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Identification of the human prostatic carcinoma oncogene PTI-1 by rapid expression cloning and differential RNA display

(DNA cotransfection/CREF-Trans 6/reverse transcription–PCR/*in vitro* translation/mutated EF-1α)

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ABSTRACT **Elucidating the relevant genomic changes mediating development and evolution of prostate cancer is paramount for effective diagnosis and therapy. A putative dominant-acting *nude* mouse prostatic carcinoma tumor-inducing gene, PTI-1, has been cloned that is expressed in patient-derived human prostatic carcinomas but not in benign prostatic hypertrophy or normal prostate tissue. PTI-1 was detected by cotransfecting human prostate carcinoma DNA into CREF-Trans 6 cells, inducing tumors in *nude* mice, and isolating genes displaying increased expression in tumor-derived cells by using differential RNA display (DD). Screening a human prostatic carcinoma (LNCaP) cDNA library with a 214-bp DNA fragment found by DD permitted the cloning of a full-length 2.0-kb PTI-1 cDNA. Sequence analysis indicates that PTI-1 is a gene containing a 630-bp 5′ sequence and a 3′ sequence homologous to a truncated and mutated form of human elongation factor 1α. *In vitro* translation demonstrates that the PTI-1 cDNA encodes a predominant ≈46-kDa protein. Probing Northern blots with a DNA fragment corresponding to the 5′ region of PTI-1 identifies multiple PTI-1 transcripts in RNAs from human carcinoma cell lines derived from the prostate, lung, breast, and colon. In contrast, PTI-1 RNA is not detected in human melanoma, neuroblastoma, osteosarcoma, normal cerebellum, or glioblastoma multiforme cell lines. By using a pair of primers recognizing a 280-bp region within the 630-bp 5′ PTI-1 sequence, reverse transcription–PCR detects PTI-1 expression in patient-derived prostate carcinomas but not in normal prostate or benign hypertrophic prostate tissue. In contrast, reverse transcription–PCR detects prostate-specific antigen expression in all of the prostate tissues. These results indicate that PTI-1 may be a member of a class of oncogenes that could affect protein translation and contribute to carcinoma development in human prostate and other tissues. The approaches used, rapid expression cloning with the CREF-Trans 6 system and the DD strategy, should prove widely applicable for identifying and cloning additional human oncogenes.**

The American Cancer Society estimates that more than 200,000 American men will be diagnosed with prostate cancer in 1995 and more than 38,000 afflicted men will die of this disease. Current methods for detecting early prostate cancer are limited in both sensitivity and specificity (1). These methods include physical examination that might easily miss small or centrally located tumors, serum prostate-specific antigen (PSA) determination that is not specific to malignant prostate disease, and tissue biopsy, in which sampling error may lead to erroneous benign diagnosis (2). Predictors and early detection of therapeutic relapse, such as monitoring PSA levels, ultrasound, and bone scans, are also unsatisfactory, as these require fairly bulky tumor regrowth before discovery (3). With current approaches, 40–50% of patients considered to have clinically localized disease actually contain understaged diseases subsequent to radical surgery (4). Surgical intervention is not considered the appropriate treatment protocol for patients with progressive disease.

A primary objective of investigators studying cancer etiology is the identification of gene(s) within tumor cells with oncogenic potential. One approach involves the transfer of high molecular weight (HMW) DNA from established tumor cell lines or primary tumors into appropriate acceptor cell lines by DNA transfection (5). Target cells are then examined for morphological transformation—i.e., focus formation. A modification of this approach involves cotransfection of target cells with HMW DNA plus a selectable antibiotic-resistance gene-containing plasmid, such as pSV2neo, selection for antibiotic resistance, and then injection of pooled antibiotic-resistant cells into *nude* mice to identify clones of cells with tumorigenic potential (6). Most studies using these approaches have relied on the immortal murine cell line NIH 3T3 (5, 6). Unfortunately, NIH 3T3 cells generally prove unsuccessful in identifying dominant-acting oncogenes from human tumor cell lines or clinical samples, and even when successful, subsequent cloning indicates genetic elements not relevant to the majority of human cancers.

Recent studies using a cotransfection/*nude* mouse tumor assay with HMW DNA from a human prostatic carcinoma cell line, LNCaP (7), and a DNA-acceptor cell line, CREF-Trans 6 (8), indicate the presence of a dominant-acting, tumor-inducing gene (8). We have now cloned and characterized prostate carcinoma tumor-inducing gene 1 (PTI-1) by using the differential RNA display (DD) technology (9, 10), library screening strategies (11, 12), and the rapid amplification of cDNA ends (RACE) procedure (11).† The full-length PTI-1 cDNA consists of 2123 bp and contains a 630-bp region that shares sequence similarity with bacterial ribosomal 23S RNA fused to a sequence that is a truncated and mutated form of human elongation factor 1α (EF-1α). PTI-1 is expressed in LNCaP tumor-derived CREF-Trans 6 cells transfected with LNCaP DNA, human prostate carcinoma cell lines, patient-derived prostate carcinomas, and additional human carcinomas, including breast, colon, and lung. These observations indicate that PTI-1 is a genetic element displaying expression in specific human carcinomas and implicates mutagenic changes in EF-1α as a potential contributor to the carcinogenic process.

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Abbreviations: DD, differential RNA display; RT–PCR, reverse transcription–PCR; PSA, prostate-specific antigen; BPH, benign prostatic hypertrophy; HMW, high molecular weight; EF-1α, elongation factor 1α; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

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†The sequence reported in this paper has been deposited in the GenBank data base (accession no. L41490).

MATERIALS AND METHODS

Cell Lines. This study incorporates the following human cell lines: prostate carcinoma (LNCaP, DU-145), endometrial carcinoma (HTB-113), small-cell lung carcinoma (NCI-H69), neuroblastoma (NB-11, IMR-32), nasopharyngeal carcinoma (KB 3-1), breast carcinoma (MCF7, T47D), colon carcinoma (WiDr, HT 29, SW480, LS 174T), normal cerebellum, glioblastoma multiforme (GBM-18), melanoma (C8161, HO-1), and osteosarcoma (Saos-2) (7, 11–14). Also included were CREF-Trans 6 cells and *nude* mouse tumor-derived CREF-Trans 6 cells transfected with LNCaP DNA (CREF-Trans 6:4 NMT) (8). Conditions for growing the various cell types were as described (8, 11–14). Cultures were mycoplasma free.

RNA Preparation, DD, and Reverse Transcription–PCR (RT–PCR). Total cytoplasmic RNA was isolated from logarithmically growing cell cultures as described (11, 12). Tissue samples from normal prostates and patients with prostatic carcinomas or benign prostatic hypertrophy (BPH) were frozen in liquid nitrogen, and RNA was isolated using the Trizol reagent as described by GIBCO/BRL. Tissue samples were supplied by the Cooperative Human Tumor Network. Normal prostate tissue was obtained from autopsies of males <40 years of age. All tissues were histologically confirmed as being normal, hypertrophic, or carcinoma of the prostate. The DD procedure was performed essentially as described by Liang and Pardee (10). Two micrograms of mRNA from CREF-Trans 6 and CREF-Trans 6:4 NMT cells was reverse transcribed with 300 units of Moloney murine leukemia virus reverse transcriptase (BRL) in the presence of 2.5 μM primer T12GC (5′-TTTTTTTTTTTTGC-3′) and 20 μM dNTP mix (BRL) for 60 min at 35°C. Two micrograms of the cDNA was amplified by PCR in the presence of 2 μM T12GC and 2 μM 5′-primer J Bgl24 (5′-ACCGACGTCGACTATCCATGAACA-3′) (15) under low-annealing conditions. The conditions for PCR included a total of 40 cycles, with a melting temperature of 94°C for 30 sec, an annealing temperature of 42°C for 1 min, and an extension temperature of 72°C for 30 sec. Samples were resolved in parallel lanes on a 5% denaturing sequencing gel and differentially expressed bands were removed from the gel and electroeluted in 0.2× TBE (1× TBE = 89 mM Tris base/89 mM boric acid/2 mM EDTA, pH 8.0) solution. The same pair of primers was used for PCR amplification of the differentially expressed sequences, which were subsequently cloned (TA cloning kit; Invitrogen). Plasmids containing inserts of the predicted size were sequenced by the Sanger method (Sequenase kit, version 2.0; United States Biochemical) or the inserts were isolated and used to probe Northern blots (10–12). RT–PCR was performed with appropriate primers as described (12).

cDNA Library Construction, Screening, and Sequencing. A cDNA library of LNCaP mRNA was constructed in the Uni-ZAP XR vector (Stratagene) and screened as described (11, 16). A 1.8-kb PTI DNA fragment was isolated by using a multiprime ^{32}P-labeled DNA fragment obtained after amplification of a 214-bp DNA fragment with a 20-mer oligonucleotide (5′-AACTAAGTGGAGGACCGAAC-3′) as a primer. Plasmids containing the largest PTI-1 inserts were tested by probing Northern blots of appropriate RNA samples and sequenced by using the Sanger method with an Applied Biosystems (model 373A, version 1.2.1) DNA sequencer and oligonucleotide primers synthesized from both ends of the gene inserts.

RACE Procedure. To identify the 5′-extended region of PTI-1, a 22-mer (I; 5′-CCTTGCATATTAACATAACTCG-3′) and a 19-mer (II; 5′-AAGTCGCCCTATTCAGACT-3′), representing antisense sequences corresponding to 262–283 bp and 317–336 bp, respectively, of PTI-1 were synthesized. The RACE protocol was performed by using the 5′ RACE system (GIBCO/BRL) as described (14).

***In Vitro* Translation of PTI-1.** Plasmid containing PTI-1 cDNA was linearized by digestion with *Xho* I and used as a template to synthesize mRNA by using the mCAP mRNA capping kit (Stratagene). *In vitro* translation of PTI-1 mRNA was performed with a rabbit reticulocyte lysate translation kit with conditions as described by GIBCO/BRL.

RESULTS

Identification and Properties of PTI-1. The rapid expression cloning system identified a potential oncogenic element in LNCaP cells (8). DD was used to identify genes displaying differential expression in CREF-Trans 6 cells and CREF-Trans 6:4 NMT cells (9, 10). A problem often encountered when using DD is the identification of amplified sequences not displaying differential expression when tested by Northern blotting (9, 10)—e.g., the band present in CREF-Trans 6:4 NMT RNA directly below the arrow in Fig. 1, lane B. The frequency of false signals can be significantly reduced by using subtraction hybridization prior to PCR amplification and DD (data not shown). By using DD, a 214-bp (194 bp from DD PCR, plus 10 bp on each side from PCR II vector) DNA fragment (PTI-1) was identified in CREF-Trans 6:4 NMT cells that was not present in parental CREF-Trans 6 cells (Fig. 1, lane B, arrow). The PTI-1 fragment was isolated, cloned, sequenced, and used to probe Northern blots containing RNAs from normal and tumor-derived cell lines (Fig. 2). The 214-bp PTI-1 DNA fragment hybridizes to RNAs (three to four) present in CREF-Trans 6:4 NMT, LNCaP and the hormone-independent prostate carcinoma cell line DU-145 (Fig. 2).

PTI-1 consists of 2123 bp, the 5′-flanking region (1–215 bp) was obtained by RACE 5′-extension, and the remainder of the gene (216–2123 bp) was determined by direct sequencing of the PTI-1 cDNA clone. Primer extension analysis and RT–PCR of LNCaP mRNA confirm that PTI-1 is a full-length cDNA (data not shown). The 3′ region of PTI-1, extending from bp 630 to bp 2123, displays 97% sequence similarity to a truncated human EF-1α gene. The 5′ region of PTI-1 displays no sequence similarity to eukaryotic genes, but instead is ≈85% similar to the prokaryotic 23S ribosomal RNA gene from *Mycoplasma hyopneumoniae.* This region of PTI-1 contains the 214-bp DNA marker (core) sequence obtained by DD (Fig. 1). The 5′ region also contains a large number of stop codons (TAA, TGA, and TAG sequences) (data not shown). These data suggest that PTI-1 is a fusion gene consisting of two regions: a 630-bp 5′ region, together with a 3′ truncated and mutated EF-1α gene.

PTI-1 contains an open reading frame from bp 621 to bp 1814 and encodes a protein of 398 amino acids (Fig. 3). A comparison of the amino acid sequence of PTI-1 (aa 1–398) and human EF-1α (aa 65–462) is presented in Fig. 3. The nucleotide sequences coding for PTI-1 and the truncated human EF-1α are 98.4% similar and 97.7% identical. PTI-1 contains the same C terminus as human EF-1α. The N terminus of PTI-1 is different from human EF-1α and consists of a deletion of 67 amino acids normally found in human EF-1α

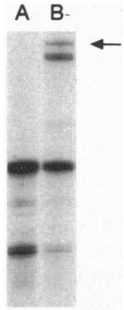

FIG. 1. DD of CREF-Trans 6 and CREF-Trans 6:4 NMT mRNAs. DD was performed as described in *Materials and Methods.* The arrow indicates the PTI-1 band appearing only in mRNA from CREF-Trans 6:4 NMT (lane B), but not in CREF-Trans 6 cells (lane A). The length of this PTI-1 DNA fragment is 214 bp.

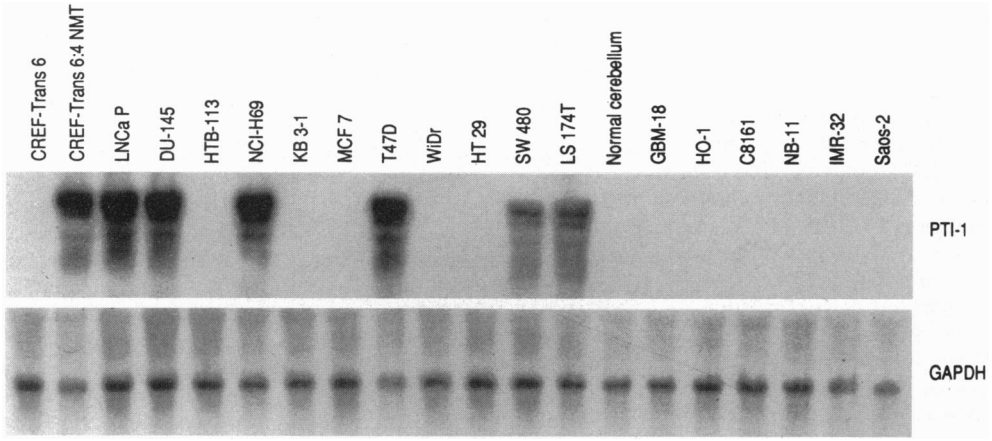

FIG. 2. Expression of PTI-1 in normal and tumor cell lines. RNAs from CREF-Trans 6, CREF-Trans 6:4 NMT, and various normal and tumor-derived human cell lines were transferred to nylon membranes and probed with a ^{32}P-labeled 280-bp PTI-1 DNA fragment (317–596 bp). Membranes were stripped and reprobed with a ^{32}P-labeled glyceraldehyde-3-phosphate (GAPDH) gene probe.

and an insertion of 3 amino acids (MQS) in PTI-1 that differs from the original N terminus (MGK) of human EF-1α. In addition, six in-frame amino acid changes are present in PTI-1 (Fig. 3). The loss of 67 amino acids in the N terminus and changes in specific amino acids, from positively charged to nonpositively charged amino acids and from hydroxyl group-containing to nonhydroxyl group-containing amino acids, can be anticipated to impact on the three-dimensional structure and functionality of this mutant EF-1α protein.

On the basis of sequence analysis, PTI-1 should encode a protein of 43.8 kDa. To confirm this prediction, *in vitro* translation analyses of proteins encoded by the PTI-1 cDNA were determined. A predominant protein present after *in vitro* translation of PTI-1 has molecular mass of ≈46 kDa (Fig. 4). This value is larger than predicted and may result from protein modification—i.e., phosphorylation—in the rabbit reticulocyte lysate system. Four additional minor proteins (41–30.5 kDa) are also present after *in vitro* translation (Fig. 4). These minor proteins may result from incorrect downstream initiation, or alternatively, these minor proteins may represent proteolysis products of the full-length protein.

Expression of PTI-1 in RNA Samples from Patient-Derived Tissues and Cell Lines. RNA was isolated from quick-frozen prostate samples from patients obtained during operations and confirmed as prostatic carcinomas or BPH histologically. RNAs were also extracted from normal prostates obtained at autopsy from men less than 40 years of age and histologically normal. By RT–PCR with primers (A and L) synthesized from the 630-bp 5′ PTI-1 sequence, expression is apparent in seven of eight human prostatic carcinomas (Fig. 5 and data not shown). In contrast, PTI-1 is not expressed in four normal prostates or three BPH patient samples (Fig. 5). The LNCaP sample and all prostate samples, including normal, BPH, and carcinoma, are positive for PSA expression, whereas CREF-Trans 6, CREF-Trans 6:4 NMT, and DU-145 do not express PSA (Fig. 5). All samples were positive for GAPDH expression (Fig. 5). These results indicate that PTI-1 is expressed in human prostate carcinomas but not in normal prostates or BPH.

To determine the pattern of PTI-1 expression in additional cell types, RNAs from various cell lines were analyzed by Northern blotting by using PTI-1 fragments and GAPDH fragments as probes (Fig. 2). In addition to being expressed in CREF-Trans 6:4 NMT, LNCaP, and DU-145, PTI-1 expression is evident in other human carcinomas, including NCI-H69 (small-cell lung), T47D (breast), and SW480 and LS174T (colon). In contrast, PTI-1 expression is not detected in HTB-113 (endometrial adenocarcinoma), KB 3-1 (nasopharyngeal carcinoma), MCF7 (breast carcinoma), WiDr and HT-29 (colon carcinoma), normal cerebellum, GBM-18 (glioblastoma multiforme), H0-1 and C8161 (melanoma), NB-11 and IMR-32 (neuroblastoma) or Saos-2 (osteosarcoma) cells.

A

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(E)1                                    MGKEKTHINIVVIGH      15

(E)16 VDSGKSTTTGHLIVKCGGIDKRTIEKFEKEAAEMGKGSFKYAWVLDKLKAER  67
(P)1                                    MQS                   3
                                        *
(E)68 ERGITIDISLWKFETSKYYVTIIDAPGHRDFIKNMITGTSQADCAVLIVAAGV 120
(P)4  ERGITIDISLWKFETSKYYVTIIDAPGHRDFIQNMITGTSQADCAVLIVAAGV  56

(E)121 GEFEAGISKNGQTREHALLAYTLGVKQLIVGVNKMDSTEPPYSQKRYEEIVKE 173
(P)57  GEFEAGISKNGQTREHALLAYTLGVKQLIVGVNKMDSTEPPYSQKRYEEIVKE 109

(E)174 VSTYIKKIGYNPDTVAFVPISGWNGDNMLEPSANMPWFKGWKVTRKDGNA 223
(P)110 VSTYIKKIGYNPDTVAFVPISGWNGDNMLEPSANMPWFKGWKVTRKDGNA 159
                        *
(E)224 SGTTLLEALDCILPPTRPTDKPLRLPLQDVYKIGGIGTVPVGRVETGVLKPGM 276
(P)160 SGTTLLEALDCILPPTRPTDKPLGLPLQDVYKIGGIGTVPVGRVETGVLKPGM 212
       *                *
(E)277 VVTFAPVNVTTEVKSVEMHHEALSEALPGDNVGFNVKNVSVKDVRRGNV 325
(P)213 VVTFGPVNVTTEVKSVEMHHEALGEALPGDNVGFNVKNVSVKDVRRGNV 261
                  *
(E)326 AGDSKNDPPMEAAGFTAQVIILNHPGQISAGYAPVLDCHTAHIACKFAELK 376
(P)262 AGDSKNDPPMEAAGFPAQVIILNHPGQISAGYAPVLDCHTAHIACKFAELK 312
                                *
(E)377 EKIDRRSGKKLEDGPKFLKSGDAAIVDMVPGKPMCVESFSDYPPLGRFAVRD 428
(P)313 EKIDRRSGKKLEDGPKFLKSGDAAIVDMVPGKPMCVESFSDYPPLGCFAVRD 364

(E)429 MRQTVAVGVIKAVDKKAAGAGKVTKSAQKAQKAK                  462
(P)365 MRQTVAVGVIKAVDKKAAGAGKVTKSAQKAQKAK                  398
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B

Human EF-1α	Amino Acid	K (100)	R (247)	A (261)	S (300)	T (341)	R (423)
	Codon	AAA	CGC	GCT	AGT	ACT	CGC
	Nucleotide	A	C	C	A	A	C
PTI-1	Amino Acid	- Q (36)	G (183)	G (217)	G (236)	P (277)	C (359)
	Codon	CAA	GGC	GGT	GGT	CCT	TGC
	Nucleotide	C	G	G	G	C	T

FIG. 3. Peptide sequence of the PTI-1 gene and comparison with the human EF-1α gene. (*A*) Peptide sequence comparison of EF-1α (E) and PTI-1 (P). The underlined region (67 amino acids) of EF-1α indicates the amino acids missing in the PTI-1 gene. Boldface letters (with an *) indicate the mutated amino acids in the PTI-1 peptide. (*B*) Differences in amino acids, codons, and nucleotides between EF-1α and PTI-1. Six single-base mutations give rise to specific amino acid changes in the PTI-1 gene. In the column of amino acids, the numbers in parentheses refer to the position of the amino acid in the peptide, and codon refers to the sequence of three nucleotides encoding the specific amino acid. The specific nucleotide change is also indicated.

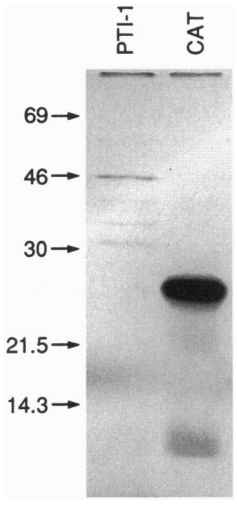

FIG. 4. *In vitro* translation of the PTI-1 gene. Lane CAT is the *in vitro* translation of the chloramphenicol acetyltransferase gene (24 kDa) used as a positive control. Lane PTI-1 contains the translated products of this cDNA. Rainbow protein standards (Amersham) were used to determine the sizes of the *in vitro* translated products.

These observations indicate that PTI-1 expression is not restricted to human prostate carcinoma, but also occurs in ≈50% of the human carcinomas analyzed.

DISCUSSION

Cancer is a progressive disease in which tumor cells manifest continuous genetic changes that correlate with increasing frequencies of chromosomal abnormalities and mutations (17–19). Recent studies suggest that mutations in genes involved in maintaining genomic stability, including DNA repair, mismatch repair, DNA replication, and chromosomal segregation, may result in acquisition of a mutator phenotype by cancer cells, predisposing them to further mutations resulting in tumor progression (18). In leukemias, as well as specific solid tumors, improved cytogenetic techniques and molecular approaches indicate that specific translocations result in the activation of protooncogene products and the creation of tumor-specific fusion proteins (19). A common observation is that both types of oncogenic elements are often transcription factors, suggesting that alterations in transcriptional control may directly contribute to cancer development and evolution (19, 20). Modifications in the translational machinery of cells, including changes in both eukaryotic initiation factors and elongation factors, can also result in susceptibility to transformation and the acquisition of transformed and oncogenic properties in specific target cells (21, 22). For example, overexpression of a normally rate-limiting eukaryotic protein synthesis initiation factor, eIF-4E, can cooperate with both the v-*myc* and adenovirus *E1A* genes in inducing transformation of primary rodent fibroblasts (23), induce tumorigenic transformation in both NIH 3T3 and Rat 2 cells (24), and induce in combination with transcription factor Max both a tumorigenic and metastatic phenotype in Chinese hamster ovary (CHO) cells (25). Enhanced expression of EF-1α, a nucleotide exchange protein that binds GTP and aminoacyl-tRNA and results in codon-dependent placement of this aminoacyl-tRNA at the A site of the ribosome (21, 22), confers susceptibility to carcinogen- and ultraviolet light-induced transformation to mouse and Syrian hamster cell lines (26). Elevated levels of wild-type EF-1α also occur in tumors of the pancreas, colon, breast, lung, and stomach relative to normal tissue (27). Moreover, enhanced expression of EF-1γ, a nucleotide exchange protein that mediates transport of aminoacyl-tRNAs to 80S ribosomes during RNA translation, is found in a high proportion of pancreatic tumors (78%), colorectal tumors (86%), and colorectal adenomas (56%) relative to normal-appearing adjacent tissue (28–30). These findings indicate that alterations in both gene transcription and protein synthetic processes contribute to cancer development and evolution.

EF-1α is analogous to bacterial elongation factor-Tu (EF-Tu), both members of the GTPase superfamily of proteins (31, 32). A primary function of EF-Tu/EF-1α is the process of kinetic proofreading that results in appropriate codon–anticodon binding interactions (32). Mutations in specific regions of EF-Tu result in altered biological function, including a dominant-negative inhibition of protein synthesis by mutational replacement of Lys-136 by glutamate or glutamine in the G4 GTPase region that interacts with guanine nucleotide release proteins (GNRPs) (33). EF-Tu mutants in *Escherichia coli* and Salmonella exhibit increases in missense error rates (34, 35). Mutations in EF-1α can directly affect the frequency of frameshifting and amino acid misincorporations in *Saccharomyces cerevisiae* (36). Single amino acid substitutions in EF-1α alter the selection or proofreading of the codon–anticodon match (36). Moreover, altering the level of EF-1α in *S. cerevisiae* directly affects suppression of nonsense mutations, further indicating a critical involvement in trans-

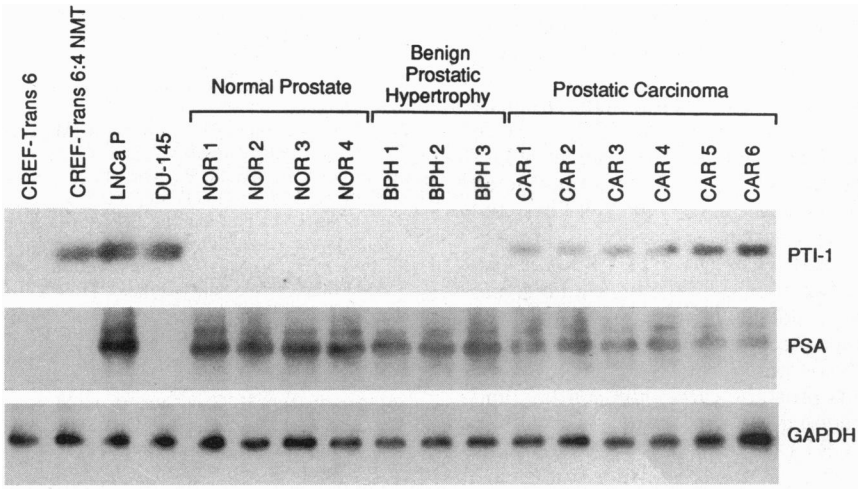

FIG. 5. RT–PCR analysis of PTI-1, PSA, and GAPDH expression in cell lines and tissue samples of normal prostate, BPH, and prostate carcinoma. RT–PCR of PTI-1 uses two 20-mers: primer L, 5′-GAGTCTGAATAGGGCGACTT-3′ (sense orientation); and primer A, 5′-AGTCAGTACAGCTAGATGCC-3′ (antisense orientation). RT–PCR of PSA uses the following primers: A, 5′-AGACACAGGCCAGGTATTTCAGGTC-3′; and B, 5′-CACGATGGTGTCCTTGATCCACTTC-3′. RT–PCR of GAPDH uses a pair of primers with the sequences 5′-TCTTACTCCTTGGAGGCCATG-3′ and 5′-CGTCTTCACCACCATGGAGAA-3′. The PCR-amplified products were blotted on nylon membranes and probed with a ^{32}P-labeled DNA fragment of PTI-1, PSA, or GAPDH, respectively.

lational fidelity (37). In this context, the mutated EF-1α protein encoded by PTI-1 could modify normal EF-1α function resulting in decreased protein translational fidelity and an inability to suppress specific mutations in carcinomas. If this "translational infidelity" hypothesis is correct, PTI-1 may represent a mutated "genomic stability" gene (18) and an important contributor to the mutator phenotype of cancer cells and tumor progression.

An important early event in carcinogenesis may involve mutations that confer immortality or an enhanced cellular life span (17, 18). During cellular senescence the levels and catalytic activity of EF-1α decrease (38). Forced expression of EF-1α in *Drosophila melanogaster* extends life-span in comparison with control flies (39). The reduction in proliferative capacity associated with senescence correlates with a reduced capacity for mitosis. In this respect, the recent demonstration that EF-1α may be an important element in mitotic spindle formation (40) may be relevant. As demonstrated in this report, the EF-1α sequence in PTI-1 contains a deletion of 67 amino acids and six point mutations in comparison with wild-type human EF-1α (Fig. 3). Although the relevance of these alterations to EF-1α activity is unknown, it is possible that this gene undergoes a series of step-wise mutations during prostate cancer development. If this hypothesis is correct, changes in the structure of the PTI-1 gene and its protein product could represent a genetic and biochemical marker for prostatic carcinoma development and progression.

A previous limitation preventing the identification and cloning of oncogenes was the absence of a sensitive transfectable indicator cell line. This problem has been ameliorated with the identification of the CREF-Trans 6 clone (8). By using rapid expression cloning with the CREF-Trans 6 acceptor cell line and the DD technology, the putative oncogene PTI-1 displaying expression in human prostate, breast, lung, and colon carcinomas has been identified and cloned. In comparative studies using NIH 3T3 cells, cotransfection of HMW DNA from LNCaP cells and the antibiotic-resistance plasmid pSV2neo DNA did not result in tumors following injection of G418-resistant cells into *nude* mice (8). Rapid expression cloning with CREF-Trans 6 also results in the transfer of tumor-inducing oncogenes from a human breast carcinoma, a glioblastoma multiforme, a small-cell lung carcinoma cell line, and a patient-derived metastatic colon carcinoma lesion (data not shown). Although the identities of the dominant-acting genetic elements present in these human tumor DNA-transfected CREF-Trans 6 clones are not known, these exciting preliminary results suggest that this acceptor cell line could prove useful for identifying and cloning human oncogenes involved in the development of diverse human cancers.

The present study implicates a gene, PTI-1, that contains a sequence linked to a truncated and mutated EF-1α gene in oncogenic transformation and carcinoma development. PTI-1 is expressed in tumor-derived CREF-Trans 6 cells transfected with LNCaP DNA, human prostatic carcinoma cell lines, and patient-derived carcinomas, whereas expression is not detected in normal prostate or BPH tissues. PTI-1 RNA is also found in additional human carcinomas of the breast, lung, and colon. These results indicate that PTI-1 expression may be a common alteration in human carcinomas. The direct cloning of PTI-1 from an LNCaP cDNA library indicates that this gene is originally present in this prostatic carcinoma cell line and does not develop as a consequence of mutation resulting during introduction into CREF-Trans 6 cells or selection for tumor formation in *nude* mice. Although further studies are required with a larger number of human tissue samples, PTI-1 could have significant diagnostic implications, and, if appropriate specificity is achieved, PTI-1 may be amenable for cancer therapeutic applications.

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