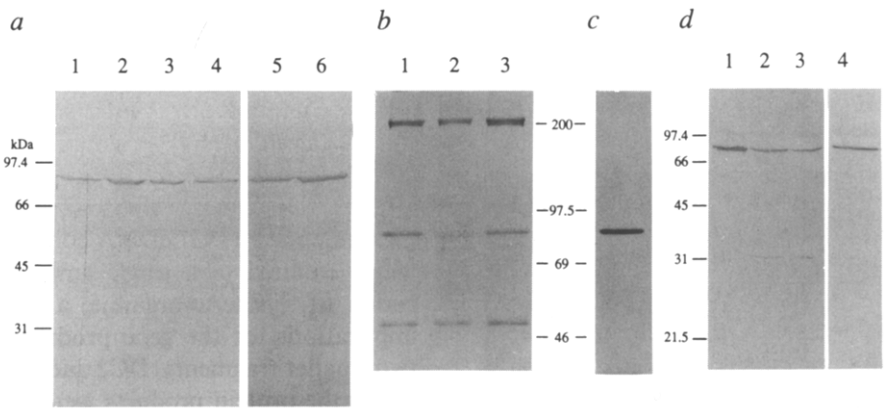

Fig. 4. Characterization of polyclonal antibodies to PTPG1 and the immunoreactive protein in various cell lines. Total cellular protein lysates from various cell lines were prepared and examined for PTPG1 protein expression by Western blotting and immunoprecipitation. The positions of protein markers are indicated in kilodaltons. (a) Cell lysates from HeLa (lanes 1 and 5), K562 (lanes 2 and 6), CV-1 (lane 3), NIH3T3 (lane 4) were separated by SDS-polyacrylamide gel electrophoresis, blotted to PVDF filter, and detected using the anti-PTPG1 N-terminal antibody (lanes 1–4) or anti-PTPG1 C-terminal antibody (lanes 5 and 6). (b) Immunoprecipitation of PTPG1 from ^{35}S-labeled cell extracts of K562 (lanes 1–3). Cells were labeled for 4 h with [^{35}S]methionine and then lysed in lysis buffer. The PTPG1 proteins were immunoprecipitated with antibodies against GST-PTPG1-N and analyzed by SDS-PAGE followed by fluorography. Antibodies were blocked by preincubation with the fusion polypeptides used for immunization (lane 2) or GST alone (lane 3). (c) Immunoblotting of PTPG1 protein immunoprecipitated from lysates of K562. Immunoprecipitated PTPG1 proteins were separated on SDS-PAGE, and then detected by imunoblotting using anti-PTPG1 N-terminal antibody. (d) Western blot analysis of truncated PTPG1 protein in DLD1 cells. The extract of K562 cells was electrophoresed as a control (lane 1). The truncated PTPG1 proteins were detected at 32 kDa in both clones of DLD1 (lane 2; clone A, lane 3; clone D), using anti-PTPG1 N-terminal antibody. Whereas the proteins of 32 kDa were not detected in DLD-1 using anti-PTPG1 C-terminal antibody (lane 4).

PTPG1 cDNA was used as a probe (data not shown). These results suggest that structural rearrangement of the PTPG1 gene does not occur in DLD-1 cells, although further genomic analysis of PTPG1 will be required to confirm it.

3.4. *Abnormal expression of the PTPG1 gene products in DLD-1 cells*

We raised polyclonal antibodies against two bacterial fusion proteins, the N-terminal half (amino acids 1–493) and the C-terminal half (amino acids 515–780) of the PTPG1 protein, to examine the translation products of PTPg1 directly. First, Western blot analysis was performed to test whether these two antibodies specifically detected the PTPG1 protein. Both antibodies detected a single molecular species of 88 kDa from extracts of various cell lines (Fig. 4a), which was equivalent with that predicted from PTPG1 cDNA nucleotide sequence [10].

To further confirm the specificity of the antibodies, we examined whether they could immunoprecipitate the PTPG1 proteins. Antibodies against the N-terminal region precipitated the 88 kDa protein from extracts of K562 cells labeled with [^{35}S]methionine (Fig. 4b). Furthermore, recognition of the 88 kDa band was strongly blocked by preincubation of the antibodies with an excess of the fusion protein used for immunization, glutathione-*S*-transferase-PTPG1-N-terminal (GST-PTPG1-N), but not by preincubation with the glutathione-*S*-transferase (GST) protein alone (Fig. 4b). Several other bands seen in Fig. 4b were non-specific, since these were not found when immunoprecipitated proteins were analyzed by immunoblotting with the same antibody (Fig. 4c). These results indicate that these antibodies are specific to the PTPG1 protein.

Western blot analysis was carried out to characterize the protein product of the PTPG1 gene in DLD-1 cells. K562 cells express a single molecular species of 88 kDa, whereas two immunoreactive bands of 88 kDa and 32 kDa, respectively, were detected in lysates from DLD-1 cells by using the antibodies against the N-terminal region (Fig. 4d). While the antibody against the C-terminal region was used as the probe, the band of 88 kDa, but not the band of 32 kDa, was detected in DLD-1 cells (Fig. 4d). These data suggest that the C-terminal truncated PTPG1 protein may be expressed in DLD-1.

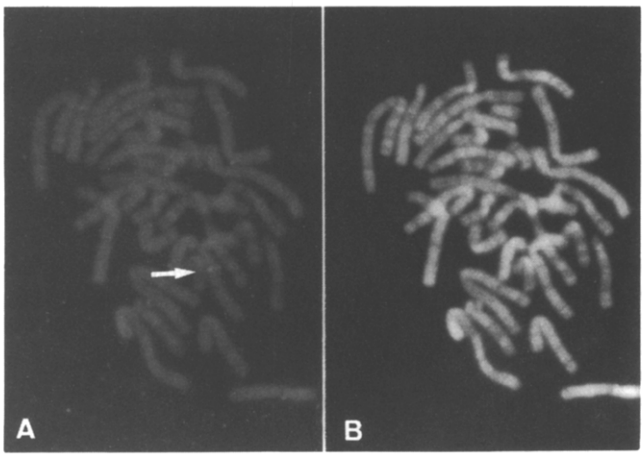

Fig. 5. (A) Fluorescence in situ hybridization of the PTPG1 gene. Double fluorescent spots were detected on R-banded chromosome 7 at the q11.23 region. (B) The same metaphase chromosomes after G-banding.

3.5. *Chromosomal mapping of the human PTPG1 gene*

Fluorescence in situ hybridization was performed using pG1 containing the 3.0 kb PTPG1 cDNA [10] to determine the chromosomal localization of the PTPG1 gene. When 50 metaphase spreads were examined, 42 of them (84%) showed either single or double spots of fluorescent signals on the proximal portion of the long arm of human chromosome 7 (Fig. 5A).. No constant fluorescence signal was seen with any other chromosomes. By comparison with the G-banding patterns (Fig. 5B), the human PTPG1 gene was assigned to chromosome 7q11.23.

4. Discussion

The human PTPG1 gene is one of the intracellular protein tyrosine phosphatases, which are believed to play a crucial role in signal transduction [10,11]. Various lines of evidece suggest that PTP may act as a tumor suppressor gene [18–21]. We have previously shown that PTPG1 mRNA was expressed in various cell lines using Northern blot analysis [10]. RT-PCR was employed here to further investigate the alterations of the PTPG1 expression in human cancer. We found the aberrant PTPG1 transcripts in colon cancer cells to reveal its primary structure. It is of interest that three altered transcripts (here designated DC1, DC2 and DC3) were detected within the phosphatase consensus region of PTPG1 cDNA from DLD-1 cells.

The DC1 transcript whose size was equal to that from the authentic PTPG1 cDNA contained a point-mutation which resulted in a single amino acid substitution at codon 61, lysine-to-arginine, a finding that may have implications for the gene product function. The order two smaller fragments, DC2 and DC3, had deletions for which the protein products were predicted to be out of frame and prematurely truncated in the phosphatase domain. Interestingly, in both cases of DC2 and DC3, the deletions of these two transcripts started at the same nucleotide 228. In addition, no gross rearrangement or amplification of PTPG1 gene could be found in DLD-1 by Southern blot analysis using the cDNA probes. These results raise the possibility that the truncated transcripts are generated by abnormal splicing, rather than by deletion or recombination. Since point-mutations revealed in the DC1 were not detected in DC2 or DC3, the latter two

transcripts may originate via abnormal splicing in one allele and DC1 transcript in the other. Interestingly, recent studies have demonstrated that point-mutations around the splice junctions in the murine protein phosphatase gene, HCP, leading to abnormal splicing in the phosphatase domain and the src homology 2 domain, which are associated with severe combined immunodeficiency and systemic autoimmunity in moth-eaten and visable moth-eaten mice [22,23]. Furthermore, mutations at analogous splice site positions in some tumor suppressor genes, including p53, APC, MCC, etc., have been shown to alter RNA processing in vivo and in vitro [24–26]. Actually, a sequence at the end of the deleted region in DC2 is $A_{-3}A_{-2}G_{-1}G_{-1}$, which matches the conserved 3′ splice site consensus sequence (C/$T_{-3}A_{-2}G_{-1}G_{+1}$) at three out of four positions [27], and therefore potentially acts as the appropriate sequence context for use of the AG dinucleotide as a 3′ acceptor site. It is plausible that DLD-1 could have other abnormalities associated with the splicing signal sequences, such as mRNA species missing the 77 bp or the 173 bp segment.

The truncated proteins translated from aberrant transcripts DC2 and DC3 would be expected to consist of 98 or 78 amino acids. To confirm their expression at the protein level, Western blot analysis was performed. Two immunoreactive bands of 88 kDa and 32 kDa were detected in lysates from DLD-1 cells, using antibodies against the N-terminal region, whereas the 32 kDa band was not detected in lysates from control cells or using the antibodies against the C-terminal region. These results suggest that the C-terminal truncated PTPG1 protein of 32 kDa could be expressed in DLD-1. Although we cannot exclude the possibility that the 32 kDa band is seen by cross-reaction of the antibodies because the sequence predicted 98 or 78 amino acid polypeptides with predicted molecular sizes of approximately 10 kDa, there appears to be at least two possibile explanations for this discrepancy: (i) mobility shifts resulted from a post-translational modification such as phosphorylation, glycosylation, etc.; or (ii) additional abnormal polypeptides translated at the further upstream site of the PCR-amplified region. Further studies on the protein alterations of PTPG1 in DLD-1 must be required to confirm it.

The human colon carcinoma cell line DLD-1 was established from a moderately to poorly differentiated adenocarcinoma of the sigmoid colon [14]. It was previously indicated that both the DLD-1 cell line and the original tumor were heterogeneous and the former could be divided into two clones, A and D. Clone A is characterized by poorly differentiated histology, hyperploid karyotype and fuzzy star-shaped colony formation in soft agar, and clone D, by moderately differentiated histology, diploid karyotype and small spheroid colony formation in soft agar [14]. Since aberrant trandscripts and C-terminal truncated protein were detected in both clones, genetic alterations might occur in the original primary tumor as somatic mutations. The pathological meaning of the abnormal transcripts is still unclear, but if alterations in enzymatic activity among them exist, the regulation of certain specific substrates participating in molecule to molecule interaction might be disturbed. Defective PTPG1 expression in cancer cells would be expected to result in altered levels of phosphorylated tyrosine residues, which might be involved in tumorigenesis.

Fluorescence in situ hybridization using PTPG1 cDNA revealed that the human PTPG1 gene is located at 7q11.23. This region is of particular interest because it is a common site of chromosomal rearrangements in malignant melanoma [28,29] and resides near the recurrent breakpoints in some hematopoietic disorders including acute lymphoblastic leukemia, malignant lymphoma, etc. [30]. Moreover, this locus is known to be aphidicolin-induced fragile sites, which are frequently located at or near breakpoints of chromosomal rearrangements found in tumor cells [31]. Allelic deletions of 7q have been reported in some solid tumors including colorectal carcinomas [32,33]. Interestingly, the gene for Zellweger syndrome, an autosomal recessive disorder that affects generation of the peroxisomal membrane, has been located in the same region [34,35]. Abnormalities of this locus may be associated with these disorders as a result of the alteration of the PTPG1 gene. Recently, a human transmembrane PTPζ was assigned to chromosome 7q31-33 [36], suggesting that there may be a family of PTPs on chromosome 7.

A previous study revealed that abnormal sized mRNAs of a transmembrane PTPγ were expressed in a human lung cancer cell line and a osteosarcoma cell line using Northern blot analysis [37]. However, RNase protection assay and PCR-SSCP of the cytoplasmic region which contains phosphatase domains showed no nucleotide changes in these cancer cell lines [37]. Therefore, the colon carcinoma cells described here eppear to be first known case of a human tumor in which the protein tyrosine phosphatase is altered at the nucleotide sequence and protein levels. Analysis of the altered PTPG1 gene may prove helpful in studying the function of the normal gene product. However, the molecular genetic events that have occurred during the formation of tumor are now providing a new framework within which to consider the etiology of human cancer. Our results raise the possibility that the PTPG1 gene is a target of the mutation in colorectal carcinoma. Further mutational analysis in clinical primary tumors as well as experiments to evaluate the biological effect of altered PTPG1 genes on the colorectal tumor cells will be required.

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