[CANCER RESEARCH 61, 8122–8126, November 15, 2001]

Advances in Brief

T-Cell Receptor γ Chain Alternate Reading Frame Protein (TARP) Expression in Prostate Cancer Cells Leads to an Increased Growth Rate and Induction of *Caveolins* and *Amphiregulin*

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Abstract

Previously, we showed that prostate and prostate cancer cells express a truncated *T-cell receptor γ chain* mRNA that uses an alternative reading frame to produce a novel nuclear T-cell receptor γ chain alternate reading frame protein (TARP). TARP is expressed in the androgen-sensitive LNCaP prostate cancer cell line but not in the androgen-independent PC3 prostate cancer cell line, indicating that TARP may play a role in prostate cancer progression. To elucidate the function of TARP, we generated a stable PC3 cell line that expresses TARP in a constitutive manner. Expression of TARP in PC3 cells resulted in a more rapid growth rate with a 5-h decrease in doubling time. cDNA microarray analysis of 6538 genes revealed that *caveolin 1*, *caveolin 2*, *amphiregulin*, and *melanoma growth stimulatory activity α* were significantly up-regulated, whereas *IL-1β* was significantly down-regulated in PC3 cells expressing TARP. We also demonstrated that *TARP* expression is up-regulated by testosterone in LNCaP cells that express a functional androgen receptor. These results suggest that TARP has a role in regulating growth and gene expression in prostate cancer cells.

Introduction

Prostate cancer is second only to skin cancer as the most commonly diagnosed cancer, and is the second leading cause of death for men in the United States (1). The highest morbidity and mortality rates are in patients with hormone-refractory disease (2). The development of androgen-resistant prostate cancer in many patients has motivated researchers to investigate the molecular mechanisms behind the development of androgen resistance (2). In prostate cancer, it is hypothesized that altered gene expression in the epithelial cells results in abnormal epithelial-stromal cell signaling, leading to epigenetic changes in the stroma and subsequent uncontrolled proliferation of the epithelium (3). To better understand this process, it is important to identify genes that are specifically expressed in prostate cancer that may be involved in the oncogenic transformation of prostate cells.

Previously, we identified the expression of the *TCRγ*[3] gene in normal prostate and prostate cancer cells (4). By RNA *in situ* hybridization, we showed that *TCRγ* mRNA is highly expressed in epithelial cells of the prostate. In addition, the *TCRγ* transcripts found in prostate are different in size from those found in the thymus, spleen, and blood leukocytes (4). Prostate *TCRγ* transcripts encode a protein using an open reading frame different from that used to make the TCRγ chain. We therefore named this protein, "TCRγ alternate reading frame protein" (or "TARP"; Ref. 5). TARP was detected in the androgen-sensitive LNCaP prostate cancer cell line but not in the androgen-independent PC3 prostate cancer cell line (5). This result was intriguing, because prostate cells often lose their androgen sensitivity as their malignancy progresses. TARP is located in the nucleus of LNCaP cells (5). Analysis of the TARP amino acid sequence reveals that it contains five leucines in heptad repeats, suggesting that TARP contains a leucine zipper motif. In addition, TARP contains a region of basic amino acids, suggesting a possible DNA-binding motif (5). A protein BLAST search against GenBank indicates that TARP shares some homology to *Saccharomyces cerevisiae* thymidine uptake 1, a yeast protein involved in the repression of genes regulated by glucose, oxygen, and DNA damage (6). Hence, the nuclear localization of TARP, potential DNA-binding and dimerization motifs, and homology to a yeast transcriptional repressor suggests that TARP is involved in regulating gene expression in normal prostate and some prostate cancer cells. In this report, we demonstrated that expression of TARP in PC3 cells resulted in an increased growth rate and changes in expression levels of genes involved in regulating prostate cancer cell growth. We also demonstrated that *TARP* expression in LNCaP cells is regulated by androgens. These data suggest a potential role for TARP in prostate cancer progression.

Materials and Methods

Primers. The primers used were: TARP-forward (5′-CCCAAGCTTATGCAGATGTTTCCC-3′) and TARP-reverse (5′-AAACTCGAGTCATGGTGTTCCCC-3′); and β-actin forward (5′-ATCTGGCACCACACCTTCTACAATGAGCTGCG-3′) and β-actin reverse (5′-CTTCATACTCCTGCTTGCTGATCCACATCTGC-3′). Primers were synthesized by Lofstrand Labs Limited (Gaithersburg, MD).

Constructs. pcDNA5/FRT/TARP and pcDNA5/FRT/TARP were generated by inserting the TARP open reading frame into the pcDNA5/FRT (Invitrogen) vector in the sense and antisense (AS) orientations respectively.

Cell Culture. PC3 and LNCaP cells were maintained as described previously (5). For analysis of gene expression in response to androgen stimulation, 5×10^6 LNCaP cells were grown in the presence of steroid-depleted culture medium, phenol red-free RPMI supplemented with 5% charcoal/dextran-treated fetal bovine serum (HyClone), 2 mM L-glutamine, 10 mM HEPES, and 1 mM sodium pyruvate for 48 h. Cells were then treated with 0.1 or 10 mM DHT for the indicated time points and harvested, and mRNA was isolated using the Micro-FastTrack 2.0 kit (Invitrogen).

Generation of the PC3 Flp-In Host Cell Line and Respective Stable Cell Lines. The PC3 Flp-In host line and the respective stable cell lines were generated according to the Flp-In System manual (7). PC3-Vector, PC3-CAT, PC3-TARP, and PC3-TARP(AS) refers to the PC3 stable cell line harboring the pcDNA5/FRT, pcDNA5/FRT/CAT, pcDNA5/FRT/TARP, or pcDNA5/

Received 8/2/01; accepted 10/4/01.

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[3] TCRγ, T-cell receptor γ chain; TARP, TCRγ alternate reading frame protein; AS, antisense; DHT, dihydrotestosterone; CAT, chloramphenicol acetyl transferase; RT-PCR, reverse transcription-PCR; NCI, National Cancer Institute; PI, propidium iodide; CAV1, caveolin 1, CAV2; caveolin 2; AREG, amphiregulin; GRO1, melanoma growth stimulatory activity α; IL, interleukin; PSA, prostate-specific antigen; EGF, epidermal growth factor.

FRT/TARP(AS) construct, respectively. Similar stable cell lines were made using the 293 Flp-In host line, purchased from Invitrogen.

Preparation of Cell Extracts and Western Blot Analysis. Whole cell, nuclear, membrane, and cytoplasmic protein extracts were prepared as described previously (5). Western blot analyses were performed as described previously (5) using either 10 μg/ml ΔPE-TARP antiserum (5) or 1 μg/ml CAT antiserum (Sigma Chemical Co.).

RT-PCR. RT-PCR was performed using conditions described previously (5) using the TARP-forward, TARP-reverse, β-actin forward, and β-actin reverse primers.

Cell Growth Study. Cells (5×10^4) of each respective stable cell line were seeded into 100-cm^2 plates in triplicate for each day of a 5-day growth study. Every 24 h after the initial seed, the cell number from each plate was determined using a Coulter Z1 cell counter. During the study, the growth medium was not changed.

Flow Cytometry. Growing cells (1×10^6) derived from each stable cell line were fixed for 24 h in absolute methanol at -20°C, treated with 100 units of RNase (Qiagen) for 20 min at room temperature, and then stained with 20 μg/ml PI for 24 h at 4°C. The cell cycle profile of the PI-stained cells was then determined by a FACSCalibur reader and analyzed using the CellQuest and ModFitLT V2.0 software. Cells (2×10^4) from each stable line were analyzed.

cDNA Microarray Analysis. cDNA microarray analyses were performed using protocols found on the NCI/Center for Cancer Research microarray website.[4] ATC-6.4k-v6p11–030601 human array chips, containing 6538 genes, were purchased from the NCI. Hybridized arrays were scanned using an Axon GenePix 4000 scanner and processed using the GenePix software. The results were analyzed using tools found on the NCI/Center for Cancer Research microarray website.[4]

Northern Blot Hybridization. Northern blot hybridizations using either 20 μg of total RNA or 2 μg of mRNA were performed as described previously (4).

Results

Generation of PC3 Stable Cell Lines. The fact that TARP is expressed in the androgen-sensitive LNCaP cell line but not the androgen-independent PC3 cell line indicates that TARP may have a role in prostate cancer progression. To investigate the physiological function of TARP, we expressed TARP in a stable manner in PC3 cells using the Flp-In System. This system produces isogenic cell lines in which all transfected clones produce equivalent levels of the protein of interest, thereby eliminating the need to analyze multiple subclones (7). To ensure that any detected phenotype was not caused by an integration effect, we generated a cell line harboring the vector without any inserts. To ensure that any detected phenotype was not caused by the nonspecific overexpression of a protein, we generated a cell line that expresses CAT. To ensure that any detected phenotype was not caused by the expression the *TARP* sequence in general, we generated a cell line that expresses the *TARP* gene in the antisense (AS) direction. Because TARP is a nuclear protein (5), we tested for the presence of TARP in nuclear extracts derived from each cell line. Fig. 1*A* shows that TARP is expressed in nuclear extracts from the PC3-TARP cell line, but not in the three other control cell lines. Fig. 1*B* shows that CAT was only detected in whole cell extracts derived from the PC3-CAT cell line. Because the TARP(AS) line will not produce a detectable protein, we verified the production of *TARP(AS)* RNA by RT-PCR using primers that amplify *TARP* sense and antisense transcripts. Fig. 1*C* shows that *TARP* was detected in the PC3-TARP and PC3-TARP(AS) cell lines, but not in the other two cell lines. Because *actin* was detected in all cell lines, the absence of a *TARP* signal in the PC3-Vector and PC3-CAT cells was not attributable to differences in RNA amounts or RNA quality.

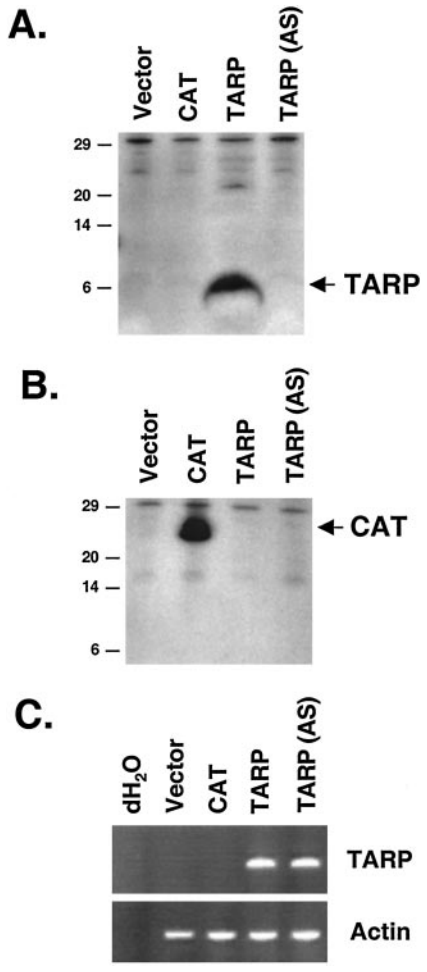


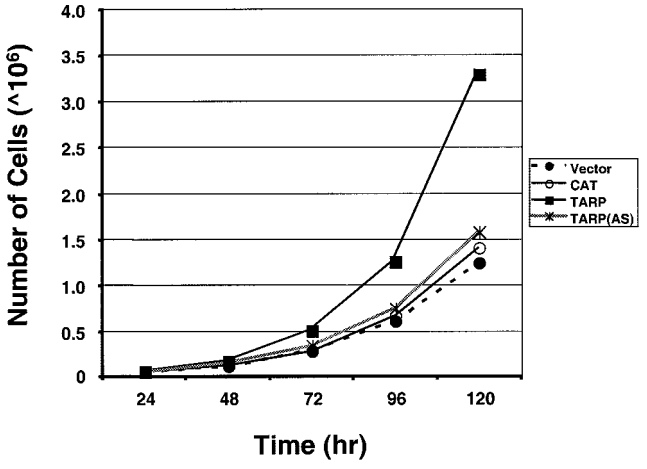
Fig. 1. Characterization of the PC3 stable cell lines. *A*, Western blot of nuclear extracts derived from each respective PC3 stable cell line. Forty μg of nuclear extract were run on a Tris/Tricine 16.5% polyacrylamide gel and probed with an antibody against TARP. Size markers in kDa are indicated on the *left*. An *arrow* on the *right* indicates TARP. *B*, Western blot of whole cell extracts derived from each respective PC3 stable cell line. Forty μg of protein extract were run on a Tris/Tricine 16.5% polyacrylamide gel and probed with an antibody against CAT. Size markers in kDa are indicated on the *left*. An *arrow* on the *right* indicates CAT. *C*, RT-PCR analysis of *TARP* mRNA expression. PCRs with primers that will recognize *TARP* expression in the sense and antisense directions (*top*) or *actin* primers (*bottom*) were performed with cDNAs derived from each respective PC3 stable cell line. RT-PCRs performed without templates are indicated as *dH$_2$O*. PCR products (20%) were run on a 1% agarose gel and visualized by ethidium bromide staining.

PC3 Cells Expressing TARP Have a Shorter Doubling Time. One common phenotypic change of cells undergoing oncogenic transformation is an increase in growth rate. To analyze the growth rates, cells derived from each PC3 stable cell line were seeded in triplicate for each time point, and the cell numbers were determined 24, 48, 72, 96, and 120 h after seeding. Cells were seeded at a density such that they would not reach confluence by 120 h. The growth medium was not changed to prevent the loss of mitotic cells. Total cell numbers at each time point were determined for each cell line, and their respective doubling times were calculated.

As shown in Fig. 2*A*, PC3-Vector, PC3-CAT, and PC3-TARP(AS) cells grew similarly. However, a dramatic increase in growth rate was observed for PC3-TARP cells. PC3-TARP cells had an average doubling time of 16.9 $\pm$ 1.3 h, whereas the PC3-Vector, PC3-CAT, and PC3-TARP(AS) cells had average doubling times of 22.6 $\pm$ 1.5, 22.5 $\pm$ 1.7, and 21.5 $\pm$ 1.4 h, respectively. Hence, expression of TARP in PC3 cells resulted in a markedly increased growth rate by decreasing their doubling time by more than 5 h. The increase in

[4] Internet address: http://nciarray.nci.nih.gov/.

A.



B.

	Vector	CAT	TARP	TARP(AS)
G_0/G_1	31.6	33.4	42.2	34.8
S	42.3	39.0	19.6	40.0
G_2/M	26.1	27.6	38.2	25.2

Fig. 2. PC3 cells expressing TARP have a shorter doubling time. *A*, cell growth study. Cells (5×10^4) of each respective PC3 cell line were seeded for each time point in triplicate. At each time point, the cells were harvested, and the total number of cells was determined. A representative of three experiments is shown. *B*, flow cytometry analysis. Asynchronous populations of each respective PC3 stable line were harvested, stained by PI, and analyzed using a FACSCalibur reader. 20,000 cells from each stable cell line were analyzed. Numbers indicate the percentage of cells found in that stage of the cell cycle. A representative of two experiments is shown.

growth rate seems to be prostate specific, because expression of TARP in 293 human embryonic kidney cells, cells lacking endogenous TARP expression, did not result in an increased growth rate (data not shown).

Because PC3-TARP cells have a shorter doubling time relative to the control cell lines, we investigated whether PC3-TARP cells have a different cell cycle profile compared with the other cell lines. Fig. 2*B* shows that the cell cycle profile of PC3-TARP cells was dramatically different from the profiles PC3-Vector, PC3-CAT, and PC3-TARP(AS) cells. One difference between the PC3-TARP cells and control cells is the percentage of cells found in S phase: only 20% of PC3-TARP cells were found in S phase, whereas ~40% of the PC3-Vector, PC3-CAT, or PC3-TARP(AS) cells were found in S phase. Hence, 2-fold fewer PC3-TARP cells were found in S phase as compared with the other lines. In conjunction with the increased growth rate, the data in Fig. 2*B* suggests that PC3-TARP cells have a shortened S phase.

Expression of TARP in PC3 Cells Results in Altered Gene Expression. To understand the molecular mechanisms behind the increased growth rate observed in PC3-TARP cells, we investigated whether the expression of TARP in PC3 cells alters gene expression. To do this, we compared the RNA expression profiles of PC3-TARP cells to PC3-Vector cells by cDNA microarray analysis. Because the fluorescently labeled Cy dyes are incorporated into newly synthesized cDNA with different efficiencies, analyses using cDNAs labeled in both combinations were performed to rule out the possibility of a labeling effect. In addition, we compared the RNA expression profiles of PC3-CAT with PC3-Vector cells to rule out the possibility that nonspecific overexpression of a protein in PC3 cells could result in the alteration of specific genes. Because there were no phenotypic differences between PC3-CAT and PC3-TARP(AS) cells, PC3-TARP(AS) cells were not analyzed in these studies. Genes that were either up- or down-regulated ≥2.5-fold in PC3-TARP cells, but not in PC3-CAT cells, were identified.

Fig. 3*A* shows the results of two complementary labeling experiments. In the *left panel*, cDNAs derived from PC3-Vector cells were labeled with Cy3 (*green*), and cDNAs derived from PC3-TARP cells were labeled with Cy5 (*red*). Genes up-regulated in PC3-TARP cells appear *red* on the array, and genes down-regulated in PC3-TARP cells appear *green*. In the *right panel*, cDNAs derived from PC3-Vector cells were labeled with Cy5 (*red*) and cDNAs derived from PC3-TARP cells were labeled with Cy3 (*green*). Thus, genes up-regulated in PC3-TARP cells appear *green*, and genes down-regulated in PC3-TARP cells appear *red*. Hence, the genes that appear red in the left panel should appear green in the right panel, and *vice versa*. Each spot on the array represents a unique cDNA fragment. Of the 6538 genes tested, 15 were up-regulated and 3 were down-regulated in PC3-TARP cells. The five most affected genes are shown. *CAV1*, *CAV2*, *AREG*, and *GRO1* were up-regulated in PC3-TARP cells, whereas *IL-1β* was down-regulated. Northern blots were used to verify the results from the cDNA microarray analysis. As shown in Fig. 3*B*, each cDNA microarray result was confirmed by its respective Northern blot. In addition, the altered expression of these genes was not caused by nonspecific protein expression, because no genes were as strongly altered in PC3-CAT cells as they were in PC3-TARP cells (Fig. 3*B* and data not shown). These results indicate that the changes in gene expression observed were specifically attributable

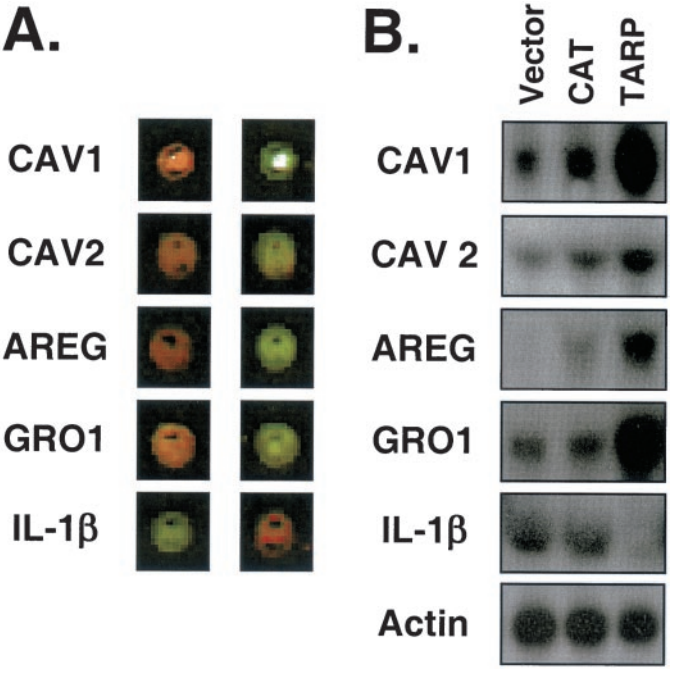


Fig. 3. Expression of *TARP* in PC3 cells results in altered gene expression. *A*, cDNA microarray analysis of gene expression in PC3-Vector cells *versus* PC3-TARP cells. Fluorescently labeled cDNAs derived from PC3-Vector and PC3-TARP total RNA were generated using Cy3- (*green*) or Cy5- (*red*) conjugated nucleotide analogues. These cDNAs were hybridized to a human ATC-6.4k-v6p11–030601 gene chip and analyzed using an Axon GenePix 4000 scanner. *Left panel: Vector*, Cy3; *TARP*, Cy5. *Right panel, Vector*, Cy5; *TARP*, Cy3. The hybridized spot relevant to each gene is shown. A representative of two experiments is shown. *B*, Northern blot analysis. Twenty μg total RNA for each PC3 cell line were run on a formaldehyde denaturing gel, blotted, and hybridized with a cDNA probe for each respective gene. The same filter was stripped and reprobed for all genes shown. The human *β-actin* RNA probe was used to verify equal loading. Exposure times for each gene are as follows: *CAV1*, 24 h; *CAV2*, 72 h; *AREG*, 24 h; *GRO1*, 24 h; *IL-1β*, 72 h; and *actin*, 4 h.

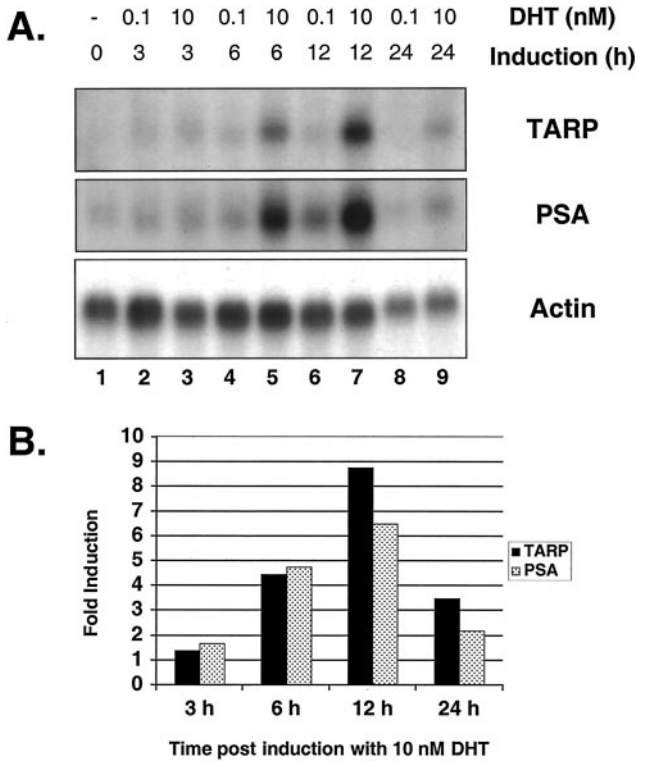


Fig. 4. TARP expression is regulated by androgens. Steady-state mRNA levels of *TARP*, *actin*, and *PSA* in LNCaP cells after 48 h of androgen deprivation (0 h) and at the specified time points (3, 6, 12, and 24 h) after being treated with 0.1 nM DHT (*Lanes 2, 4, 6*, and *8*) or 10 nM DHT (*Lanes 3, 5, 7*, and *9*). A representative of two experiments is shown. *A*, Northern blot analysis. Two μg poly(A) RNA for each time point were run on a formaldehyde denaturing gel, blotted, and hybridized with a cDNA probe for each respective gene. The same filter was stripped and reprobed for all genes shown. The human β-*actin* RNA probe was used to verify equal loading. Exposure times for each gene are as follows: *TARP*, 24 h; *PSA*, 6 h; and *actin*, 2 h. *B*, quantitation of the Northern blots. The signals in *A* were quantitated using the NIH Image 1.62 software. *TARP* and *PSA* signals were normalized against *actin*, and the fold induction by 10 nM DHT for each time point is shown.

to the presence of TARP in the cell. In addition, these results seem to be prostate specific, because no changes in expression were detected for these genes in similar experiments using TARP-expressing 293 stable cell lines (data not shown).

TARP Expression Is Regulated by Androgens. Two of the genes found to be induced in the PC3-TARP cells, *AREG* and *CAV1*, have been implicated in mediating androgen-stimulated cell growth in prostate cancer cells. AREG is involved in mediating androgen-stimulated proliferation of prostate cancer cells (8) whereas CAV1 is involved in promoting metastatic growth in prostate cancer cells (9). Because the expression of *AREG* and *CAV1* has been shown to be regulated by androgens (8, 9), we investigated whether the expression of *TARP* is also regulated by androgens. To do this, we used the androgen-sensitive LNCaP cell line. LNCaP cells were grown in androgen-depleted media for 48 h and then treated with either 0.1 nM or 10 nM DHT for the indicated time points. As shown in *Lane 1* of Fig. 4*A*, *TARP* is not expressed in LNCaP cells that were grown in androgen-depleted media. Because TARP is expressed in LNCaP cells grown under normal conditions (5), this result suggests that *TARP* expression is regulated by androgens. To support this claim further, we demonstrated that *TARP* is induced by DHT in a dose- and time-dependent manner. As shown in Fig. 4, *TARP* was maximally induced 12 h after 10 nM DHT treatment. As a positive control for the induction, we probed the same filter with a probe against *PSA*. Similar to *TARP*, *PSA* RNA was also induced by DHT in a dose- and time-dependent manner, with its levels being highest 12 h after 10 nM DHT treatment (Fig. 4). Because this experiment measures steady-state RNA, it does not determine whether androgens regulate *TARP* expression by increasing *TARP* RNA synthesis, decreasing *TARP* RNA degradation, or both. Preliminary experiments using a luciferase reporter gene driven by the TARP promoter indicate that DHT induces *TARP* RNA synthesis (data not shown).

Discussion

TARP is a nuclear protein found in normal prostate and in most, but not all, prostate cancers (5). To investigate the role of TARP in prostate cancer, we introduced TARP into the androgen-independent PC3 prostate cancer cell line and found that the cells grew more rapidly and had a specific increase in expression of *CAV1*, *CAV2*, and *AREG* and a decrease in the expression of IL-1β. Among the 6538 genes analyzed by cDNA microarray analysis, these genes were previously shown to have a role in prostate cancer growth and metastasis (8, 10, 11). We also found that testosterone treatment increased *TARP* mRNA levels in the androgen-responsive LNCaP cell line. These data indicate that TARP may have an important role in prostate cancer and raises many interesting questions.

The PC3 cell line is considered to be a very undifferentiated prostate cancer cell line. It was originally isolated from the bone marrow of a patient with prostatic adenocarcinoma (12). PC3 cells do not make PSA or respond to androgens, and they do not express TARP (5). Presumably, PCS cells have accumulated many mutations and epigenetic changes. Nevertheless, TARP expression produced changes in growth rate and gene expression in these cells, changes that have been previously described to be predictive of human prostate cancer and to be associated with an increased metastatic potential.

CAV1 is overexpressed in prostate cancer cells (13) and is considered to be a good prognostic marker for clinically confined human prostate cancer (14). CAV1 expression promotes survival of prostate cancer cells under conditions that can lead to cell death, including those encountered during metastasis and those generated by androgen withdrawal (11). Interestingly, suppression of CAV1 converted androgen-insensitive prostate cancer cells to an androgen-sensitive phenotype (11), implying that CAV1 is involved in promoting an androgen-insensitive, more malignant prostate cancer cell.

Androgen-stimulated autocrine loops are understood to contribute to hormone responsive growth and may provide a way for cancer cells to survive after steroid deprivation (8). One important autocrine loop in prostate cancer cells is the interaction between the EGF receptor and its ligands. The EGF receptor is the receptor for AREG, which induces its own expression through the EGF receptor pathway (8). Furthermore, AREG is expressed at increased levels in the androgen-independent PC3 and DU145 prostate cancer cell lines grown in androgen-depleted media (8). These data imply that AREG helps prostate cancer cells survive during androgen deprivation by inducing a self-sustaining autocrine pathway.

IL-1β has been shown to inhibit the growth of some prostate cancer cell lines. It partially suppresses growth of LNCaP cells when grown by themselves and completely inhibits the growth of LNCaP cells grown in coculture with WI-38 cells (10). However, IL-1β does not inhibit PC3 cell growth either in monoculture or coculture (10). This information has relevance to our study because IL-1β was found to be repressed in PC3-TARP cells, which grew faster than the control cell lines. We speculate that a possible mechanism for the increased growth rate in PC3-TARP cells involves repressing IL-1β expression. However, it is presently unclear why IL-1β levels would be repressed in PC3-TARP cells, and additional investigation is required.

Another interesting and unexpected finding was that TARP induced the expression of *GRO1*, a gene previously implicated in melanocyte

tumor progression (Ref. 15 and references therein). Treatment of Hs294T malignant melanoma cells with GRO1 resulted in a significant increase in growth rate (Ref. 16 and references therein), whereas treatment of melanoma tumor cells with antibodies against GRO1 inhibited growth in nude mice (17). The fact that GRO1 expression was elevated in PC3-TARP cells suggests that GRO1 may contribute to the increased growth rate in these cells. Currently, there is no documented relationship between GRO1 expression and prostate cancer progression. Interestingly, IL-1β increases the stability of *GRO1* by inhibiting the deadenylation of *GRO1* mRNA (18). However, in PC3-TARP cells, levels of *GRO1* increased, whereas levels of *IL-1*β decreased. This result suggests that *GRO1* levels in PC3-TARP cells are not dependent on the presence of IL-1β.

One question for future studies is whether TARP directly or indirectly affects the up-regulation of *CAV1*, *CAV2*, *AREG*, and *GRO1* and the down-regulation *IL-1*β in PC3-TARP cells. Several lines of evidence suggest that TARP probably affects gene expression. First, TARP is a nuclear protein expressed in LNCaP cells (5). Second, IL-1β is expressed in PC3-Vector cells but not in PC3-TARP cells. Importantly, IL-1β is not expressed in LNCaP cells that express TARP endogenously (19). The correlation of these two pieces of evidence suggests that IL-1β may be a direct target of TARP. Third, TARP contains five leucines in heptad repeats, indicative of a leucine zipper dimerization motif (5). In addition, a region of basic amino acids comes after the potential leucine zipper motif, suggesting that TARP contains a DNA-binding motif (5). The basic region in TARP may function as a nuclear localization signal, allowing TARP to interact with other DNA-binding proteins and thereby help regulate transcription.

Some lines of evidence suggest that the changes in gene expression observed in PC3-TARP may not be a direct, but instead an indirect effect of TARP expression. For example, TARP is expressed in normal prostate and LNCaP cells (4). However, CAV1 is expressed at very low to undetectable levels in normal prostate and LNCaP cells (Ref. 9 and data not shown), and AREG is not expressed in normal prostate (20). If the induction of *CAV1* and *AREG* in PC3-TARP cells were a direct effect of TARP expression, one would expect to see *CAV1* and *AREG* expression in normal prostate and *CAV1* expression in LNCaP cells. It is not yet known whether TARP expression results in *CAV1* or *AREG* induction in other prostate cancer cell lines. It is possible that the induction of *CAV1* and *AREG* by TARP may be specific to PC3 cells. Clearly, the molecular mechanisms behind the alteration of gene expression observed in PC3-TARP need additional study.

On the basis of the current results, it is not yet possible to establish the role of TARP in prostate cancer cell growth or normal cell growth. However, the results presented in this paper propose a pathway that links TARP expression to the modulation of genes involved in generating a malignant phenotype in prostate cancer cells. The question that remains is, why are the downstream components of this pathway inactive or blocked in normal prostate and LNCaP cells but active in PC3 cells? As discussed earlier, PC3 cells are very undifferentiated and presumably have accumulated many mutations and epigenetic changes. These changes would therefore cause PC3 cells to have a different cellular context than normal prostate and LNCaP cells. Because of the small size of TARP, it is unlikely that TARP regulates gene expression by itself. It must interact with other transcription factors or cofactors to regulate the expression of those genes involved in metastasis. It is conceivable that PC3 cells contain some factors and are missing others that are present in LNCaP and normal prostate epithelial cells, and *vice versa*. These factors might account for the results presented in this study. It is our goal to identify these factors to understand the possible role of TARP in regulating the growth of normal and prostate cancer cells.

In summary, we demonstrated that TARP is androgen-inducible and that its expression in PC3 prostate cancer cells results in an increased growth rate and altered expression of several genes that have an important role in prostate cancer.

Acknowledgments

We thank Drs. Byungkook Lee, Tapan Bera, Janendra Batra, and Rangan Maitra for their helpful comments and Lori Zbar for technical assistance.

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