Molecular Cloning of a Human Transmembrane-type Protein Tyrosine Phosphatase and Its Expression in Gastrointestinal Cancers*

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Takashi Matozaki, Toshiya Suzuki, Tohru Uchida, Johji Inazawa, Takeshi Ariyama, Kohei Matsuda, Kazutsugu Horita, Hitoshi Noguchi, Hisashi Mizuno, Choitsu Sakamoto, and Masato Kasuga

From the Second Department of Internal Medicine, Kobe University School of Medicine, Kusunoki-cho, Chuo-ku, Kobe 650 and the Department of Hygiene, Kyoto Prefectural University of Medicine, Kamigyo-ku, Kyoto 602, Japan

To determine the expression of various protein-tyrosine phosphatases (PTPs) in human gastric cancers, cDNAs encoding conserved PTP domains were amplified by reverse transcriptase polymerase chain reaction from KATO-III cell mRNA and sequenced. Among 72 polymerase chain reaction clones, one of the cDNA sequences encoded a novel potential PTP (stomach cancer-associated PTP, SAP-1). The full length (3.9 kilobases) of the SAP-1 cDNA was further isolated from the KATO-III cell cDNA library and the WiDr cell cDNA library. The predicted amino acid sequence of the SAP-1 cDNA showed that mature SAP-1 consisted of 1093 amino acids and a transmembrane-type PTP, which possessed a single PTP-conserved domain in the cytoplasmic region. The extracellular region of SAP-1 consisted of eight fibronectin type III-like structure repeats and contained multiple N-glycosylation sites. These data suggest that SAP-1 is structurally similar to HPTPβ and that SAP-1 and HPTPβ represent a subfamily of transmembrane-type PTPs. SAP-1 was mainly expressed in brain and liver and at a lower level in heart and stomach as a 4.2-kilobase mRNA, but it was not detected in pancreas or colon. In contrast, among cancer cell lines tested, SAP-1 was mainly expressed in pancreatic and colorectal cancer cells. The bacterially expressed SAP-1 fusion protein had tyrosine-specific phosphatase activity. Immunoblotting with anti-SAP-1 antibody showed that SAP-1 is a 200-kDa protein. In addition, transient transfection of SAP-1 cDNA to COS cells resulted in the predominant expression of a 200-kDa protein recognized by anti-SAP-1 antibody. SAP-1 is mapped to chromosome 19 region q13.4 and might be related to carcinoembryonic antigen mapped to 19q13.2.

The phosphorylation of tyrosine residues of protein is a crucial event in the regulation of normal cellular processes such as proliferation and differentiation and is also involved in the malignant transformation of cells (1, 2). In gastric cancers and colon cancers, it has been shown that the expression of genes encoding growth factor receptors such as erbB2 (3) and K-sam (4), a gene encoding a fibroblast growth factor receptor family, is elevated (5). In addition, the level of tyrosine phosphorylated proteins is increased in gastric cancer cells (6). Since the level of tyrosine phosphorylation is determined by the balance between the actions of both protein-tyrosine kinases and PTPs (7, 8), not only the unregulated activation of protein-tyrosine kinases but also the inactivation of PTPs may be involved in the malignant transformation of gastrointestinal cells. In fact, it has been suggested that the inactivation of HPTPβ may be involved in the pathogenesis of small cell lung cancer, since the deletion of chromosome 3p21, where HPTPβ is mapped, is often associated with this type of cancer (9). However, a recent study (10) has indicated that the contribution of PTPs to tumor suppression may not be simple, since it has been shown that the overexpression of HPTPα, a transmembrane-type PTP, induces cell transformation through the dephosphorylation of pp60c-src (10), suggesting that malactivation of PTP may be one of the steps to oncogenic transformation. Thus, various types of PTPs may be involved in the oncogenesis of various cancers in different manners. Since little is known of the role of PTPs in the pathogenesis of gastric cancers, we have studied the PTPs expressed in a gastric cancer cell line, KATO-III, by PCR amplification of cDNAs with oligonucleotide primers to the conserved regions of the known PTPs. Among PCR clones amplified from KATO-III cDNA, we have identified a gene encoding a novel transmembrane-type PTP, SAP-1 (stomach cancer-associated PTP). This PTP is a 200-kDa glycosylated protein with a single PTP catalytic domain in the cytoplasmic region and FN type III-like structures in the extracellular region. It is of interest that this PTP is highly expressed in colon cancers and pancreatic cancers but not in the corresponding normal tissues. Further, we have shown that human SAP-1 is located on the long arm of chromosome 19 at 13.4.

MATERIALS AND METHODS

Cloning of cDNAs for Human SAP-1—PCR amplification of cDNA was used to obtain portions of potential PTPs with PCR primers to conserved sequences of known PTPs, as described previously (11, 12). Briefly, total RNA was extracted from KATO-III by the standard guanidium thiocyanate/cesium chloride method (13). Complementary DNA (cDNA) was then synthesized by incubating ~2 μg of total RNA of KATO-III with 100 pmol of an antisense PCR primer and 60 units of avian myeloblastosis virus reverse transcriptase (Boehringer

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EMBL Data Bank with accession number(s) D15049.

‡ To whom correspondence should be addressed. Tel.: 011-81-78-341-7451 (ext. 5226); Fax: 011-81-78-382-2080.

1 The abbreviations used are: PTP, protein-tyrosine phosphatase; Ab, antibody; PCR, polymerase chain reaction; GST, glutathione S-transferase; pNPP, p-nitrophenyl phosphatase; FN, fibronectin; MES, 4-morpholineethanesulfonic acid; SAP, stomach cancer-associated PTP; CEA, carcinoembryonic antigen; kb, kilobase(s).
Mannheim) for 60 min at 42 °C (13). PCR amplification was performed in a 100-μl reaction volume using one-tenth of the cDNA and 100 pmol of each oligonucleotide primer for 30 cycles, as described (13, 14). Each cycle included denaturation at 94 °C for 1.5 min, annealing at 37 °C for 3 min, and primer extension at 72 °C for 3 min. A 5′-PCR primers were 5′-CGCGAGCTCATGCGAGCCAAATCCCGACTGCAGTG, respectively. The primers containing flanking sequences for EcoRI or PstI sites to facilitate subsequent cloning. The PCR products were then digested with EcoRI and PstI, ligated to pUC 19 (Takara), and sequenced by dyeoxy termination methods (15) using the Sequase kit US (U.S. Biochemical Corp.). An overnight 72 °C incubation. Among 72 independent PCR clones sequenced, one novel cDNA encoding a PTP domain was isolated (SAP-1). To isolate full-length cDNA of each PTP, a 200-nucleotide cDNA fragment of SAP-1 was excised, labeled with [α-32P]dCTP (3000 Ci/mmol) (Amersham Corp.) by random primer methods (16), and used to screen a cDNA library of KATO-III cells (generously provided by Dr. Terasa, National Cancer Research Institute). Three independent cDNA clones were subcloned to a β-luciferase vector, and the nucleotide sequence was determined in both directions as described above. Because the cDNA clones initially isolated did not contain the complete coding sequence, further cDNA clones were isolated from the cDNA library of WiDr cells, a colon cancer cell line (Japanese Cancer Research Resources Bank) in which SAP-1 mRNA was abundantly expressed.

Northern Blotting—For Northern blotting, poly(A) RNA (2-5 µg) extracted from human organs or cultured cells was electrophoresed on a 12% agarose/formaldehyde gel and transferred to nitrocellulose as described previously (14). The blot was hybridized with a 32P-labeled 3.0-kb fragment of SAP-1 cDNA in 50% formamide, 5× SSPE, 5 mM sodium pyrophosphate, 100 mM NaCl, 100 µM dithiothreitol, and 100 µg/ml salmon sperm DNA overnight at 42 °C. The blots were then washed as described previously (14). The filter was then rehybridized with a 32P-labeled mouse β-actin probe.

Expression and Purification of SAP-1 and PTP Assays—For amplification of cDNA encoding the PTP domain of SAP-1, PCR amplification was performed essentially as described previously (14) by using a sense primer 5′-ATTGGATCCCAGGGGACATCCCGATATCACCATCCCTGTTCCCTCGG-3′ and an antisense primer 5′-AATTCTCTATACGGAATCGTTCTCCCTG-3′ (nucleotides 3310-3333), and a 3-kb SAP-1 cDNA as a template. The amplified PCR fragment was digested with BamHI and EcoRI and inserted into in-frame BamHI and EcoRI sites of pGEX-2T (Pharmacia LKB Bio-technology Inc.). Glutathione S-transferase (GST) fusion protein expression and purification were performed as described previously (14, 17). Fresh overnight culture of Escherichia coli (JM 109) transformed with pGEX-2T or pGEX-2T-SAP1 recombinant was diluted 1:20 in LB medium containing ampicillin (100 µg/ml) and incubated at 37 °C. After a 1.5-h incubation, isopropyl β-D-thiogalactoside was added to a final concentration of 0.1 mM, and this was followed by a 5-h incubation. For fusion protein recovery on glutathione-Sepharose beads (Pharmacia), 10 ml of bacterial culture was centrifuged at 10,000×g for 15 min at 4 °C, and the resultant supernatants were used for immunoprecipitation and immunoblotting. Immunoprecipitation was performed by incubating 500 µl of cell lysate with 5 µg of either anti-SAP-1 Ab or Tween. After centrifugation, the supernatants were subjected to pGEX-2T fusion protein beads (Pharmacia) (20 µl) for 4 h at 4 °C. The beads with immunoprecipitated proteins were washed twice with 1 ml of washing buffer (50 mM Hepes pH 7.6, 150 mM NaCl, 2 mM EDTA, 1% Triton X-100, 0.5% deoxycholate, 0.1% SDS, 10% glycerol) containing 1 mM PMFS and 10 µg/ml aprotinin. The lysates were centrifuged at 10,000 × g for 15 min at 4 °C, and the resultant supernatants were used for immunoprecipitation and immunoblotting. Immunoprecipitation was performed by incubating 500 µl of cell lysate with 5 µg of either anti-SAP-1 Ab or Tween. After centrifugation, the supernatants were subjected to pGEX-2T fusion protein beads (Pharmacia) (20 µl) for 4 h at 4 °C. The beads with immunoprecipitated proteins were washed twice with 1 ml of washing buffer (50 mM Hepes pH 7.6, 150 mM NaCl, 0.1% Triton X-100), and then solubilized in SDS-polyacrylamide gel electrophoresis sample buffer. Gel electrophoresis and immunoblotting were performed with a polyclonal anti-SAP-1 Ab as described previously (14, 17), except that visualization of immunoreactive protein bands was carried out by using ECL detection kit (Amersham Corp.).

Transient Expression of SAP-1 in COS Cells—For transient expression studies, a full-length SAP-1 cDNA was inserted into the EcoRI site of pSRα (originally distributed by Dr. N. Arai). Semiconfluent COS-7 cells in 6-cm dishes were transfected with 3 µg of pSRα containing SAP-1 cDNA, or a vector alone as a control, by the calcium phosphate method (15). After transfection, the cells were cultured in Dulbecco's modified Eagle's medium containing 10% fetal calf serum for 3 days. Cells were then lysed as described above, and lysates were used for immunoprecipitation and immunoblotting.

Chromosomal Mapping of SAP-1 by Fluorescence in Situ Hybridization—Determination of chromosomal location of SAP-1 was performed as described previously (21, 22). Briefly, normal male metaphase chromosomes were obtained by thymidine synchronizing procedure. The chromosomes were obtained by thymidine synchronizing procedure (22). Briefly, normal male metaphase chromosomes were obtained by thymidine synchronizing procedure. The chromosomes were obtained by thymidine synchronizing procedure. The chromosomes were then fixed and denatured in 70% ethanol. The metaphase chromosomes were then debanded with 20% formamide. The metaphase chromosomes were then debanded with 20% formamide. The metaphase chromosomes were then debanded with 20% formamide. The metaphase chromosomes were then debanded with 20% formamide. The metaphase chromosomes were then debanded with 20% formamide.
(3 clones) (28), SAP-2/SHTPTp3 (3 clones) (29), HPTPa (2 clones) (30), HPTPy (4 clones) (30), LAR (3 clones) (31), and a novel putative PTP (SAP-1) (2 clones). The amplified cDNA for SAP-1 detected a 4.2-kb transcript in KATO-III cells and was used to isolate cDNA clones from the KATO-III cell cDNA library (provided by Dr. M. Terada, National Cancer Research Institute). Since cDNA clones initially isolated from the KATO-III cDNA library did not contain the complete coding sequence, further cDNA clones were isolated from the cDNA library of WiDr cells, a colon cancer cell line.

The complete nucleotide sequence of cloned cDNAs extended to 3925 nucleotides and contained a single long open reading frame of 1118 amino acid residues (nucleotides 42–3395) (Fig. 1). The first ATG codon, found in this open reading frame at position 42, matched the Kozak consensus sequence (32) for a translation initiation site. Therefore, the methionine at position 42–44 is presumed to be the translation initiation codon. The 3'-noncoding region contains a typical polyadenylation signal (ATTAAA) followed by a poly(A) tail. The amino-terminal 25 amino acids are highly hydrophobic and are a likely signal peptide (33). The predicted mature SAP-1 protein is composed of a 728-amino acid extracellular domain, a 25-amino acid transmembrane region, and a 340-amino acid cytoplasmic region. The cytoplastic region of the predicted protein sequence contains the conserved sequence motifs found in all PTPs so far (7, 8) (Figs. 1 and 2A).

Comparison of the amino acid sequence of the SAP-1 PTP with other PTPs, HPTPP was found to be most closely related to SAP-1, with 49.6% identity. Furthermore, comparison of nucleotide sequences reveals that the SAP-1 PTP domain is similar to those of human LAR and HPTP, with highest degree of similarity 58.8% and 59.3%, respectively. In contrast to the PTP domain, comparison of each FN III-like repeat of SAP-1 reveals that 24% are similar to HPTP, suggesting that the extracellular domains of SAP-1 and HPTP may be functionally different.

Preferential Expression of SAP-1 Transcripts in Pancreatic and Colon Cancer Cells—To examine the pattern of expression of SAP-1, 3.0-kb SAP-1 cDNA was used as a probe in Northern blotting with various tissues and cultured cell lines. In tissues and cells tested so far, a major transcript of approximately 4.2 kb was observed. Among normal human tissues tested, SAP-1 was not ubiquitously expressed but was primarily present in brain and liver and at a low level in heart and stomach (Fig. 3). In lung, pancreas, colon, placenta (Fig. 3), and peripheral mononuclear cells (data not shown), SAP-1 transcripts were not detected. Although SAP-1 transcripts were readily detected in KATO-III cells, a low level was observed in MKN45 and JR-1 cells, and no detectable level was observed in other gastric cancer cells. In contrast, SAP-1 was highly expressed in two pancreatic cancer cell lines, Panc-1 and MIA-PaCa2, and in three colon cancer cell lines, WiDr, SW837, and COLO205 (Fig. 4). In addition, two other colon cancer cell lines, COLO320 and SW480, showed a detectable level of SAP-1 transcripts (Fig. 4). Among other cells tested, SAP-1 was expressed in the glioblastoma cell line, A172, and at a low level in T97G, another glioblastoma cell line (Fig. 4). In both A431 cells, an epidermoid carcinoma cell line, and ZR-75-1 cells, a breast cancer cell line, a low level of transcripts was observed, while no SAP-1 transcripts were observed in Huh-7, a hepatoma cell line, or in Hela cells. In hematopoietic cells, such as HL-60, SKM, Raji, and SKNO-1, SAP-1 transcripts were not detected (Fig. 4).

SAP-1 cDNA Encodes an Active PTP of 200 kDa—To determine the catalytic activity of SAP-1, the gene encoding a putative PTP domain of SAP-1 was amplified by PCR. The amplified DNA was inserted into the pGEX-2T vector, and then the GST-SAP-1 fusion protein was bacterially expressed; this was followed by purification. As shown in Fig. 5, GST-SAP-1 protein was expressed as a 65-kDa protein, with an apparent molecular mass consistent with the size of the PTP domain encoded by the cDNA insert of SAP-1 plus the 26 kDa contributed by the GST leader sequence. Although GST alone had no PTP activity, the GST-SAP-1 showed a significant PTP activity when either pNPP or 32P-labeled Ray tide was used as a PTP substrate (Fig. 5). To further characterize SAP-1 protein, an affinity-purified polyclonal Ab against the GST-SAP-1 fusion protein was generated. Immunoblotting of WiDr cell lysate with anti-SAP-1 Ab recognized a single protein of 200 kDa (Fig. 6, lane 1). In contrast, SAP-1 protein was not detected in HL-60 cell lysates (Fig. 6, lane 2). These data correspond well with results showing that SAP-1 mRNA was highly expressed in WiDr cells but not in HL-60 cells (Fig. 4). Since the protein product predicted by SAP-1 cDNA would have an apparent M, of 120 after the removal of the signal peptide, the apparent size of SAP-1 seen in immunoblotting suggests a potential post-translational modification. In addition, significant PTP activity against either pNPP or labeled Ray tide was detected in the immunoprecipitates from WiDr cells (data not shown). To further confirm whether the isolated gene encoded SAP-1 protein, we transiently transfected COS-7 cells with a SAP-1 expression vector by the calcium phosphate method. The immunoblotting of transfected COS cell lysates with anti-SAP-1 Ab showed the expression of a 200-kDa protein, the size of which corresponded well to that of the SAP-1 protein detected in WiDr cells (Fig. 6, lanes 3 and 4).

Chromosomal Localization of the Human SAP-1 Gene—To determine the chromosomal location of the SAP-1 gene, fluorescence in situ hybridization was performed using pBluescript bearing SAP-1 cDNA. A total of 100 metaphase cells were examined. Of them, 62% exhibited specific hybridization signals on 19q13.4 (Fig. 7). The distribution of the signals was as follows: double spots on both homologs of chromosome 19 (12.9%), double spots on one homolog and a single spot on the other (45.2%), and a single spot on both or one homolog (41.9%). Such double-spot signals were not detected on any other chromosome region. These results localized the human SAP-1 gene to 19q13.4.

DISCUSSION

In the present study, we have isolated a novel transmembrane-type PTP that contains a single PTP domain in the cytoplasmic region. Among transmembrane-type PTPs identified so far, only human HPTPy and DPTP 10 D of Drosophila contain a single PTP domain, whereas others have duplicated PTP domains. When the amino acid sequence of the PTP domain of SAP-1 was compared with those of known PTPs, HPTPy was found to be most closely related to SAP-1. In addition, SAP-1 shows FN III-like repeated structure but possesses no immunoglobulin-like domains in its extracellular region as do HPTPy and DPTP 10 D. Thus, SAP-1
appears to be structurally similar to HPTPβ and DPTP 10 D and may belong to the Class I1 of PTP described by Krueger et al. (30). However, the amino acid sequence of each tandem repeat in the SAP-1 extracellular region is different from that seen in HPTPp, indicating that SAP-1 and HPTPp may have different functions and act at different sites. The size of SAP-1 protein predicted by isolated cDNA is 120 kDa, whereas immunoblotting with anti-SAP-1 Ab showed that SAP-1 is a 200-kDa protein. Since there are multiple putative N-glycosylation sites in the extracellular region of SAP-1, it might be post-translationally modified and highly glycosylated. Although the extracellular region of SAP-1 might act as an adhesion molecule according to the predicted structure, the role of the intracellular PTP domain is largely unknown. The binding of the specific ligand to SAP-1 or cell-to-cell contact might regulate the PTP activity, thereby influencing the phosphorylation state of SAP-1 target protein.

It is interesting that SAP-1 is predominantly expressed in colon cancer cells and pancreatic cancer cells, whereas SAP-1 transcripts are undetectable in normal colon and pancreas.
Southern blotting of genomic DNA extracted from these cancer cells showed that the SAP-1 gene was not amplified in cancer cells; suggesting that the amplification of the SAP-1 gene may not be involved in their elevated levels of SAP-1 transcripts. Thus, alterations in the regulation of transcription or degradation rate of mRNA of SAP-1 gene may occur in these cells. It has been demonstrated that the p53 gene, a suppressor oncogene, is mutated and deleted in KATO-II cells (13) and most colon cancer cells (36). The p53 has also been demonstrated that the tyrosine-specific protein kinase activity in colon tumor cells is associated with an apparent involvement of SAP-1 in the oncogenesis of these cells. It has been demonstrated that Northern hybridization using 32P as the probe. Two μg of poly(A)+ RNA isolated from different tissues was used (upper panel). The 1.2-kb mouse β-actin probe was used to examine the same blot (lower panel).
A.
Two \( \mu \)g of poly(A)\(^+\) RNA extracted from various cell lines as indicated was used for Northern blotting as described under "Materials and Methods." The cell lines include a series of gastric cancer cells (KATO-III, KWS, TMK-1, MKN1, MKN28, MKN45, MKN74, AGS, JR-1), a pancreatic cancer cell line (Panc-1, MIA PaCa-2), colon cancer cell lines (COLO330, COLO320, SW480, SW137, WiDr), glioblastoma cell lines (A172, T97G), a neuroblastoma cell line (Jonathan, KWS), a B-lymphoid cell line (Raji), an epidermoid cancer cell line (A431), a cervical carcinoma cell line (HeLa), and a breast cancer cell line (ZR-75-1). The mouse \( \beta \)-actin probe was used to examine the same blots.

B.

FIG. 4. Expression of SAP-1 in various human cell lines. Two \( \mu \)g of poly(A)\(^+\) RNA extracted from various cell lines as indicated was used for Northern blotting as described under "Materials and Methods." The cell lines include a series of gastric cancer cells (KATO-III, KWS, TMK-1, MKN1, MKN28, MKN45, MKN74, AGS, JR-1), a pancreatic cancer cell line (Panc-1, MIA PaCa-2), colon cancer cell lines (COLO330, COLO320, SW480, SW137, WiDr), glioblastoma cell lines (A172, T97G), a neuroblastoma cell line (Jonathan, KWS), a B-lymphoid cell line (Raji), an epidermoid cancer cell line (A431), a cervical carcinoma cell line (HeLa), and a breast cancer cell line (ZR-75-1). The mouse \( \beta \)-actin probe was used to examine the same blots.

Fig. 5. PTP activity of the GST-SAP-1 fusion protein. A, GST-SAP-1 fusion protein or GST alone was expressed and purified as described under "Materials and Methods." Proteins were then electrophoresed on 10% polyacrylamide gel and visualized by Coomassie Blue staining. Molecular sizes are indicated in kDa. Lane 1, GST alone; lane 2, GST-SAP-1. B, PTP activities of GST alone and GST-SAP-1 fusion protein were assayed by using pNPP or \(^{32}\)P-labeled Raytide as a substrate as described under "Materials and Methods." The results are the mean \( \pm \) S.E. from three separate experiments.

unregulated elevation of PTP activity may contribute to malignant transformation by activation of pp60\(^{src}\). Other src family PTKs such as p56\(^{la}\) have also been shown to be activated by the dephosphorylation of their carboxyl-terminal region (42). Taken together, the elevated SAP-1 activity might result in the dephosphorylation of pp60\(^{src}\) or src family PTKs, thereby inducing malignant transformation of cells.

Chromosomal localization of the SAP-1 gene was assigned to 19q13.4. Although SAP-1 expression was not observed in most gastric cancer cell lines, deletion of 19q13 has not been reported in gastric cancer samples so far (43). On the other hand, CEA, a high level of which is usually associated with

Fig. 6. Immunoblotting and transient expression of SAP-1. WiDr cell (lane 1) and HL-60 cell (lane 2) lysates (50 \( \mu \)g) were immunoblotted with anti-SAP-1 Ab as described under "Materials and Methods." COS-7 cells were transfected with the pSRa vector alone (lane 3) or the vector containing a full length of SAP-1 cDNA (lane 4) by the calcium phosphate precipitation method as described under "Materials and Methods." Cells were lysed 3 days after transfection and immunoblotted with anti-SAP-1 Ab. Molecular weights are indicated. Arrows indicate the position of SAP-1 protein.

Fig. 7. Fluorescence in situ hybridization of the human SAP-1 gene. Fluorescence in situ hybridization was performed as described under "Materials and Methods." Double fluorescence spots were detected on R-banded chromosome 19 at the q13.4 region. Colon cancers and pancreatic cancers (44), is also mapped to 19q13.2 (45). In addition, CEA-related genes are also clustered near this region (46). CEA as well as SAP-1 has been shown to be a highly glycosylated protein potentially acting as an adhesion molecule (44); these structural and genetic similarities between the CEA family and SAP-1 seem to be interesting. On the other hand, DCC (47), a gene which encodes a putative adhesion molecule, has been shown to be often deleted in human colon cancers. The significance of amplification and deletion of genes encoding adhesion molecules in human colon cancer cells needs further elucidation.

In summary, we have isolated a novel transmembrane type PTP having a FN III-like structure in the extracellular region and a single PTP catalytic domain in the cytoplasmic region. The high level of expression of this PTP in both pancreatic and colon cancers suggests the possibility that the elevated PTP activity of SAP-1 may induce dephosphorylation of src family protein-tyrosine kinases and subsequent cellular transformation of gastrointestinal cells, in concert with the activation of other oncogenes and inactivation of tumor suppressor genes.

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REFERENCES

1. Cantley, L. C., Auger, K. R., Carpenter, C., Duckworth, B., Graziani, A., Kapeller, H. and Soloff, S. (1991) Cell 64, 231-232
2. Ullrich, A. and Schlessinger, J. (1990) Cell 61, 205-212
3. Kameyama, T., Yasui, W., Yoshihara, K., Tanino, T., Nakayama, H., Ito, M., Ito, H. and Tabara, E. (1990) Cancer Res. 60, 8002-8009
4. Hattori, Y., Oda, H., Nakata, K., Miyawaki, H., Naito, K., Sakaumioto, H., Katoh, Y., Yoshihara, T., Sugimura, T. and Tsuruda, M. (1990) Proc. Natl. Acad. Sci. U.S.A. 87, 5893-5897
5. Hirota, S. and Sugimura, T. (1991) Cancer Cells 3, 49-52
6. Takahashi, E., Hamaguchi, M., Watanabe, T., Akiyama, S., Katozaki, M., Ohnishi, Y., Xiao, H., Negai, Y. and Takagi, H. (1991) Proc. Jpn. Cancer Res. 82, 1428-1435
7. Fisher, E., Charbonneau, H. and Tonks, N. K. (1991) Science 253, 401-406
8. Trowbridge, I. S. (1991) J. Biol. Chem. 266, 23317-23320
9. LaFoglia, S., Morse, B., Barnea, G., Cannizzaro, L. A., Li, F., Nowell, P. C., Boghosian-Sell, L., Glick, J., Westo, A., Harris, C. C., Drabkin, H., Patterson, D., Croce, C. M., Schlessinger, J. and Huebner, K. (1991) Proc. Natl. Acad. Sci. U.S.A. 88, 5036-5040
10. Zheng, X. M., Wang, Y. and Pallee, C. J. (1992) Nature 359, 336-339
11. Adachi, M., Sekiya, M., Artimura, Y., Takekawa, M., Itoh, F., Hirota, Y., Imai, K. and Yachi, A. (1992) Cancer Res. 52, 737-740
12. Vi, T., Cleveland, J. and Ilihe, J. N. (1991) Blood 78, 2222-2228
13. Matozaki, T., Sakamoto, C., Matsuda, K., Suzuki, T., Kondo, Y., Nakano, O., Wada, K., Uchida, T., Niishichi, H., Nagao, M. and Kasuga, M. (1992) Biochem. Biophys. Res. Commun. 182, 215-223
14. Matozaki, T., Sakamoto, C., Suzuki, T., Matsuda, K., Uchida, T., Nakano, O., Wada, K., Niishichi, H., Kondo, Y., Nagao, M. and Kasuga, M. (1993) Cancer Res. 53, 4535-4541
15. Sambrook, J., Fritch, E. F. and Maniatis, T. (1989) Molecular Cloning, A Laboratory Manual, p. 540, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
16. Smith, D. B. and Johnson, K. S. (1988) Gene (Amst.) 71, 31-40
17. Kornblihtt, A. R., Umezawa, K., Vibe-Pedersen, K. and Baralle, F. E. (1985) Nature 318, 244-246
18. Ito, H., Yamaoka, M., Yamada, K., Yasunii, H., Abe, T., Doh, H., Hirano, J. and Fukushima, S. (1992) J. Biol. Chem. 267, 17858-17863
19. Inazawa, J., Fukushima, K., Seto, Y., Nakagawa, H., Misawa, S., Abe, T. and Nagata, S. (1991) Genomics 10, 1075-1078
20. Vingoe-pequinot, E. and Duttilius, B. (1978) Ann. Genet. 21, 122-124
21. Finegold, S., Strum, T. and Gray, J. W. (1986) Proc. Natl. Acad. Sci. U.S.A. 83, 2934-2938
22. Yang, Y. and Tonks, N. K. (1991) Proc. Natl. Acad. Sci. U.S.A. 88, 9494-9498
23. Charbonneau, H., Tonks, N. K., Kumar, S., Ditto, C. D., Harrylock, M., Cool, D. E., Krebs, E. E., Fischer, E. H. and Walsh, K. A. (1989) Proc. Natl. Acad. Sci. U.S.A. 86, 5223-5226
24. Finegold, S., Strum, T. and Gray, J. W. (1986) Proc. Natl. Acad. Sci. U.S.A. 83, 2934-2938
25. Vingoe-pequinot, E. and Duttilius, B. (1978) Ann. Genet. 21, 122-124
26. Finegold, S., Strum, T. and Gray, J. W. (1986) Proc. Natl. Acad. Sci. U.S.A. 83, 2934-2938