PDEF, a Novel Prostate Epithelium-specific Ets Transcription Factor, Interacts with the Androgen Receptor and Activates Prostate-specific Antigen Gene Expression*

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Prostate cancer, the most frequent solid cancer in older men, is a leading cause of cancer deaths. Although proliferation and differentiation of normal prostate epithelia and the initial growth of prostate cancer cells are androgen-dependent, prostate cancers ultimately become androgen-independent and refractory to hormone therapy. The prostate-specific antigen (PSA) gene has been widely used as a diagnostic indicator for androgen-dependent and -independent prostate cancer. Androgen-induced and prostate epithelium-specific PSA expression is regulated by a proximal promoter and an upstream enhancer via several androgen receptor binding sites. However, little progress has been made in identifying androgen-independent regulatory elements involved in PSA gene regulation. We report the isolation of a novel, prostate epithelium-specific Ets transcription factor, PDEF (prostate-derived Ets factor), that among the Ets family uniquely prefers binding to a GGAT rather than a GGAA core. PDEF acts as an androgen-independent transcriptional activator of the PSA promoter. PDEF also directly interacts with the DNA binding domain of androgen receptor and enhances androgen-mediated activation of the PSA promoter. Our results, as well as the critical roles of other Ets factors in cellular differentiation and tumorigenesis, strongly suggest that PDEF is an important regulator of prostate gland and/or prostate cancer development.

Prostate cancer, the most common solid cancer in older men, is one of the most frequent causes of cancer deaths. The lack of effective therapies for advanced prostate cancer reflects in part the lack of knowledge about the molecular mechanisms involved in the development and progression of this disease (1, 2). In particular, little is known about the mechanisms that trigger the conversion of an initially androgen-dependent cancer to androgen independence (1, 2). Prostate cancer development can be divided into different steps of epithelial cell transformation (3). Proliferation within the normal and hyperplastic prostate epithelium is restricted to the basal cells and is regulated by autocrine or paracrine growth factors. Differentiation from basal cells to secretory luminal epithelial cells is androgen-dependent, and androgen remains crucial for the initial growth of prostate cancer cells (3, 4). Nevertheless, prostate cancers ultimately become androgen-independent and refractory to hormone therapy. PSA has been used widely as a diagnostic indicator for prostate cancer (5). PSA is expressed by normal and cancerous luminal epithelial cells of the prostate, and its expression is under the control of androgens acting through the androgen receptor (6). However, even in hormone-refractory prostate cancer, PSA is expressed, suggesting an androgen-independent component in PSA regulation as well (7). Both regulatory regions contain binding sites for androgen receptor and are essential for androgen-induced transcriptional activation of the PSA gene. However, little progress has been made in identifying androgen-independent regulatory elements involved in PSA gene regulation.

We report here the characterization of a novel prostate epithelial-specific Ets transcription factor, PDEF, that is involved in PSA gene regulation and acts as a co-regulator of AR. Ets factors play a crucial role in the regulation of genes involved in hematopoiesis, angiogenesis, organogenesis, and specification of neuronal connectivity (10–12), and several distinct chromosomal translocations involving various Ets factors have been discovered in human cancer (10–12). The recent isolation of three epithelial-specific Ets factors, ESE-1 (ESX/ELF3/ERT/JEN), ESE-2 (ELF5), and ESE-3 (EHF), has demonstrated the relevance of Ets factors in epithelial cells (13–21). Our results now support the notion that PDEF is involved in prostate epithelium-specific gene expression and possibly in prostate cancer development or progression.

MATERIALS AND METHODS

Cell Culture—Human foreskin keratinocytes, HaCAT (keratinocyte line), Hek293 (fetal epithelial kidney), C-33A (cervical carcinoma), HeLa (cervical carcinoma), H157 (large cell lung carcinoma), H249 (small cell lung carcinoma), HuVEC (endothelial), U-937 (monocytes), 1

1 The abbreviations used are: PSA, prostate-specific antigen; AR, androgen receptor; DHT, dihydrotestosterone; PDEF, prostate-derived Ets factor; EMSA, electrophoretic mobility shift assay; EST, expressed sequence tag; RT, reverse transcriptase; PCR, polymerase chain reaction; GST, glutathione S-transferase; kb, kilobase pair(s); MAP, mitogen-activated protein; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.
human synovial fibroblasts, and human chondrocytes were grown as described (15, 22). LNCap (prostate cancer) cells were grown in T-medium (Life Technologies, Inc.) with 10% fetal calf serum.

**Isolation of PDEF cDNA**—A human expressed sequence tag (EST) from a subtracted prostate benign hyperplasia cDNA library with significant homology to the ETS domain was identified in the Human Genome Sciences EST data base using the tBLASTN program (NCBI). To isolate the entire coding region for PDEF, we performed the rapid amplification of cDNA ends method using human adult prostate Marathon Ready cDNA (CLONTECH) as described (15, 23).

**RT-PCR Analysis and Northern Blot Analysis**—mRNAs were isolated as described (20). Northern blots containing poly(A)+ mRNA (CLONTECH) were hybridized with random prime-labeled PDEF, ESE-1, PSA promoter oligonucleotide WT Ets site A (5'-TCAGGAGCCAGATTAAAGCAG-3'), antisense, 5'-TGACGTCTTGGCTCGAGGAGTTCAGC-3', and were hybridized with random prime-labeled PDEF, ESE-1, and GAPDH cDNA probes in QuickHyb solution (Stratagene) (15). cDNAs were generated from 1 μg of mRNA. RT-PCR reactions were performed and analyzed as described (15).

The sequences of the PDEF primers were: sense, 5'-GACCAGTGAGGAGACCTGAGCAGCGG-3'; antisense, 5'-TGACCTTGGGCTCGAGGAGTTCAGC-3'. The full-length PDEF cDNA as well as other Ets factor cDNAs as the pGL3 luciferase vector (pGL3/PSA). The PSP954 promoter and the mvitro transcription/translation product (PSP954) were cloned into the EcoRI site of the pCI (Promega) eukaryotic expression vector downstream of the cytomegalovirus and T7 promoters (pC/MPLES). Coupled in vitro transcription/translation reactions of full-length PDEF and other Ets factors were performed (Promega) as described (26). The human AR expression vector pAR0 was kindly provided by Dr. Albert Brinkmann (Erasmus University, Rotterdam, The Netherlands) (27).

**Electrophoretic Mobility Shift Assay (EMSA)**—EMSA were performed as described (22, 26) using 2 μl of in vitro translation product and 0.2 ng of 32P-labeled double-stranded oligonucleotide probes (20000 cpm) and run on 4% polyacrylamide gels, containing as buffer 0.5× TGE as described (15).

**Oligonucleotides used as probes are as follows.**

1. **PSA promoter oligonucleotide WT Ets site A** (5'-TCAGGAGCCAGATTAAAGCAG-3'); 3'-CCGGCTCTACTTCTGACAGT-5'.
2. **PSA promoter oligonucleotide WT Ets site B** (5'-TCAGGAGCCAGATTAAAGCAGT-3'); 3'-CCGGCTCTACTTCTGACAGT-5'.
3. **PSA promoter oligonucleotide WT Ets site C** (5'-CCCGGCTCTTAAAGGCTACAGT-3'); 3'-CCGGCTCTACTTCTGACAGT-5'.
4. **PSA promoter oligonucleotide WT Ets site D** (5'-CCCGGCTCTTAAAGGCTACAGT-3'); 3'-CCGGCTCTACTTCTGACAGT-5'.
5. **PSA promoter oligonucleotide WT Ets site E** (5'-CCCGGCTCTTAAAGGCTACAGT-3'); 3'-CCGGCTCTACTTCTGACAGT-5'.
6. **PSA promoter oligonucleotide WT Ets site F** (5'-CCCGGCTCTTAAAGGCTACAGT-3'); 3'-CCGGCTCTACTTCTGACAGT-5'.
7. **PSA promoter oligonucleotide WT Ets site G** (5'-CCCGGCTCTTAAAGGCTACAGT-3'); 3'-CCGGCTCTACTTCTGACAGT-5'.
8. **PSA promoter oligonucleotide WT Ets site H** (5'-CCCGGCTCTTAAAGGCTACAGT-3'); 3'-CCGGCTCTACTTCTGACAGT-5'.
9. **PSA promoter oligonucleotide WT Ets site I** (5'-CCCGGCTCTTAAAGGCTACAGT-3'); 3'-CCGGCTCTACTTCTGACAGT-5'.
10. **PSA promoter oligonucleotide WT Ets site J** (5'-CCCGGCTCTTAAAGGCTACAGT-3'); 3'-CCGGCTCTACTTCTGACAGT-5'.
11. **PSA promoter oligonucleotide WT Ets site K** (5'-CCCGGCTCTTAAAGGCTACAGT-3'); 3'-CCGGCTCTACTTCTGACAGT-5'.
12. **PSA promoter oligonucleotide WT Ets site L** (5'-CCCGGCTCTTAAAGGCTACAGT-3'); 3'-CCGGCTCTACTTCTGACAGT-5'.

**DNA Transfection Assays**—Co-transfections of 3 × 10⁵ CV-1 (green monkey kidney) cells were carried out with 3.5 μg of pGL3/PSA DNA, 2.5 μg of pR0 AR expression vector, and 1.5 μg of pCI/PDEF DNA using 12.5 μl of LipofectAMINE (Life Technologies, Inc.) as described (15). Cells grown in medium containing charcoal-stripped serum for 24 h were incubated in the absence or presence of 10⁻⁸ M dihydrotestosterone (DHT) for an additional 16 h and harvested for luciferase activity as described (28). Co-transfections of 3 × 10⁵ LNCap cells were carried out with 1.75 μg of pGL3/PSA DNA and 0.75 μg of pCI/PDEF DNA using 4 μl LipofectAMINE Plus (Life Technologies, Inc.) as described (15). Cells were grown in medium containing charcoal-stripped serum and assayed 36 h later for luciferase activity as described (28). Transfections were performed independently in triplicate and repeated three times with two different plasmid preparations with similar results. Cotransfection of a second plasmid for determination of transfection efficiency was omitted because potential artifacts with this technique have been reported (29).

**In Situ Hybridization**—Tissues fixed in 4% paraformaldehyde in phosphate-buffered saline, pH 7.4, for 2–4 h at 4 °C were transferred to 30% sucrose in phosphate-buffered saline overnight at 4 °C, frozen in OCT compound (Miles Diagnostics, Elkhart, IN), and stored at −70 °C. In situ hybridization was performed on 6-μm frozen sections with 35S-labeled riboprobes as described (15).

**GST Pull-down Assay**—A series of GST-AR fusion proteins were generated by PCR with specific primers to contain in frame restriction enzyme sites (30) and sequenced to confirm that there were no mutations introduced by the PCR. GST fusion proteins were prepared as described before (31). 5 μl of [35S]methionine labeled in vitro translated full-length PDEF protein was incubated with equal amounts of GST-AR fusion proteins or GST on agarose beads in 200 μl of NETN (0.5% Nonidet P-40, 1 mM EDTA, 20 mM Tris-HCl, pH 8.0, 100 mM NaCl) for 3 h at 4 °C with gentle shaking. PDEF protein that bound to the GST-AR fusion proteins was eluted after three washings with NETN buffer and analyzed on a 12% SDS-polyacrylamide gel.

**RESULTS**

**Isolation of PDEF and Sequence Comparison with Other Members of the Ets Family**—We have isolated full-length cDNA clones from human prostate encoding a novel member of the Ets transcription factor/oncogene family, PDEF (Fig. 1A). Sequence analysis revealed an open reading frame encoding a 335-amino acid protein with a predicted molecular mass of 37.5 kDa. The deduced amino acid sequence of PDEF predicts a protein rich in glutamic acid (8%), alanine (8%), serine (11%), leucine (10%), and proline (8%). Two putative PEST domains, common to rapidly degraded proteins, are located between amino acids 19 and 53 and between 161 and 186. Potential phosphorylation sites present in PDEF include a protein kinase C site, two AKT phosphorylation sites, two tyrosine kinase phosphorylation sites, and eight c-Jun N-terminal kinase/p38/extracellular signal-regulated kinase kinase phosphorylation sites. Five of these MAP kinase phosphorylation sites are clustered at the amino terminus of PDEF, and one of these sites conforms to the optimal MAP kinase phosphorylation site PKS/TYP (Fig. 1A). One of the tyrosine kinase phosphorylation sites contains the consensus sequence for interaction with phosphoinositide 3-kinase, YXXM (32).

Ets transcription factors share a highly conserved DNA binding domain, the ETS domain (10–12). Alignment of the carboxyl-terminal ETS domain of PDEF with that of other Ets family members reveals highest homology to one particular subclass of Ets factors that includes Drosophila D-ets-4 (75%) and sea urchin SpETS4 (76%) (Fig. 1B). SpETS4 plays a role in establishing the animal-vegetal axis of the sea urchin embryo at late cleavage/later blastula stages (33). D-Ets-4 is expressed highest in the pole cells, suggesting an involvement in gene expression differentiation (34). Upstream of the Ets domain, PDEF contains a region with significant homology to the Pointed domain present in several other members of the Ets family including ESE-1, ESE-2, ESE-3, Tel, Tel-2, yan, pointed, Ets-1, Ets-2, fli-1, erg-2, Elg, and GABP-α and weakly homologous to the SAM protein-domain interaction domain of polycomb proteins and Ebp receptors (35) (Fig. 1C). In contrast to all other Ets factors where the Pointed domain is directly at the amino acid sequence, PDEF conforms to the optimal MAP kinase phosphorylation site PKS/TYP (Fig. 1A). One of the tyrosine kinase phosphorylation sites contains the consensus sequence for interaction with phosphoinositide 3-kinase, YXXM (32).

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PDEF, a Prostate-specific Ets Transcription Factor

PDEF Expression Is Restricted to the Epithelial Layers of the Prostate and to a Lower Extent to Other Hormone-regulated Tissues—Expression of PDEF was analyzed by Northern blot hybridization of poly(A)$^+$ mRNAs derived from human adult and fetal tissues, indicating the presence of one predominant PDEF transcript of 2.0 kb (Fig. 2A). PDEF is almost exclusively expressed in the prostate gland. Low levels of PDEF transcripts were also found in ovary (Fig. 2A) and in mammary and salivary gland (data not shown), all tissues regulated by androgens or steroids. No PDEF transcripts were detected in brain, heart, gastrointestinal tract, liver, spleen, thymus, kidney, lung, and skeletal muscle or in fetal tissues. These results suggest that PDEF is expressed in a very restricted set of primarily hormone-regulated epithelial tissues with particularly high expression in prostate, an expression pattern strikingly different from other Ets factors such as ESE-1 (Fig. 2A).

More detailed analysis of PDEF expression in different cell types by RT-PCR revealed that only the prostate epithelial cancer cell line, LNCaP, expressed PDEF mRNA, whereas

terminus and the Ets domain at the opposite carboxyl terminus, PDEF has the Pointed domain immediately upstream of the Ets domain.

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**A**

**A**(POINTED)

**B**(ETS)
other epithelial cells and non-epithelial cells were completely devoid of PDEF mRNA (Fig. 2B). To further test the hypothesis that PDEF expression is restricted to epithelial cells within the prostate gland, we performed in situ hybridization on frozen sections of human prostate. Diffuse strong expression was only noted in luminal epithelium of the prostate, but not in other cell types (Fig. 2C). These results most vividly demonstrate that PDEF is exclusively expressed in epithelial cells and particularly strong in the prostate.

**PDEF Binds Specifically to Several Sequences in the PSA Promoter/Enhancer—** In the prostate, PDEF expression is restricted to luminal epithelial cells, the exact cells that express PSA. The PSA gene is regulated by an androgen-responsive promoter and an androgen-responsive upstream enhancer (Fig. 3A). At least eight high and low affinity androgen receptor binding sites have been identified in the promoter and enhancer region and implicated in androgen-mediated regulation of the PSA gene. Inspection of the PSA promoter and enhancer sequence revealed the existence of at least 11 putative Ets binding sites. Some of these sites are in close proximity to androgen receptor binding sites (Fig. 3A). To determine whether the PSA promoter contains binding sites for PDEF, we tested the ability of in vitro translated PDEF to bind specifically to oligonucleotides containing a variety of Ets related binding sites derived from the PSA promoter. EMSA analysis revealed that the PSA B site and G site oligonucleotides formed two major complexes with proteins present in the PDEF extract (Fig. 3B), which were not formed by the control extract (Fig. 3B). A third strong complex was nonspecific and was present in both PDEF and control reticulocyte lysates. Two additional PSA sites, site H and to a lesser extent site C (Fig. 3C), formed weak complexes with PDEF, whereas other potential Ets sites in the PSA promoter did not significantly interact with PDEF. Strikingly, the high affinity binding sites for PDEF diverge from the binding sites found for other Ets factors showing an apparent preference for GGAT versus GGAA in the core of the binding site (Fig. 3C). EMSA analysis of additional canonical Ets sites from other promoters did not reveal any significant PDEF binding.
FIG. 2. Expression of PDEF in different human fetal and adult tissues. A, Northern blot analysis of poly(A)⁺ mRNA from human fetal and adult tissues. The blots were sequentially probed with a PDEF (upper panel), ESE-1 (middle panel), and a GAPDH cDNA probe (lower panel) under
suggested strong DNA binding selectivity for PDEF.\(^2\)

The DNA Binding Specificity of PDEF Is Uniquely Distinct from Other Members of the Ets Family—To directly assess the relative DNA binding specificity and affinity of PDEF and other members of the Ets family, we compared the ability of PDEF and several other Ets factors to bind to the GGAT containing PSA promoter E site and to the same site containing a single nucleotide change to GGAA in the core of the binding site. PDEF bound with high affinity only to the GGAT-containing oligonucleotide, but not to the GGAA-containing oligonucleotide (Fig. 4). Some low affinity binding of ESE-3 to the GGAT oligonucleotide was also observed. In striking contrast

![Diagram of the human PSA promoter/enhancer region. The locations of putative regulatory elements including the androgen receptor binding sites and the Ets binding sites are indicated. The letters within the symbols correspond to the Ets sites as described under "Material and Methods." B, several PSA promoter Ets sites are targets for PDEF. Panel shows DNA binding of PDEF to PSA promoter Ets sites (see "Material and Methods" and panel C), and DNA binding of full-length PDEF in an EMSA using synthetic oligonucleotides coding for the PSA promoter Ets sites using either reticulocyte lysate programmed with the empty pCI expression vector (pCI) or reticulocyte lysate programmed with the PDEF pCI expression vector (PDEF). C, relative DNA binding affinity of PDEF toward a variety of putative Ets binding sites within the PSA promoter as measured by EMSA analysis. A putative high affinity consensus PDEF binding site is shown at the bottom.

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stringent conditions. B, expression of PDEF in various cell types (see "Materials and Methods") by RT-PCR analysis of poly(A)\(^+\) mRNA from indicated cells using primers specific for PDEF (upper panel) or GAPDH (lower panel). C, in situ hybridization studies. Panel shows paired bright field (a and c) and corresponding polarized fluorescence (b and d) photomicrographs. Intense labeling of prostate epithelium in normal prostate is seen with antisense probe to PDEF mRNA (a and b). No labeling is seen with control sense probe (c and d).
PDEF, a Prostate-specific Ets Transcription Factor

Fig. 4. PDEF, in contrast to other Ets factors, preferentially binds to GGAT containing DNA sequences. Figure shows binding of full-length in vitro translated ETS factors as indicated above the figure in an EMSA to synthetic oligonucleotides coding for either the wild type PSA promoter E Ets site (GGAT) containing GGAT in the core of the binding site or to a mutant PSA promoter E Ets site (GGAA) containing GGAA in the core of the binding site.

PDEF Enhances Transcription of the PSA Promoter—To assess whether the PSA gene is indeed a target for PDEF and to determine whether PDEF acts as a repressor or enhancer of transcription, full-length PDEF inserted into the eukaryotic expression vector pCI was co-transfected into PDEF- and AR-negative CV-1 cells together with a reporter gene construct containing the luciferase gene under the control of the 7-kb PSA promoter. Co-transfection with pCI/PDEF resulted in a ∼4-fold transcriptional stimulation of the PSA promoter construct compared with the parental pCI vector (Fig. 5). The specificity of this effect was evaluated in co-transfections with other epithelial-restricted members of the Ets family that are expressed in the prostate. Neither ESE-1 nor ESE-2 enhanced PSA promoter activity. On the contrary, ESE-1, and to a lesser extent ESE-2, repressed basal PSA promoter activity (Fig. 5).

To confirm that the PSA promoter is a target for PDEF in prostate epithelium, the same transfection experiment was repeated in LNCaP cells, which are widely used as a model for prostate cancer, since these cells are androgen-sensitive, secrete PSA, and form androgen-sensitive tumors in nude mice (36–41). PDEF enhanced PSA promoter activity 11-fold in LNCaP cells grown with charcoal-stripped serum in the absence of androgen, whereas ESE-1 and ESE-2 repressed the PSA promoter (Fig. 5). The specificity of this transactivation was confirmed in co-transfections with other epithelial-restricted members of the Ets family that are expressed in the prostate. Neither ESE-1 nor ESE-2 enhanced PSA promoter activity. On the contrary, ESE-1, and to a lesser extent ESE-2, repressed basal PSA promoter activity (Fig. 5).

Enhances Androgen-mediated Transactivation of the PSA Promoter—The AR binding sites in the PSA gene have been shown to be critical for androgen inducibility, and both PDEF and AR are co-expressed in the same cells. A characteristic feature of all Ets factors is their ability to interact with transcription factors of other gene families, suggesting that interaction of PDEF with factors binding to other regulatory elements within the PSA promoter may be an important mechanism of transcriptional control.

To evaluate whether PDEF cooperates with AR, PDEF and AR expression vectors were co-transfected together with pGL3/PSA into AR-negative CV-1 cells and incubated either in the absence or presence of androgen DHT. PDEF in the absence of androgen enhanced PSA promoter activity ∼4-fold. Androgen in the absence of PDEF enhanced PSA promoter transcription ∼27-fold (Fig. 6). In the presence of both PDEF and androgen, a significant synergistic effect of PSA promoter transactivation was observed leading to a ∼57-fold activation, indicating that PDEF and AR cooperate in the regulation of the PSA promoter and that PDEF-mediated PSA promoter transcription contains both an androgen-independent and androgen-dependent component.

To evaluate whether functional interaction between PDEF and AR correlates with physical interaction, GST pull-down experiments were performed with GST fusion proteins containing different domains of AR and in vitro translated [35S]methionine-labeled full-length PDEF. Specific retention of PDEF was only observed, when GST/AR fusion proteins containing the DNA binding domain (amino acids 559–624) were used, but not with GST alone or with other domains of AR (Fig. 7, A and B). These results strongly suggest that PDEF interacts with the DNA binding domain of AR and that cooperativity between PDEF and AR may be due to direct interaction between these two factors.
Functional domains of the AR are denoted by GST fusion proteins and of the GST pull-down results with PDEF.

Promoter. Figure shows transcriptional activation of the PSA promoter and luciferase constructs containing the PSA promoter. Cells were cotransfected with the indicated PDEF pCI expression vector construct or the parental pCI expression vector together with an AR expression vector and luciferase constructs containing the PSA promoter. Cells were incubated in the absence or presence of DHT.

FIG. 6. PDEF cooperates with AR in transactivation of the PSA promoter. Figure shows transcriptional activation of the PSA promoter by PDEF in the absence or presence of androgen. CV-1 cells were co-transfected with the indicated PDEF pCI expression vector construct or the parental pCI expression vector together with an AR expression vector and luciferase constructs containing the PSA promoter. Cells were incubated in the absence or presence of DHT.

FIG. 7. PDEF interacts with AR. A, PDEF binds to the AR DNA binding domain in GST pull-down assays. GST fusion proteins with different domains of AR are indicated above Fig. 4A and schematically in Fig. 4B. B, schematic representation of the AR fragments used as GST fusion proteins and of the GST pull-down results with PDEF. Functional domains of the AR are denoted above the diagram of full-length AR.

DISCUSSION

Normal function of the prostate gland requires proper differentiation of epithelial cells along a tightly controlled developmental pathway. This process is regulated by distinct sets of transcription factors, leading to a strategically timed switch on or off of specific sets of genes. Deregulation of this pathway leads to aberrant differentiation, immortalization, uncontrolled proliferation, and eventually prostate cancer formation.

While some aspects of prostate epithelium-specific gene expression have been elucidated, only a limited number of prostate epithelial cell-restricted transcriptional regulators have been characterized. The Ets transcription factor family plays a particularly important role in epithelial cells, as demonstrated by the isolation of three highly related epithelial-specific Ets factors, ESE-1, ESE-2, and ESE-3 (13–21). Each of these Ets factors, nevertheless, expresses unique expression patterns as well as distinct functions. PDEF is the factor with the most restricted expression pattern, and, whereas ESE-1, ESE-2, and ESE-3 are all members of the same subclass of Ets factors, PDEF is the sole mammalian member of its subclass. The sequence of the PDEF Ets DNA binding domain is significantly different from all other members of the Ets family, which is also reflected in its distinct DNA binding specificity. EMSA analysis of potential Ets binding sites within the PSA promoter demonstrated a significant preference for binding to sites containing a GGAT core rather than the preferred GGAA core typical for other members of the Ets family (12). Direct comparison of the relative DNA binding affinity of PDEF to other members of the Ets family using two oligonucleotides differing only in a single nucleotide change (GGAT versus GGAA) confirmed that PDEF strongly prefers interaction with GGAT containing binding sites, whereas other Ets factors such as ESE-1, ESE-2, ESE-3, NERF-2, ELF-1, and PU.1 have a striking preference for GGAA. This DNA binding specificity of PDEF is unique among the Ets family, although some Ets factors have been shown to interact with lower affinity with GGAT binding sites (19, 42). The distinct DNA binding specificity of PDEF also predicts that PDEF recognizes regulatory elements that are distinct from binding sites for other Ets factors, which is in contrast to the majority of Ets factors which all recognize very similar DNA sequences. The distinct DNA binding specificity of PDEF might be due to amino acid differences within helix 3 of the Ets DNA binding domain (Fig. 1B) (12). Helix 3 in PU.1, SAP-1, and Ets-1 has previously been shown to directly interact with DNA, and PDEF contains several unique amino acid substitutions that may influence DNA binding specificity (12). Further support for the difference between PDEF activity and other Ets factors is provided by the transactivation experiments demonstrating that PDEF, but not other Ets factors can transactivate the PSA promoter. ESE-1 and to lesser extent ESE-2 actually repress the PSA promoter. Since both PDEF and ESE-1 are expressed in prostate epithelium, PDEF and ESE-1 may play opposing roles in the regulation of prostate epithelium-specific gene expression. Distinct protein-protein interactions, subcellular location, and phosphorylation may modulate the activity of PDEF and ESE-1 and ultimately determine whether a gene is positively or negatively regulated by PDEF and ESE-1. Indeed, both PDEF and ESE-1 contain putative MAP kinase phosphorylation sites as well as sites for other types of kinases. In PDEF, the majority of MAP kinase phosphorylation sites are clustered at the amino terminus. Similar clusters of MAP kinase phosphorylation sites have been observed in the transactivation domains of other Ets factors such as ELK-1 and SAP-1, where they play a critical role in the regulation of transactivation capacity of ELK-1 and SAP-1 (10).

Northern blot analysis indicates the presence of high levels of PDEF transcripts almost exclusively in the prostate and significantly lower amounts in other hormone regulated tissues such as mammary gland, salivary gland, and ovary. Analysis of the public Est data base revealed in addition to prostate multiple entries for PDEF in libraries derived from breast, ovarian, lung, colon, and uterine cancer as well as oligodendroglioma, indicating that PDEF might be overexpressed in several types of cancer including from tissues whose normal counterparts do not express PDEF.

Our in situ hybridization and PCR experiments demonstrate that PDEF expression is restricted to the luminal epithelial cells within the prostate as well as LNCaP prostate cancer cells, the same cells that express PSA and AR. This unique expression pattern of PDEF indicates a function for PDEF in prostate gland development and possibly prostate cancer, and,
furthermore, suggests a connection between PDEF and androgen. The level of PSA expression is being widely used as a marker for prostate cancer, and its expression is restricted to epithelial cells of the prostate. The PSA gene is activated in the presence of androgen in normal prostate epithelium as well as in early stages of prostate cancer, and androgen ablation therapy is used at these stages to control prostate cancer growth. Therapeutic efficacy of androgen ablation-mediated prostate cancer regression is being monitored by the decrease in PSA expression, and it is the eventual rise of PSA gene expression that indicates the conversion of a previously androgen-sensitive prostate cancer to an androgen-resistant prostate cancer. Regulation of PSA gene expression in the androgen-sensitive phase of prostate cancer is at least partially regulated by an androgen-responsive regulator of the AR that can act both synergistically with the AR as well as androgen-independent in enhancing PSA gene expression. Our data support the notion that PDEF function may exemplify one mechanism of androgen-independence upon prostate cancer progression and the concomitant reactivation of PSA gene expression.

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