

TEP1, Encoded by a Candidate Tumor Suppressor Locus, Is a Novel Protein Tyrosine Phosphatase Regulated by Transforming Growth Factor β^1

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Abstract

It has long been postulated that protein tyrosine phosphatases may act as tumor suppressors because of their ability to counteract the oncogenic actions of protein tyrosine kinases. Here we report the cloning and characterization of a novel human protein tyrosine phosphatase, TEP1. TEP1 contains the protein tyrosine phosphatase signature motif, and we show that it possesses an intrinsic protein tyrosine phosphatase activity. TEP1 also shares extensive homology with tensin, a cytoskeletal protein localized to focal adhesions, and with auxilin, a protein involved in synaptic vesicle transport. Immunofluorescence studies show that TEP1 is a cytoplasmic protein. The abundance of TEP1 transcription is altered in many transformed cells. In the transforming growth factor β -sensitive cells, TEP1 expression is rapidly down-regulated by transforming growth factor β , a cytokine shown to be involved in regulating cell adhesion and cell motility. We have also mapped the gene encoding TEP1 to chromosome 10q23, a locus that is frequently deleted in a variety of human cancers. TEP1 protein is identical to the protein encoded by the candidate tumor suppressor gene *PTEN/MMAC1*. Our functional studies of the TEP1 protein suggest that its tumor suppressor function may associate with its intrinsic protein tyrosine phosphatase activity and its cytoplasmic localization.

Introduction

The proper levels of protein tyrosine phosphorylation inside the cells are regulated by the dynamic actions of both protein tyrosine kinases and protein tyrosine phosphatases (1, 2). Because many of the protein tyrosine kinases are encoded by proto-oncogenes, it has long been postulated that some of the protein tyrosine phosphatases may act as tumor suppressors. Although protein tyrosine phosphatases are diverse in their structure and cellular localization, they all contain a conserved signature motif (HCXXGXGRXG; Ref. 3), which constitutes the active site in the phosphatase catalytic domain. A critical cysteinyl residue is required for the formation of a thiophosphate intermediate during the phosphate transfer reaction (4). This motif is also conserved by the dual-specificity phosphatases, a subfamily of protein tyrosine phosphatases that can dephosphorylate both phosphotyrosyl and phosphoserine/threonine residues. Protein tyrosine phosphatases have been shown to act as either positive or negative regulators during signal transduction, cell cycle progression, and cellular transformation. For example, CDC25 is shown to specifically dephosphorylate and thus activate the CDKs³ (5), whereas MKP-1/CL100 acts to dephosphorylate and consequently inactivate the MAPKs (6, 7).

Received 4/16/97; accepted 4/20/97.

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¹ This work was supported in part by the Yale Cancer Research Award and the Pew Charitable Trust. H. S. is a Pew Scholar in the Biomedical Sciences.

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³ The abbreviations used are: CDK, cyclin-dependent kinase; MAPK, mitogen-activated protein kinase; TGF, transforming growth factor; FBS, fetal bovine serum; RCML, reduced, carboxyamidomethylated, and maleylated lysozyme.

In addition to growth factor receptor tyrosine kinase-mediated signaling events, tyrosine phosphorylation also plays an important role in regulating the cell-cell or cell-extracellular matrix interactions that affect cell migration, attachment, and communication between neighboring cells (8). The signaling proteins at focal adhesions, which mediate cell attachment to substratum, include both focal adhesion kinase and Src tyrosine kinases. Furthermore, cytoskeletal proteins such as tensin and paxillin are tyrosine phosphorylated and localized to focal adhesions during cell attachment to extracellular matrix. Tensin, a protein that contains an SH2 domain and binds to actin, has been implicated in the assembly of the signaling complexes at focal adhesions (9).

Here we report the cloning and characterization of a novel member of protein tyrosine phosphatase TEP1 (TGF- β -regulated and epithelial cell-enriched phosphatase).⁴ This protein contains the tyrosine phosphatase signature motif and also shares extensive homology to tensin and auxilin. TEP1 is identical to the protein encoded by *PTEN/MMAC1*, a candidate tumor suppressor gene at chromosome 10q23 (10, 11). Inactivation of this gene by deletions or mutations has been found recently in many primary tumors or in tumor cell lines. Here we report that TEP1/*PTEN/MMAC1* possesses intrinsic protein tyrosine phosphatase activity and its gene transcription is highly regulated.

Materials and Methods

Cell Lines. WI38 (normal human foreskin fibroblasts), Saos2 (human osteosarcoma), 293 (human embryo kidney carcinoma), HeLa (cervical epitheloid carcinoma), and A431 (epidermoid carcinoma) cells were maintained in DMEM supplemented with 10% FBS. PC12 (rat pheochromocytoma) cells were cultured in DMEM with 10% FBS together with 15% horse serum. HepG2 (human hepatocellular carcinoma) cells were maintained in MEM with 10% FBS. NIH3T3 (mouse fibroblasts) cells were maintained in DMEM with 10% calf serum. All of the above cell lines were obtained from American Type Culture Collection. HaCaT (human keratinocytes) and ML1 (human myeloblastic leukemia) cells were obtained from Hui Zhang at Yale University and were maintained in DMEM or RPMI with 10% FBS, respectively. TGF- β 1 treatment of asynchronously growing HaCaT cells were performed as described (12).

Cloning of the TEP1 Gene. EST clone N48030 was obtained from Research Genetics, Inc. (St. Louis, MO). The cDNA fragment in this clone was excised and used as a probe to screen a ML1 λ ZAPII cDNA library (kindly provided by Hui Zhang at Yale University). Among one million phages screened, 15 positive clones were identified. The size of cDNA inserts were analyzed by restriction mapping. Clone 13-1 was found to contain the longest insert and was chosen for further studies. DNA sequencing was performed on both DNA strands. Inspection of clone 13-1 sequence indicated that it contained a full-length cDNA. The coding region of the TEP1 cDNA was amplified by PCR with a *Bam*HI linker added immediately before the initiating codon, and the PCR fragment was subcloned into the pBlueScript vector (Stratagene). Site-directed mutagenesis was performed to generate the C124S mutant (substitution of cysteine at codon 124 with serine). To construct a plasmid expressing a histidine-tagged TEP1 in bacteria, a 1.3-kb *Bam*HI and *Xho*I fragment was transferred from the pBlueScript vector into pQE31 vector

⁴ The GenBank accession number for TEP1 is U96180.

(Qiagen). Flag-epitope tagged TEP1 was constructed by subcloning the 1.3-kb *Bam*HI and *Kpn*I fragment from the pBlueScript vector into pFlagCMV4, a mammalian expression vector (Kodak-IBI).

RNA Analysis. Total RNAs were isolated using RNazol (Tel-Test, Inc.) according to the manufacturer's recommendation. Northern blot hybridization was performed in 250 mM NaPO₄ (pH 7.2), 7% SDS, and 1 mM EDTA (13) at 65°C, and the filters were washed extensively with 40 mM NaPO₄ (pH 7.2), 1% SDS and 1 mM EDTA at 65°C. For TEP1 detection, a 1-kb *Bgl*III-*Xho*I fragment spanning nucleotides 1122-2095 was used as a probe. For the detection of p15^{INK4B} RNA, a PCR fragment corresponding to the first coding exon of the p15 gene (12) was used. For the detection of CDK2, a 2-kb fragment containing the entire CDK2 cDNA was used as the probe.

Recombinant Protein Production and Purification. *Escherichia coli* (strain BL21/DE3) cells harboring the plasmid pQE31-TEP1 were grown to late log phase ($A_{600} = 1.0$), and expression of the histidine-tagged TEP1 protein was induced with 0.4 mM isopropyl-1-thio- β -D-galactopyranoside for 4 h at room temperature. Histidine-tagged TEP1 (His₆-TEP1) was purified from the *E. coli* lysates using nickel-agarose beads as the affinity resin (ProBond metal-binding resin; Invitrogen) following the conditions recommended by the manufacturer. The purified fraction contains 0.1 mg/ml His₆-TEP1 protein and was ~90% pure as judged by SDS-PAGE analysis and Coomassie Blue staining. Similar methods were used to purify the histidine-tagged TEP1(C124S) mutant protein.

Protein Tyrosine Phosphatase Assay. Phosphotyrosyl RCML was phosphorylated using a recombinant insulin receptor kinase catalytic domain (BIRK) and then used as substrates for assaying tyrosine phosphatase activity as described (14). Purified His₆-TEP1 protein or the C124S mutant derivative (0.5 μ g each) was assayed with 3 μ M phosphotyrosyl-RCML in a buffer containing 40 mM HEPES (pH 7.0), 2 mM DTT, and 0.1 mg/ml BSA at 30°C for the indicated time. Reactions were terminated by the addition of an activated charcoal mixture. After centrifugation, the released inorganic phosphate in the supernatant was quantitated by liquid scintillation counting.

Transfection and Immunostaining of Transfected Cells. HepG2 and NIH3T3 cells were grown on glass coverslips in DMEM supplemented with either 10% FBS or 10% calf serum, respectively. Cells were transfected by the standard calcium phosphate method with 10 μ g each of either pFlagCMV4 vector or pFlagCMV4-TEP1, together with 10 μ g of pUC18 carrier DNA. Thirty-six h after transfection, cells were fixed in 3.7% formaldehyde in PBS for 30 min at room temperature and then permeabilized with 0.1% Triton X-100 in PBS for 3 min. After blocking with 5 mg/ml BSA, coverslips were incubated with anti-Flag epitope antibody M2 (Kodak-IBI), followed by rhodamine-conjugated donkey anti-mouse IgG secondary antibody (Jackson ImmunoResearch). Antibodies were diluted in PBS containing 2 mg/ml BSA. Cells were examined and photographed using a Zeiss Axiophot epifluorescence microscope.

Results

Identification and Cloning of TEP1 cDNA. We are interested in isolating novel protein tyrosine phosphatases that are potentially involved in the signaling events during the formation of focal adhesions. Two approaches were used: (a) pairs of degenerate oligonucleotides that correspond to the conserved catalytic domain were used as primers in a PCR to amplify gene sequences from human cDNA libraries; and (b) we also used the conserved sequence motifs in the tyrosine phosphatase catalytic domain to search the GenBank EST database. These combined approaches yielded several clones that potentially encode novel protein tyrosine phosphatases.

We report here the characterization of one of the clones that we have isolated. A partial cDNA sequence (EST clone N48030) was discovered during the search of the GenBank EST database using the conserved sequences of the tyrosine phosphatases. The potential peptide encoded by this cDNA not only contains the tyrosine phosphatase signature motif but also shares homology to tensin, a cytoskeletal protein that associates with focal adhesions. To further examine the function of this gene, we have isolated its full-length cDNA from a human lambda phage cDNA library constructed from ML1, a myelo-

blastic cell line, using the partial cDNA fragment as a probe. Fifteen positive clones were identified among one million phages screened. The clones that contained the longest inserts were fully sequenced. The complete cDNA (Fig. 1A) contained an open reading frame of 1209 nucleotides that encodes a deduced protein of 403 amino acids. Several lines of evidence suggest that the methionine at nucleotide 805 is the initiating codon. The nucleotide sequence near the first methionine matches a Kozak consensus sequence, and there are several in-frame stop codons upstream of the putative initiation methionine. Consistent with the deduced protein molecular weight, a M_r close to 47,000 protein was synthesized using this cDNA as the template in an *in vitro* transcription/translation assay (data not shown). We have named this novel protein TEP1, as described below.

The full-length cDNA of TEP1 has several interesting features. The cDNA contains an unusually long 5' untranslated region (Fig. 1A), an indication that its expression may be translationally regulated. Furthermore, multiple islands of CGG repeats were present in this untranslated region. Deletion or expansion of di- or trinucleotides repeats have been reported for several disease-related genes, including the gene responsible for Huntington disease (15). Alternatively, the CpG islands may be regulated by DNA methylation. The tumor-specific, CpG methylation-induced transcriptional silencing of several human tumor susceptibility genes, including the gene encoding p16^{INK4A}, has been reported (16).

The phosphatase domain of the deduced protein shares homology with a subfamily of protein tyrosine phosphatases, the dual specificity phosphatases. These include BVP, a dual specificity phosphatase encoded by the baculovirus *Autographa californica* (17); VHR, a dual specificity phosphatase identified by expression cloning (18); MKP-1, a specific phosphatase that inactivates MAPKs (6, 14); and CDC14, a putative phosphatase involved in cell cycle regulation in yeast *Saccharomyces cerevisiae* (19). As observed previously for the members of the dual specificity phosphatases, the sequence homology is mostly confined in the region surrounding the protein tyrosine phosphatase signature motif (Fig. 1B).

In addition, the deduced protein sequence shares extensive homology along the entire protein length with two cytoplasmic proteins, tensin (20) and auxilin (21) (Fig. 1C). Tensin is a cytoskeletal protein shown to bind actin and is localized in focal adhesions (9). Auxilin is a cytoplasmic protein involved in synaptic vesicle transport (22). Very recently, GAK (23), a serine/threonine kinase shown to interact with cyclin G, has also been reported to contain a region homologous to tensin and auxilin (Fig. 1C). The sequence homology among these proteins suggest that they may be involved in related cellular processes.

TEP1 Gene Encodes a Protein Tyrosine Phosphatase. To determine whether TEP1, which contains the protein tyrosine signature motif, is indeed a protein tyrosine phosphatase, we tested whether TEP1 is capable of dephosphorylating phosphotyrosyl RCML, an *in vitro* substrate for many tyrosine phosphatases. We expressed the TEP1 protein in bacteria as a histidine-tagged protein and purified the recombinant protein using nickel-agarose beads as an affinity resin (Fig. 2A). The purified TEP1 protein displayed an intrinsic tyrosine phosphatase activity, which was abolished when the essential cysteinyl residue in the tyrosine phosphatase signature motif was mutated to serine (C124S; Fig. 2B). Because structurally TEP1 is related to the dual specificity phosphatases, we also assayed TEP1 for phosphoserine/threonine phosphatase activity. No phosphatase activity was detected when TEP1 was assayed using phosphoserine/threonine casein as substrate (data not shown).

To further characterize the tyrosine phosphatase activity of TEP1, we examined its activity in the presence of various phosphatase inhibitors (Fig. 2C). The tyrosine phosphatase activity of TEP1 was

A

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GAATTCGGCAGGAGTGGAGCGAGCGGGCTCAGGCGAGGGAGATGAGAGACGGCGCGGGCCGGCCGGAGCCCTCTCAGCGCCTG
90
TGAGCAGCCGCGGGGAGCGCCCTCGGGGAGCCGGCCCGCTCGCGCGGGCGGAGCGCGCGGTTCTCGCCTCCTTCTTTTCT
180
AACCGTGAGCCTCTTCCTCGGCTTCTCCGAAAGGGAAGGTGAAGCCGTGGGCTCGGGCGGGAGCCGGCTGAGGCGCGGGCGGG
270
CGGCACCTCCCGCTCTGAGCGGGGGGAGAAGCGCGCGGGCGGGCGGGCGGCTGCAGCTCCAGGGAGGGGGTCTGAGTCGCTG
360
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450
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540
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630
AGCAGCCGCCGAGCAGCATTACCCGGCTGCGGTCCAGAGCCAAGCGCGCGGAGGCGGAGGGGCATCAGCTACCGCAAGTCCAGAGCC
720
ATTTCCATTCCTGCAGAAGAAGCCCGCCACCCAGCAGCTTCTGCCATCTCTCTCTCTTTTCTTTCAGCCACAGGCTCCCAGACATGACA
810
M T
2
GCCATCATCAAAGAGATCGTTAGCAGAAAACAAAGGAGATATCAAGAGGATGGATTTCGACTTAGACTTGACCTATATTTATCCAAACATT
900
A I I K E I V S R N K R R Y Q E D G F D L D L T Y I Y P N I
32
ATTGCTATGGGATTTCTCGAGAAAGACTTGAAGGCGTATACAGGAACAATATTGATGATGTAGTAAGGTTTTTGGATTCAAAGCATAAA
990
I A M G F P A E R L E G V Y R N N I D D V V R F L D S K H K
62
AACCATTACAAGATATACAACTTTGTGCTGAAAGACATTTAGCACCGCCAAATTTAATTGCAGAGTTGCACAATATCTTTTGAAGAC
1080
N H Y K I Y N L C A E R H Y D T A K F N C R V A Q Y P F E D
92
CATACCACCACAGCTAGAATTTATCAAACCTTTTGTGAAGATCTTGACCAWTGGCTAAGTGAAGATGACAATCATGTTGCAGCAATT
1170
H N P P Q L E L I K P F C E D L D Q A W L S E D D N H V A A I
122
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1260
H C K A G K G R T G V M I C A Y L L H R G K F L K A Q E A L
152
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1350
D F Y G E V T R D K K G V T I P S Q A R R Y V Y Y Y S Y L L
182
AAGAATCATCTGGATTATAGACCAGTGGCACTGTTGTTTCAAGACTATGTTGAAACTATTCCAATGTTCAAGTGGCGGAACATGCAAT
1440
K N H L D Y R P V A L L F H K M M F E T I P M P S G G T C N
212
CCTCAGTTTGTGGTCTGCCAGCTAAAGGTGAAGATATATCTCCAAITTCAGACCCACAGCGGGAAGACAAGTTCATGTACTTTGAG
1530
P Q F V V C Q L K V K I Y S S N S G P T R R E D K F M Y F E
242
TTCCTCAGCCGTTACCTGTGTGTGATATCAAAGTAGAGTTCTCCACAAACAGAAAGATGCTAAAAAGGACAAAATGTTTCAC
1620
F P Q P L P V C G D I K V E F F H K Q N K M L K K D K M F H
272
TTTGGGTAATACATCTTTCATACCAGGACCAGAGGAAACCTCAGAAAAAGTAGAAAATGGAAGTCTATGTGATCAAGAAATCGATAGC
1710
F W V N T F F I P G P E E T S E K V E N G S L C D Q E I D S
302
ATTTGCAGTATAGCGCTGAGATAATGACAAGGAATATCTAGTACTTACTTTAACAATAATGATCTTGACAAGCAATAAAGACAAA
1800
I C S I E R A D N D K E Y L V L T L T K N D L D K A N K D K
332
GCCAACCGATCTTTCTCCAAATTTAAGGTGAAGCTGTACTTCAAAAAACAGTAGAGGAGCGCTCAATCCAGAGGCTAGCAGTTCA
1890
A N R Y F S P N F K V K L Y F T K T V E E P S N P E A S S S
362
ACTTCTGTAACACCAGATGTTAGTGACAATGAACCTGATCATTATAGATATTTCTGACACCCTGACTCTGATCCAGAGAATGAACCTTTT
1980
T S V T P D V S D N E P D H Y R Y S D T T D S D P E N E P F
392
GATGAAGATCAGCATACAAATACAAAGTCTGAATTTTTTTTTTATCAAGAGGATAAAAACACCATGAAAATAAACTTGAATAAACTG
2070
D E D Q H T Q I T K V *
403
AAAAAAAAAAAAAAAAAACTCGAG
2095
    
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B

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Tep1 CEDLDQLS...EDDNHVA A I H C K A C K G R V M I C H R G K F
Bvp IDTVKETE...KCPML G V H C T H G I N H Y M V C R H L G I
Vhr FERAD D Q A L A Q K N R L V H H O R E G G Y S S P T L V I M R Q K M
Mkpl FNEID D . S I K D A R R F M H O Q R G I S S A T I C L R N R V
Cdc14 VGEAETIK...R..K A M H O C K A G L G R E E C L I G H I Y Y G F
    
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C

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Tep1 DMDLTVIYPN I I A M G F P A E R L G V Y I D E V V R E L D S K H K N H Y K I Y N L C A E H D T A 81
Tensin E R D L V Y I T E R I I A V S Y P S T A E I Q S F S L R E M A H M L K S K H G D N V L F H N S . E R H D I S 124
Auxilin D L D F T V I T S R I I V M S F P L D S V H I G F Q V D D I R S F L D G R H L D H Y T V Y H L S P . K S R T A 118
Gak D L B I S V I T S R I A V K S F P A E G V S A I K I E D V R L E L D A K H P C H E A V Y N L S P . I I R A S 463

Tep1 N C A Q Y P E D H N P Q E L I K P F E D D Q S E D D N H A A I K A K G R T G M C Y L L H 141
Tensin P K L D F G P D L H T A E K I C S I K A D T N A A A H V V L N K G N G R L G V A Y M H Y 184
Auxilin S S E C S P I R Q A S H N L F A V R N Y N L Q N P K C L D L D A A S S L G M F I F 178
Gak N T E C G A V R R A H H S L Y T L R S H A R E D H R C V M D A A S A A C F L C F 523

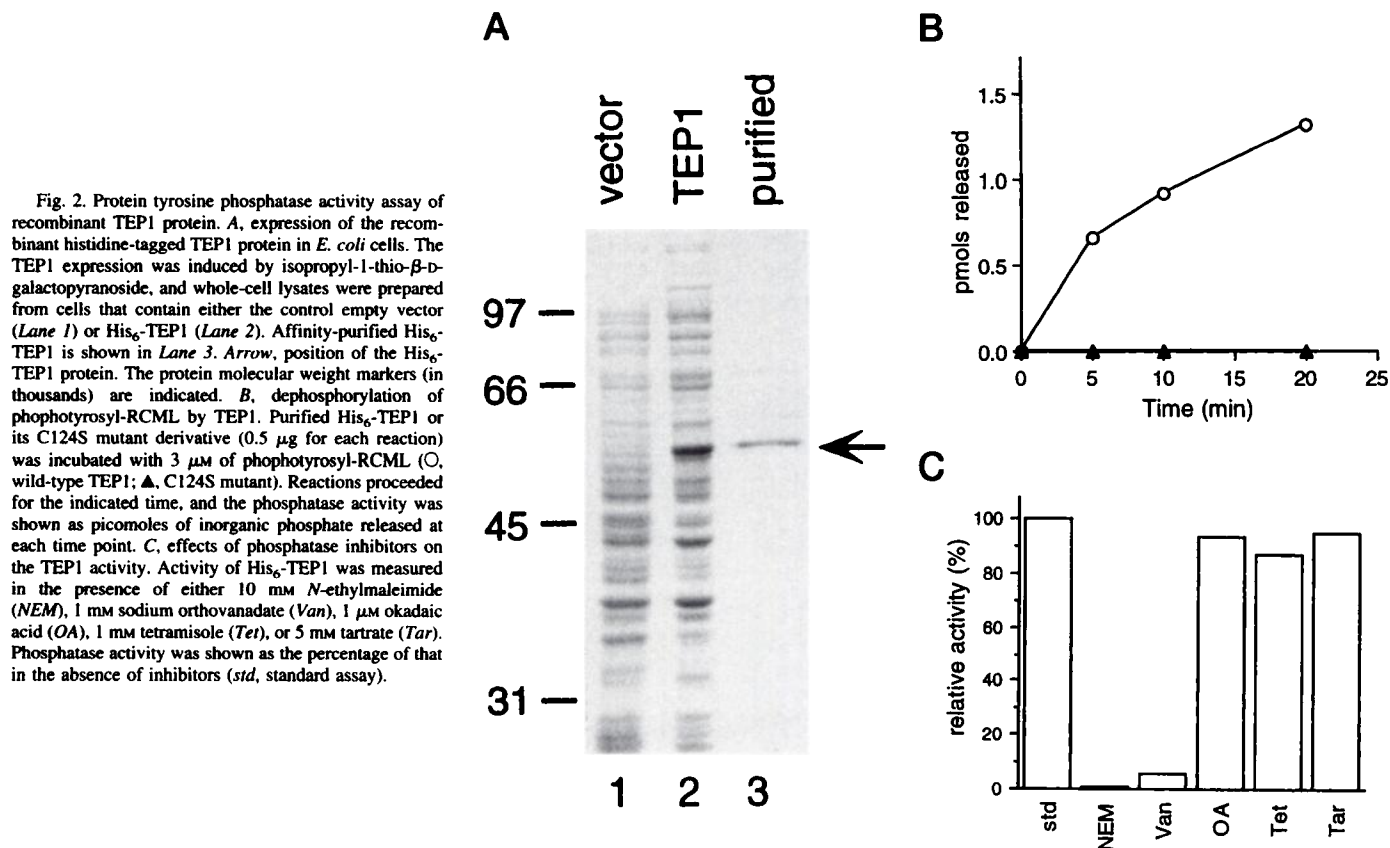
Tep1 R G K F L K Q E L D F Y G E V T R D K K G V T I . . Q R V Y Y S Y K N . H L D Y R P V A L F H K M 198
Tensin S N I S A S D Q L D R F A M F Y E D K V P V G Q Q K I H F S G S G S I K M N N F L H H V 243
Auxilin C N L Y S T P G P V R L L Y A . . . . P G I G L S H R K L G M C D A D K P Y R P H S T I K S I 233
Gak C R L F S T E A V Y M F S M . . . . C P P G I W H K I E I V C D V A E E P I T P H S L V K S V 578

Tep1 M F E T I M . S G . G T N Q F V C Q L K V K I S S N S G . . . . P T R R E . D F M Y F E P Q P L P C 250
Tensin I M H G I N E S K . G . G F L K Y Q A M Q P V S S G I Y . . . . N V Q G D S Q T G I C I T E P G L L L K 297
Auxilin T V S P V F N K Q R N . . . Y C D L I G E T K I T C A D F E R M K E Y R V Q D G I F . . L S I T Q 290
Gak V M T P V L L S K Q R N . . . F C E Y V G E E R V T T S Q E Y D R M K E F K I E D G A V . . L G I T Q 635

Tep1 I K V E F K Q N K M L K K . . . . . D K M H F W V N . . F . . . . F I P G P E E T S E K V E N G S L 295
Tensin I L L K C K K F R S T . . . . . D V I R V . . C A V H D L D I V G E D E A F R D R F 348
Auxilin V V V S M L R S T I G S L Q A K V T N T Q I Q L G F I P L D T T V L K T P E A C D V P K Y 350
Gak V L T I I A R S T L G G L Q A K M A S M K M Q I G F V P R N A T T V K A Y D A C D I Q K Y 695
    
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Fig. 1. Sequence of the TEP1 cDNA. A, nucleotide sequence of the TEP1 cDNA and the deduced amino acid sequence. Nucleotides and amino acids are numbered at the end of each sequence line. B, sequence alignment of TEP1 with several dual specificity phosphatases. Alignment was performed using the PILEUP program from the Wisconsin Genetics Computer Group. Identical amino acids are highlighted by the black boxes. C, sequence alignment of TEP1 with tensin, auxilin, and GAK. Alignment was performed using the same program as in B. The black box highlights the identical amino acids between these proteins.

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sensitive to both *N*-ethylmaleimide, a cysteinyl-modifying agent, and vanadate, a classic tyrosine phosphatase inhibitor. In contrast, the phosphatase activity of TEP1 was insensitive to okadaic acid, a potent inhibitor of mammalian types 1 and 2A phosphatases, nor was it sensitive to tetramisole or tartrate, inhibitors of bacterial alkaline and acid phosphatases, respectively (Fig. 2C). These studies demonstrate that TEP1 is indeed a protein tyrosine phosphatase.

Transcriptional Regulation of TEP1. To determine how TEP1 may be regulated, its expression in various cell lines was examined by Northern blot analysis. Multiple RNA species of TEP1 were observed (Fig. 3A). Two major transcripts, approximately 2 and 5 kb in size, were detected in several cell lines. Additional minor RNA species were also found. Because all of these RNA species were observed in the cell lines that express TEP1, it is likely that these RNA may be derived from the same gene. Whether they represent different spliced forms of TEP1 RNA remains to be determined.

The level of TEP1 transcription was compared among several cell lines. TEP1 is highly expressed in ML1 (human myeloblastic), HaCaT (human keratinocyte), and A431 (epidermoid) cells (Fig. 3A). In addition, TEP1 mRNA is present at high levels in nerve growth factor-treated PC12 (rat pheochromocytoma) cells. In contrast, TEP1 expression is greatly reduced in cells transformed by DNA tumor viruses including 293 cells (human embryonic kidney cells transformed by adenovirus *E1A* gene), HeLa cells (cervical epitheloid carcinoma cells expressing human papillomavirus *E6* and *E7* genes), or Saos2 cells (human osteosarcoma) that lack functional p53 and pRb. We are currently investigating whether inactivation of p53 or pRb is responsible for reduction of TEP1 gene transcription in these transformed cells.

TEP1 mRNA was relatively abundant in a human keratinocyte cell line, HaCaT, which is sensitive to TGF- β . TGF- β , which can regulate cell growth as well as cell adhesion properties (24, 25), has been

shown to regulate transcription of several genes in HaCaT cells. We tested whether *TEP1* gene expression was modulated by TGF- β (Fig. 3B). The addition of TGF- β 1 to actively growing cells led to the rapid down-regulation of TEP1 mRNA, occurring within 2 h after TGF- β treatment. In contrast, as described previously, TGF- β treatment caused a gradual induction of the p15^{INK4B} gene, which encodes an inhibitor of cyclin D/CDK4 (12). As a control, the expression of the CDK2 gene is marginally affected by TGF- β treatment. These studies suggest that the TEP1 gene may be a novel target for TGF- β .

TEP1 Is a Cytoplasmic Protein. To gain an insight into the roles that TEP1 may play inside of the cell, we examined its intracellular localization. We expressed a Flag-epitope tagged TEP1 in several mammalian cell lines by a transient transfection method. The localization of the Flag-epitope tagged TEP1 protein was examined by immunofluorescence microscopy. In both HepG2 and NIH3T3 cells, TEP1 was localized in the cytoplasm, and the staining had an appearance of a network-like structure (Fig. 4). Similar cytoplasmic staining pattern was also observed in HeLa or HaCaT cells (data not shown). These observations are consistent with the notion that TEP1 shares homology with cytoplasmic proteins such as tensin and auxilin, suggesting that TEP1 may exert its function in the cytosol.

Discussion

Using the TEP1 cDNA as a probe, we have mapped the gene encoding TEP1 to chromosome 10q23 by fluorescence *in situ* hybridization (data not shown). Deletion of the chromosome 10q23 region is often associated with prostate cancers or malignant gliomas (26, 27). Very recently, it has been shown that using the positional cloning method, a candidate tumor suppression gene, *PTEN/MMAC1* (10, 11), was isolated from this locus. *PTEN/MMAC1* were reported to encode a putative protein tyrosine phosphatase. The sequence of the TEP1

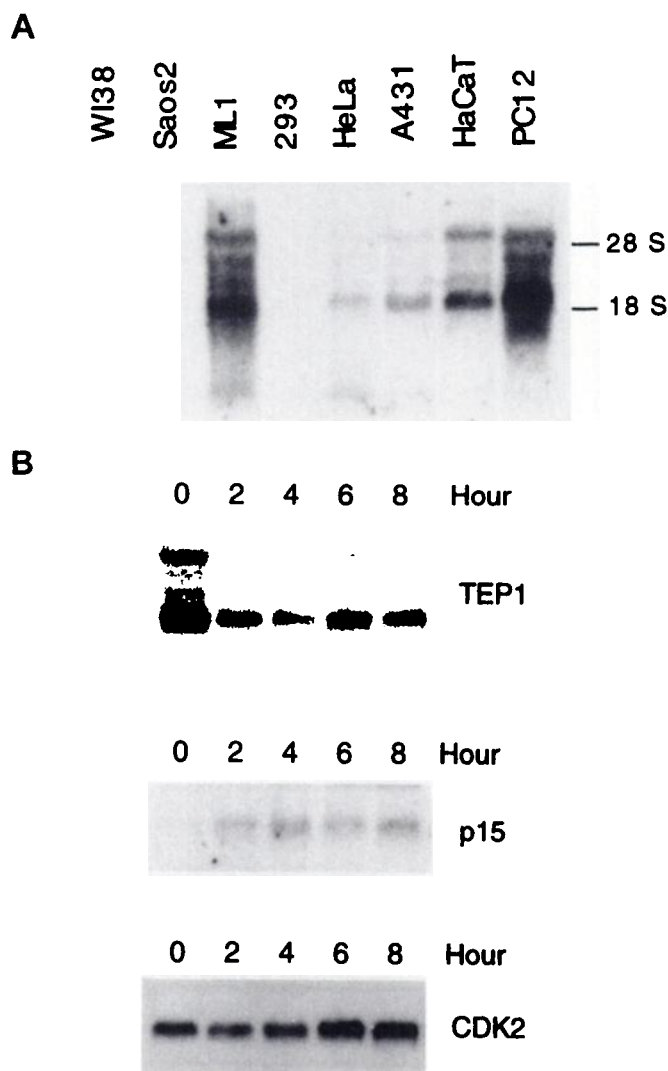


Fig. 3. Expression of the TEP1 gene. A, expression of the TEP1 gene in a variety of normal and tumor cell lines. Total RNA (5 μ g each) from the indicated cell lines was analyzed by Northern blot analysis using TEP1 cDNA as the probe. B, TEP1 gene expression in TGF- β -treated human keratinocytes. Asynchronously growing HaCaT cells were either treated with TGF- β (2 ng/ml) for the indicated times or untreated (0 Hour). Total RNA (5 μ g each) from the indicated time points were analyzed by Northern blot analysis with the TEP1 cDNA as the probe (top panel). Duplicate filters were also probed with either a p15^{INK4B} cDNA (middle panel) or a CDK2 cDNA probe (bottom panel).

protein is identical to the predicted polypeptide encoded by the *PTEN/MMAC1* gene, although at the nucleotide sequence level, there are differences at both 5' and 3' untranslated regions. Whether these differences are due to the source of the cDNA clones remains to be determined. Mutations that inactivate the *PTEN/MMAC1* gene have been identified in primary breast cancer, prostate cancer, and glioblastomas as well as in many *in vitro* cultured tumor cell lines. These observations suggest that *PTEN/MMAC1* is a good candidate for the tumor suppressor gene located on chromosome 10q23.

In this report, we demonstrated that the TEP1/*PTEN/MMAC1* protein indeed contains an intrinsic protein tyrosine phosphatase activity. Consistent with its homology to tensin and auxilin, TEP1 is located in the cytoplasm. Our demonstration of TEP1 being a tyrosine phosphatase suggests that its enzymatic activity is responsible for its tumor suppression function. Because TEP1 shares homology with tensin, a focal adhesion protein, potential targets for TEP1 may reside in the focal adhesion complexes. Identification of the substrates for TEP1 tyrosine phosphatase should help elucidate the roles it plays in tumor suppression.

Compared to other tyrosine phosphatases such as PTP1B, the specific activity of the TEP1 tyrosine phosphatase activity is relatively low toward the *in vitro* artificial substrate, phosphotyrosyl RCML. This may reflect the fact that TEP1 may have a stringent substrate specificity. Similar low tyrosine phosphatase activity toward phosphotyrosyl RCML was observed previously for MKP-1 (14). MKP-1 was later shown to be a potent and specific phosphatase for the MAPKs (6). Alternatively, it is also possible that *in vivo* TEP1 may require certain posttranslational modification or association with accessory factors to achieve a higher activity. Although we failed to detect that TEP1 has serine/threonine phosphatase activity *in vitro*, it remains possible that *in vivo* it may dephosphorylate phosphoseryl/threonyl residues in addition to phosphotyrosyl residues with its physiological substrates.

We also demonstrated that TEP1 gene transcription is down-regulated by TGF- β . This down-regulation of TEP1 may play an important role in the cellular responses to TGF- β . TGF- β can either positively or negatively regulate cell growth, depending on the cellular context (24, 25). TGF- β can affect cell adhesion properties, including stimulating extracellular matrix production and enhancing cell motility (24, 25). TGF- β has also been implicated in tumor progression, possibly through regulation of cell-cell or cell-matrix interactions. Increased TGF- β production has been observed in the advanced stages of breast cancers, particularly in invasive carcinomas with associated lymph node metastasis (28). Tumorigenicity of human breast cancer cells in

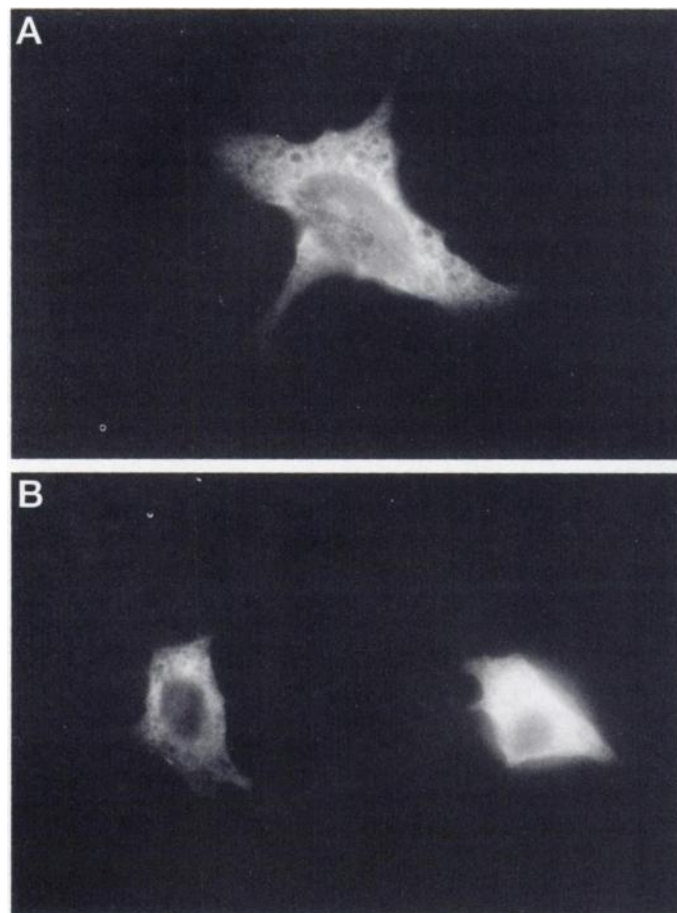


Fig. 4. Cellular localization of TEP1. Immunofluorescence staining of the expressed TEP1 protein in transfected cells. Flag-epitope tagged TEP1 carried on the mammalian expression vector pFlag-CMV4 was introduced into the HepG2 cells (A) or NIH3T3 cells (B) by transient transfection. Flag-epitope tagged TEP1 protein was detected by immunostaining with the anti-Flag epitope antibody (M2 antibody), followed by staining with a rhodamine-conjugated secondary antibody.

experimental animals was also shown to be increased by *in vitro* treatment of TGF- β (29) or by expression of the TGF- β gene (30). Our observations of TGF- β down-regulation of TEP1 raises an interesting possibility that TEP1 may normally be responsible for negatively regulating cell motility, a role consistent with its sequence homology with focal adhesion protein tensin and with its candidate tumor suppression function. By reducing TEP1 expression, TGF- β may promote cell migration during tumor invasion and metastasis.

Acknowledgments

We are grateful to Drs. Patricia Ward, Amy Banks, and David Ward for generous help with FISH mapping of the TEP1 gene, Lynn Cooley, Jerome Eisenstadt, and Stephen Meyn for kindly providing the fluorescence microscope facilities, and Douglas Robinson for assistance in fluorescence microscope photography. We would like to thank David Ward, Daniel DiMaio, Michael Reiss, and Hui Zhang for helpful discussions and critical reading of the manuscript.

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