PART-1: A Novel Human Prostate-specific, Androgen-regulated Gene that Maps to Chromosome 5q12

Biaoang Lin, James T. White, Camari Ferguson, Roger Bumgarner, Cynthia Friedman, Barbara Trask, William Ellis, Paul Lange, Leroy Hood, and Peter S. Nelson


Abstract

Genes regulated by androgenic hormones are of critical importance for the normal physiological function of the human prostate gland, and they contribute to the development and progression of prostate carcinoma. We used cDNA microarrays containing 1500 prostate-derived cDNAs to profile transcripts regulated by androgens in prostate cancer cells. This study identified a novel gene that we have designated PART-1 (prostate androgen-regulated transcript 1), which exhibited increased expression upon exposure to androgens in the LNCaP prostate cancer cell line. Northern analysis demonstrated that PART-1 is highly expressed in the prostate gland relative to other normal human tissues and is expressed as different transcripts using at least three different polyadenylation signals. The PART-1 cDNA and putative protein are not significantly homologous to any sequences in the nonredundant public sequence databases. Cloning and analysis of the putative PART-1 promoter region identified a potential binding site for the homeobox gene PBX-1a, but no consensus androgen response element or sterol-regulatory element binding sites were identified. We used a radiation hybrid panel and fluorescence in situ hybridization to map the PART-1 gene to chromosome 5q12, a region that has been suggested to harbor a prostate tumor suppressor gene. These results identify a new gene involved in the androgen receptor-regulated gene network of the human prostate that may play a role in the etiology of prostate carcinogenesis.

Introduction

The AR pathway is a critical mediator of normal and neoplastic prostate development. During human embryogenesis, the AR is expressed in mesenchymal cells of the urogenital sinus with subsequent temporal expression in epithelial cells, leading to a differentiated epithelial phenotype and the production of prostate-specific proteins (1). Testosterone and 5α-dihydrotestosterone are the primary ligands activating the AR pathway. The majority of prostate cancers initially require androgens for growth, and the elimination of AR ligands by surgical or chemical castration leads to marked tumor regression through a mechanism of programmed cell death (2). However, surviving cancer cells lose their dependence on androgens over time and are capable of proliferation in the absence of serum androgens. The molecular events leading to androgen independence have not been defined, but potential mechanisms include overexpression of the AR, mutations in the AR gene leading to promiscuous ligand binding, and activation of the AR or downstream regulatory molecules by other endocrine or paracrine growth factors (3, 4).

Despite the importance of androgens for the proliferation and differentiation of normal and neoplastic prostate epithelia, relatively few downstream targets of the AR pathway have been identified. Of the prostate genes shown to be regulated by androgens, the serine proteases PSA and hK2 have proven to be extremely interesting from a biological standpoint, due in part to their prostate-specific expression profile and their potential role in the proliferation and progression of prostate cancers (5).

Our objective in this study was to identify genes expressed in human prostate cells exhibiting transcriptional regulation by androgens. We hypothesize that such genes could be direct mediators of the AR pathway or could be involved in prostate-specific functions that could be exploited for understanding normal and neoplastic prostate growth. We used a cDNA microarray hybridization approach to simultaneously analyze the levels of transcripts corresponding to 1500 different cDNAs derived from human prostate tissues. Transcripts expressed in the androgen-sensitive LNCaP prostate tumor cell line were quantitated for comparison under conditions of androgen depletion and androgen stimulation. Here we report the cloning, chromosomal mapping, and partial characterization of a novel gene, PART-1 (prostate androgen-regulated transcript 1), which exhibits no significant homology to functional domains of known genes or proteins. PART-1 is predominantly expressed in prostate, with a low level of expression in the salivary gland, and is regulated by androgens in human prostate cancer cells. These data provide evidence for another member of the prostate androgen response program that may provide further insight into normal and neoplastic prostate development.

Materials and Methods

Cell Culture and General Methods. DNA manipulations including transformation, plasmid preparation, gel electrophoresis, and probe labeling were performed according to standard procedures (6). The prostate carcinoma cell lines LNCaP, DU145, and PC3 were cultured in RPMI 1640 supplemented with 10% FCS (Life Technologies, Inc., Rockville, MD). LNCaP cells were transferred into RPMI 1640 with 10% CS-FCS (Life Technologies, Inc.) 24 h before androgen regulation experiments. This medium was replaced with fresh CS-FCS media or CS-FCS supplemented with 1 nM of the synthetic androgen R1881 (New England Nuclear Life Science Products, Inc.). Cells were harvested for RNA isolation at the 0- and 72-h time points.

Microarray Fabrication. A nonredundant set of 1500 prostate-derived cDNA clones was identified from the Prostate Expression Database, a public sequence repository of EST data derived from human prostate cDNA libraries (7). Five hundred of the selected cDNAs were derived from LNCaP cDNA libraries. Individual clone inserts were amplified by the PCR using 2 μl of bacterial transformant culture as template with primers BL_m13F (5′-GTA- AAAACCGGCCGATGATG-3′) and BL_m13R (5′-ACACAGGAAA-CAGCTATGACATT-3′) as described previously (8). PCR products were purified through Sephacryl S500 (Pharmacia), mixed 1:1 with DMSO (Amer-
sham), and spotted in duplicate onto coated Type IV glass microscope slides (Amersham) using a Molecular Dynamics GenII robotic spotting tool. After spotting, the glass slides were air-dried and UV-cross-linked with 500 mJ of energy and then baked at 95°C for 30 min.

**Probe Construction and Microarray Hybridization.** Total RNA was isolated from LNCaP cells after 72 h of androgen depletion or supplementation using TRIzol (Life Technologies, Inc.) according to the manufacturer’s directions. Polyadenylated RNA was purified using oligodeoxynucleotidic acid magnetic beads (Dynal). Fluorescence-labeled probes were made from 1 μg of polyadenylated RNA or 30 μg of total RNA in a reaction volume of 20 μL containing 1 μL of anchored oligodeoxynucleotidic acid primer (Amersham); 0.05 mM Cy3-dCTP (Amersham); 0.05 mM dCTP; 0.1 mM each dGTP, dATP, and dTTP; and 200 units of Superscript II reverse transcriptase (Life Technologies, Inc.). Reactants were incubated at 42°C for 120 min, followed by heating to 94°C for 3 min. Unlabeled RNA was hydrolyzed by the addition of 1 μL of 5 N NaOH and heating to 37°C for 10 min. One μL of 5 M HCl and 5 μL of 1 M Tris-HCl (pH 7.5) were added to neutralize the base. Unincorporated nts and salts were removed by chromatography (Qiagen), and the cDNA was eluted in 30 μL of distilled. One μg of DAdT 12–18 (Pharmacia) and 1 μg of human Cot1 DNA (Life Technologies, Inc.) were added to the probe, denatured at 94°C for 5 min, combined with an equal volume of 2x microarray hybridization solution (Amersham), and prehybridized at 50°C for 1 h. The mixture was then placed onto a microarray slide with a coverslip and hybridized in a humid chamber at 52°C for 16 h. The slides were washed once with 1× SSC, 0.2% SDS at room temperature for 5 min and then washed twice with 0.1× SSC, 0.2% SDS at room temperature for 10 min. After washing, the slide was rinsed in distilled water to remove trace salts and dried.

**Image Acquisition and Data Analyses.** Fluorescence intensities of the immobilized targets were measured using a laser confocal microscope (Molecular Dynamics). Intensity data were integrated at a pixel resolution of 10 μm using approximately 20 pixels/spot and recorded at 16 bits. Quantitative data were obtained with the SpotFinder V 2.4 program written at the University of Washington. Local background hybridization signals were subtracted before comparing spot intensities and determining expression ratios. For each experiment, each cDNA was represented twice on each slide, and the experiments were performed in duplicate producing 4 data points/cDNA clone/hybridization probe. Intensity ratios for each cDNA clone hybridized with probes derived from androgen-stimulated LNCaP and androgen-starved LNCaP cells were calculated (stimulated intensity/stained intensity). Gene expression levels were considered significantly different between the two conditions if (a) all four replicate spots for a given cDNA demonstrated a ratio of >2 or <0.5, and (b) the signal intensity was greater than 2 SDs above the image background. We have previously determined that expression ratios less than 1.5 are not reproducible in our system (data not shown).

**Northern Analysis.** Ten μg of total RNA were fractionated on 1.2% agarose denaturing gels and transferred to nylon membranes by using the capillary method (6). The human multiple tissue and master blots were obtained from CLONTECH. Blots were hybridized with DNA probes labeled with 32P-dCTP by random priming using the Random Primers DNA labeling kit (Life Technologies, Inc.) according to the manufacturer’s protocol. Filters were imaged and quantitated by using a phosphor-capture screen and Imagequant software (Molecular Dynamics).

**RACE.** RACE reactions were performed using the human prostate Marathon-ready cDNA cloning kit (CLONTECH) following the manufacturer’s instructions. Templates for RACE reactions were prostate Marathon-ready cDNA (CLONTECH) and androgen-stimulated or androgen-starved LNCaP cDNA prepared using the Marathon cDNA amplification kit (CLONTECH). Nested 5′-RACE reactions were performed with primers 14D7-196 L (5′-GTGACGGTCTTGGA-3′) and 14D7-85 L (5′-GTC-3′) and dTTP; and 200 units of Superscript II reverse transcriptase (Life Technologies, Inc.). Reactants were incubated at 42°C for 120 min, followed by heating to 94°C for 3 min. Unlabeled RNA was hydrolyzed by the addition of 1 μL of 5 N NaOH and heating to 37°C for 10 min. One μL of 5 M HCl and 5 μL of 1 M Tris-HCl (pH 7.5) were added to neutralize the base. Unincorporated nts and salts were removed by chromatography (Qiagen), and the cDNA was eluted in 30 μL of distilled. One μg of DAdT 12–18 (Pharmacia) and 1 μg of human Cot1 DNA (Life Technologies, Inc.) were added to the probe, denatured at 94°C for 5 min, combined with an equal volume of 2x microarray hybridization solution (Amersham), and prehybridized at 50°C for 1 h. The mixture was then placed onto a microarray slide with a coverslip and hybridized in a humid chamber at 52°C for 16 h. The slides were washed once with 1× SSC, 0.2% SDS at room temperature for 5 min and then washed twice with 0.1× SSC, 0.2% SDS at room temperature for 10 min. After washing, the slide was rinsed in distilled water to remove trace salts and dried.

**Results**

**Identification of a Novel Androgen-regulated cDNA PART-I by Microarray Hybridization.** Microarrays comprised of prostate-derived cDNAs were hybridized with total cDNA probes produced from androgen-stimulated and androgen-starved LNCaP prostate cancer cells. Four independent data points for each arrayed cDNA were generated because each cDNA was spotted twice per slide, and each hybridization was performed in duplicate. The hybridization ratios of 10 different cDNAs were consistently increased by >2-fold in androgen-stimulated cells relative to androgen-starved cells. We did not observe any cDNAs with consistent hybridization ratios of <0.5, a ratio that would indicate down-regulated expression. The genes induced by androgens included hK2 and hK3, also known as PSA (Fig. IA). The regulation of hK2 and PSA expression has previously been shown to be mediated by androgens through a mechanism involving ARE binding sites in the promoter regions of these genes (11, 12). In addition to hK2 and PSA, other cDNAs transcriptionally up-regulated by androgens in the microarray analysis were the serine protease TMPRSS2 (13, 14), the lipid metabolic genes acyl-CoA synthetase 3 and 3-hydroxy-3-methylglutaryl-CoA synthase, and six anonymous ESTs. The expression level of the cDNA clone corresponding to one of these ESTs, 14D7, increased 6-fold in androgen-stimulated LNCaP cells relative to androgen-deprived cells as assayed by microarray hybridization (Fig. IA). Sequence comparison against the nonredundant nt and protein subdivision of the GenBank database revealed homology only to an uncharacterized partial-length cDNA (GenBank accession number AL050198). We have named this cDNA **PART-1**.

**Androgen-regulated and Prostate-localized Expression of **PART-1.** The androgen-regulated expression of **PART-1** was confirmed by Northern analysis using the same LNCaP RNA that was used for the microarray analysis. Phosphorimage quantitation of the Northern blot demonstrated a 3.5-fold induction of **PART-1** expression after 72 h of androgen exposure (Fig. IB). **PART-1** expression

5 http://www.cbil.upenn.edu/tess/index.html.
increased 25-fold, and the expression of the G3PDH loading control did not change significantly (Fig. 2).

The distribution of PART-1 transcripts in normal human tissues was determined by Northern analysis. Of 16 adult tissues examined, a PART-1 message of 2.1 kb was predominantly expressed in the prostate, with no expression detected in the colon, lung, liver, kidney, pancreas, spleen, thymus, testes, ovary, peripheral leukocytes, heart, brain, placenta, or skeletal muscle (Fig. 2). Additional bands of smaller molecular weight were observed on the Northern blot and may represent cross-hybridization to genes homologous to PART-1 or represent alternate PART-1 transcripts as described below. The PART-1 expression profile was confirmed using a RNA Master dot blot (CLONTECH) comprised of RNA from 50 different tissues (data not shown). PART-1 expression was detected predominantly in the prostate with a low level of expression in salivary gland tissue, an expression pattern also described for PSA and the prostate-specific membrane antigen (15).

**PART-1 Cloning and Sequence Analysis.** The original PART-1 cDNA clone contains a 1.8-kb insert with a poly(A) region located at the putative 3′-end of the clone. Two rounds of 5′-RACE from prostate and LNCaP cDNA using PART-1-specific primers produced a 300-bp fragment from both cDNA sources. Sequence analyses of eight individual RACE clones selected from both cDNA templates revealed that all overlapped with the original PART-1 cDNA, and all originated with the same 5′ base. A total of 2109 bp was obtained, which corresponds to the 2.1-kb band seen on the Northern blot (Fig. 2). The cDNA sequence was submitted to GenBank under the accession number AF163475.

---

7 Nucleotide sequence data from this report have been deposited in GenBank under accession numbers AF163474 and AF163475.
The PART-1 cDNA encodes a putative 60-amino acid protein (Fig. 3). The translational start site AagATGC conforms to the Kozak consensus motif, RNNatG (where R is a purine and Y is a pyrimidine), in an adequate context (16). Polyadenylation signals, AAUAAA, were identified at nts 633 and 1558 3' of the TAG stop codon. A common natural variant of the polyadenylation signal, AAUAAA, were identified at nts 633 and 1558 3' of the TAG stop codon (17). The translational start site ATG codon is shown in bold. The putative protein has two protein kinase C phosphorylation sites and one tyrosine kinase site. We used the full-length PART-1 nt and the putative amino acid sequences to search the National Center for Biotechnology Information nonredundant sequence database using BLAST and BEAUTY algorithms. No significant homology to any database sequence was identified. Queries against the BLOCKS protein motif database (19) revealed only weak homology to the XPG_1 motif found in the DNA damage inducible gene Din7 from yeast (20) and in the XPG DNA repair endonuclease (Ref. 21; data not shown).

Cloning of the Promoter Region of PART-1 by Genomic Walking. Genomic walking with primers 14D7RC3 and API produced a 1.3-, a 2.3-, and a 0.8-kb band from the Dral, PvuII, and SspI human GenomeWalker libraries, respectively (data not shown). The 2.3-kb band from the PvuII library was cloned and sequenced. A Dral site and a SspI site were found in the sequences, and these sites would generate the respective 1.3- and 0.8-kb genome walking PCR bands from the Dral and SspI libraries.

The 2.3-kb sequence overlaps with the 5'-end of the PART-1 cDNA and extends 1969 bp 5' of the PART-1 translational start site (Gen-Bank accession number AF163474). A TATA box (TATAAAA) was identified at nt –562 (where +1 is the A of the ATG start codon). A putative transcriptional start site (TGTCTTCAAT) is located 30 nts 3' of the putative transcriptional initiation site. We were unable to identify an ARE consensus sequence in the cloned genomic DNA fragment. However, a binding site (TTCAGGAGG) for the homeodomain containing protein Pbx-1a (22) is located at nt –978, and a binding site (AAAGGAAAA) for nuclear factor of activated T cells (23) is located at nt –588 relative to the putative transcriptional initiation site.

Chromosomal Localization of 14D7. The Stanford G3 radiation hybrid panel was used to map the chromosomal location of PART-1 using primers 14D7mapR and 14D7mapF. Analysis of the typing results indicated that PART-1 is located closest to SHGC-14390 on chromosome 5 with a lod score of 18.0 and a cR 10,000 distance of 18 cR. SHGC-14390 is mapped between markers D5S2376 and D5S427, and the region, D5S624, was inconclusively mapped either to 5q12–13 or 5q13–14. Putative polyadenylation signals from the usage of the AAUAAA signal located 1558 nts from the TAG stop codon. Our PART-1 cDNA has the longest 3'-untranslated region and would derive from the usage of the AAUAAA signal located 2054 nts from the stop codon.

Several of these sequences, accession numbers AA410580, AA411616, and AA419011, have a poly(A) stretch that would arise from the usage of the AAUAAA signal located 633 nts from the TAG stop codon. EST AL245259 has a poly(A) stretch that would use the AAUAAA signal located 644 nts from the stop codon. EST AL050198 has a poly(A) stretch that would require the usage of the AAUAAA signal 1558 nts from the stop codon.

Discussion

Androgens influence vital aspects of prostate cellular growth and function including proliferation (24), apoptosis (2), differentiation (1),

---

Fig. 3. PART-1 cDNA sequence and putative protein translation. The predicted translational start site ATG codon is shown in bold. Putative polyadenylation signals ATTAAA and ATATTTAAA are underlined. The 3' polyadenylation signal present in the original PART-1 cDNA is double underlined.
lipid metabolism (25), and secretory action (12). Although these processes involve a complex series of metabolic steps, the direct androgen response gene expression program, as assessed by the transcriptional regulation of downstream genes, is limited. Studies designed to identify genes regulated by androgens in the rat prostate estimated that androgens increase the transcription of about 56 genes and decrease the transcription of less than 10 genes (26). This estimate does not include transient alterations in transcript levels, posttranscriptional regulation, or the potential cascade of molecular interactions set in motion by the initial transcriptional alterations.

Using a cDNA microarray-based approach we identified a novel gene, PART-1, as an androgen-induced gene in prostate adenocarcinoma cells. We have also shown that PART-1 exhibits a pattern of expression essentially restricted to the prostate and salivary glands. The identification of genes with selective expression in specific organs or cell types provides an entry point for understanding biological processes that occur uniquely within a particular tissue. In addition, the identification of genes and their cognate proteins whose expression is specific for the prostate has greatly aided the diagnosis and treatment of prostate carcinoma. The most useful of these proteins to date is PSA, a serine protease expressed by the luminal epithelium of normal prostate tissue and also produced by malignant prostate cells. If the tissue expression profile of the PART-1 protein corresponds to the transcript expression profile, then PART-1 may represent an additional target for prostate cancer diagnostic and therapeutic interventions.

The mechanism of androgen-mediated PART-1 expression remains to be characterized. The lack of a consensus ARE in the 5′-upstream regulatory region suggests that either the AR is interacting with nonconsensus DNA binding sites or an ARE(s) is located further upstream than the proximal 1.9-kb promoter-containing sequence we have analyzed. The AR NH₂ terminus (amino acids 142–485) has been shown to selectively bind to the basal transcription factors TFIIF and the TATA box-binding protein (27). PART-1 contains a TATA box sequence, and it is possible that the AR can bind to TFIIF and TATA box-binding protein and potentially recruit other transcriptional factors that regulate the expression of TATA box-containing genes, such as PART-1. A more likely hypothesis is that PART-1 may be regulated indirectly by androgens through the expression of an intermediary transcription factor. Such a mechanism has been shown for the regulation of several lipid-metabolizing enzymes that are regulated by sterol regulatory element-binding proteins that in turn are regulated by the activated AR.

The identification of a binding site for the homeodomain protein PBX-1a (22) in the PART-1 sequence suggests additional regulatory mechanisms. Homeobox genes are known to control the expression of many genes involved in development and organogenesis. PBX-1a may contribute to PART-1 regulation. A human homeobox gene, NKX3.1, which encodes a protein related to the *Drosophila* NK gene family, has been cloned and is restricted in expression to the prostate and testis (28). Of further interest, NKX3.1 expression is androgen-regulated in LNCaP cells (29). Functional studies will be required to determine whether PBX-1a or NKX3.1 activates PART-1 transcription. A DNA-binding site sequence for a protein hypothesized to direct prostate-specific gene expression has recently been described (30). However, the proposed prostate-specific binding site sequence, 5′-GAAAATATGATA-3′, is not present in our cloned PART-1 sequence upstream of the transcriptional start site. Thus, factors dictating the prostate-specific expression of PART-1 remain to be defined.

We have mapped PART-1 to chromosome 5q by radiation hybrid panel mapping and, more specifically, to chromosome 5q12.1 by FISH. Cytogenetic studies have identified deletions in chromosome 5q in prostate cancer cell lines (31) and in 39% of prostate cancer metastasis (32). The introduction of chromosome 5 into metastatic PC3 prostate cancer cells by microcell-mediated transfer decreases the cellular growth rate and suppresses their tumorigenic potential (33). These findings suggest that 5q might contain one or more tumor suppressor genes for human prostate cancer development, and PART-1 should be investigated as a potential candidate.

**Acknowledgments**

We thank Steve Lasky, John Hall, and the Molecular Biotechnology Sequencing Facility for DNA sequencing. We thank Robert Vessella for helpful discussions.
References


PART-1: A Novel Human Prostate-specific, Androgen-regulated Gene that Maps to Chromosome 5q12

Biaoyang Lin, James T. White, Camari Ferguson, et al.

Cancer Res 2000;60:858-863.

Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/60/4/858

Cited articles
This article cites 31 articles, 14 of which you can access for free at:
http://cancerres.aacrjournals.org/content/60/4/858.full#ref-list-1

Citing articles
This article has been cited by 14 HighWire-hosted articles. Access the articles at:
http://cancerres.aacrjournals.org/content/60/4/858.full#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.