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ORIGINAL ARTICLE

Androgen induces expression of the multidrug resistance protein gene MRP4 in prostate cancer cells

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Multidrug resistance-associated proteins (MRPs) may mediate multidrug resistance in tumor cells. Using a gene array analysis, we have identified MRP4 as an androgen receptor (AR)-regulated gene. Dihydrotestosterone induced MRP4 expression in both androgen-dependent and -independent LNCaP cells, whereas there was little detectable expression in PC-3 or normal prostate epithelial cells. Disruption of MRP4 expression renders LNCaP cells more sensitive to the cytotoxic effects of methotrexate but not etoposide. Analysis of human tissues showed detectable MRP4 expression only in metastatic prostate cancer. These results suggest that AR induction of MRP4 mediates resistance of PC cells to nucleotide-based chemotherapeutic drugs.
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Introduction

The overexpression of drug resistance-inducing proteins in many tumors suggests a possible molecular mechanism for unexplained failures of chemotherapy. The discovery that most cancer cells express genes which are involved in intracellular drug export has provided a possible explanation for cancer drug resistance.[1] For example, the multidrug resistance-associated protein 1 (MRP1), the founding member of the MRP family, was first identified in anthracycline-resistant human lung cancer cell line[2] and has been detected in a number of tumor types.[2]

The MRPs comprise a family of nine currently identified members.[3] MRPs are transmembrane ATP-binding cassette (ABC) transporters that function as efflux pumps. This family of proteins confers resistance to a variety of structurally diverse compounds. MRP1, also known as ABCC1, is the best characterized among the MRP family. MRP1 is structurally related to MRP2 (ABCC2/c-MOAT), MRP3 (ABCC3/MOAT-D), MRP6 (ABCC6/MOAT-E) and MRP7 (ABCC10). MRP4 (ABCC4/MOAT-B), based on its structure and substrate specificity, is closely related to MRP5 (ABCC5/MOAT-C), MRP8 (ABCC11) and MRP9 (ABCC12).[4] MRP1–3 and MRP6–7 have an extra N-terminal domain that is absent in MRP4–5 and MRP8–9.[5]

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MRP4 and MRP5 have been well characterized as transporters of modified nucleotide analogs.[6] The MRP4 tissue expression profile includes lung, muscle, pancreas, kidney and testis. In contrast, MRP5, a close structural homolog of MRP4, is ubiquitously expressed.[7] The MRP family has been reported to confer resistance to a range of anticancer drugs. In contrast, MRP4 can function as an efflux pump and confer resistance to the anti-retroviral compounds 9-(2-phosphonylmethoxyethyl) adenine (PMEA)[8] and azidothymidine monophosphate (AZTMP) and to methotrexate.[8] Like other MRP family members involved in organic anion efflux, PMEA and AZTMP are organic anion drugs, making MRP4 an organic anion transporter.[7] The biological functions of MRP4 are not known, but its ability to transport cyclic adenosine monophosphate and cyclic guanosine monophosphate (cGMP) suggests a possible role in cellular signaling.[9,10]

Androgens are steroid hormones that play key physiological roles in normal prostate development and cancer pathology (reviewed by Chatterjee[11]). Through the androgen receptor (AR), androgens are known to be intimately involved in either turning on or off the transcription of important genes with diverse cellular functional roles, ranging from cell growth, differentiation, cell cycle and apoptosis.[12] The regulation of these key biological processes by androgens and AR makes the AR an attractive candidate linked in the regulatory control processes of prostate cancer (PC) initiation and progression.

Using oligonucleotide gene array analysis in LNCaP cells, we have identified a number of both known and novel androgen-regulated genes. As expected, prostate-specific antigen (PSA), a well-established androgen-induced gene,[13] was among the known genes identified. MRP4 was among the novel genes identified. Both gene and protein expression assays support the androgen

regulation of MRP4 in LNCaP cells. Although MRP4 gene expression was not detected in either PC-3 or normal prostate epithelial cells, it was found in both androgen-dependent and, more importantly, androgen-independent LNCaP cells. Analysis of human prostate tissues showed detectable MRP4 expression only in metastatic PC. Disruption of MRP4 expression makes LNCaP cells more sensitive to the cytotoxic effect of methotrexate, but not etoposide, suggesting a substrate specificity for the MRP4 cell export function in PC cells.

Materials and methods

Affymetrix gene chip assay
C14 and A103 cells[14] were grown to 60–70% confluency in 10% fetal bovine serum (FBS) and then changed to FBS-free medium. After 24 h of incubation, cells were treated with either ethanol or 100 nM dihydrotestosterone (DHT). After 48 h of incubation, total messenger RNA (mRNA) was isolated and subjected to gene chip analysis using chips purchased from Affymetrix, Santa Clara, CA, USA (GeneChip Human Genome U95Av2 Array) according to the manufacturer's protocol.

Cell culture and siRNA transfection
LNCaP, C33, C81, PC-3 and stable cell lines C14, M37 and A103 cells were cultured as described.[14] Commercial small interfering RNAs (siRNAs) were obtained for MRP4 and negative control (both from Santa Cruz Biotechnology, Santa Cruz, CA, USA). X-tremeGENE siRNA transfection reagent was used to transfect siRNA into cells following the manufacturer's protocol (Roche, Basel, Switzerland).

For DHT treatment, cells were grown to 60–70% confluency in 10% FBS-containing medium and then changed to medium containing 2% FBS extracted with dextran-coated charcoal (DCC). After 48 h of incubation, ethanol or 100 nM DHT was added to the cells. After an additional 48-h incubation, the cells were subjected to either semiquantitative reverse transcription-polymerase chain reaction (RT-PCR) or Western blotting. Note that DHT was added 24 h after siRNA transfection.

Semiquantitative RT-PCR and real-time quantitative PCR analyses
RNA isolation was performed using the TRIZOL Reagent and subjected to either semiquantitative RT-PCR, as described previously,[14] or real-time quantitative PCR using Sybr Green (iSCRIPT from Bio-Rad, Hercules, CA, USA). The upstream and downstream primers, respectively, used for each gene were: MRP1, 5′-AGGT GGACC TGTTTCGTGAC-3′ and 5′-ACCCTGTGATC CACCAG AAG-3′; MRP2, 5′-CTGGTTG GGAACCTGA CTGT-3′ and 5′-CAACAGCCACAATGTTGGTC-3′; MRP3, 5′-GGC GTCTATGCTGCTTTAGG-3′ and 5′-CCTTGGA GAAGCAGTTCAGG-3′; MRP4, 5′-ACTGCACCGTGC TAACCATT-3′ and 5′-CTTCTGCCTTGCCAAGTTGT-3′; MRP5, 5′-ACCCGTTGTTGCCATCTTAG-3′ and 5′-GCTTTGACCCAGGCATACAT-3′; PSA, 5′-GCAGCATT GA ACCAGAGGAG-3′ and 5′-CCCATGACGTGATAC CTTGA-3′; AR, 5′-CAATGAGTACCGCATGCAC-3′ and 5′-GCCCATCCACTGGAATAATG-3; EZH2, 5′-CCTCTG AAGCAAATTCTCGG-3′ and 5′-CACAACCGGTGTTT CCTCTT-3′; E-cadherin, 5′-GAACGCATTGCCACATA CAC-3′ and 5′-GTGGTCAGCGGAA ACTTGAT-3′; and glyceraldehyde-3-phosphate dehydrogenase (GAPDH), 5′-CGACCACTTTGTCAAGCTCA-3′ and 5′-AGGGGA GATTCAGTGTGGTG-3′. GAPDH was used as a control for mRNA amount.

SDS-PAGE and Western blotting
Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and Western blotting were carried out as described.[14] The antibodies used were against MRP4 (Santa Cruz Biotechnology) or E-cadherin (a gift from Dr K Johnson). E-cadherin was used as a loading control for protein amount. Samples run on SDS-PAGE were crude membrane fractions prepared from LNCaP cells. These fractions were prepared by washing cells twice with ice-cold phosphate-buffered saline (PBS) and incubating in a hypotonic solution (15 mM Tris-HCl (pH 7.4), 1.25 mM $MgCl_2$ and 1 mM ethylenediaminetetraacetic acid (EDTA)) for 10–15 min at 4°C. Cells were harvested from the plates and centrifuged at 6000 r.p.m. for 10 min and resuspended in ice-cold transmission electron microscopic (TEM) buffer (50 mM Tris (pH 7.4), 0.5 mM EDTA and 10 mM $MgCl_2$) containing 1 mM freshly added phenylmethylsulfonyl fluoride and Protease Inhibitor Cocktail (Sigma, St Louis, MO, USA). Resuspended cells were lysed on ice by sonication (seven pulses of 15 s on/off) and lysates were centrifuged at 40 000 r.p.m. for 1 h. The resulting pellet was resuspended in TEM buffer and run on an SDS-PAGE gel.

Immunocytochemistry
LNCaP cells were grown on glass coverslips in 12-well plate with 500 μl medium. Culture medium was removed and cells were rinsed with warm PBS. Cells were fixed with 3.7% formaldehyde at room temperature for 15 min and then incubated with 2% Triton X-100 for 5 min. Then, the cells were incubated with 500 μl Image-iT FX signal enhancer (Molecular Probes, Leiden, The Netherlands). After rinsing with PBS, cells were incubated with 1:100 diluted anti-MRP4 antibody (Santa Cruz Biotechnology) at 37°C for 1 h and followed by incubating with 1:500 Alexa Fluor 488 secondary antibody (Molecular Probes) at 37°C for 1.5 h. The nuclear staining was applied with 300 nM 4′, 6-diamidino-2-phenylindole at 37°C for 5 min. After rinsing with PBS, the Antifade Reagent, proLong Gold (Molecular Probes), was used to mount the specimen for fluorescence microscopy. Note that all micrographs were taken at the same microscope settings.

Cell viability/toxicity assay
The cell proliferation experiments were performed as described previously using 2% DCC-extracted FBS.[14] Note that 100 nM DHT was used, which we have previously shown to be as active in LNCaP cell proliferation as 1 nM R1881.[14] Cells were seeded in 24-well plates and 2 days later were transfected with siRNA. After 1 day, cells were treated with ethanol or 100 nM DHT and 1–1000 μM methotrexate (Sigma) or 30 μM etoposide (Sigma). Cells were then incubated for an

additional 4 (methotrexate) or 3 days (etoposide) and quantified as described previously.[14]

Results

DHT regulates the expression of MRP4 in LNCaP cells

As LNCaP cells mimic the procarcinogenic effects of androgens, these cells were used in a gene microarray study to identify novel androgen-regulated genes. PC-3 cells stably transfected with AR (called A103 cells), exhibit anticarcinogenic effects of androgens[14–16] and thus were used as a negative control. Thus, we focused on androgen-regulated genes that were expressed in C14 (LNCaP cells stably transfected with empty vector) cells but not A103 cells. Performing a microarray study using the Affymetrix human genome chip Hu95A led to the identification of several known (e.g. *PSA* and *hKLK*2) and several unknown androgen-regulated genes (data not shown). MRP4 was among the novel genes.

To confirm the gene array results, we analyzed gene expression by semiquantitative RT-PCR analysis of RNA from C14, A103 and primary prostate epithelial (PrEC) cells. As shown in Figure 1a, MRP4 mRNA was strongly expressed in C14 cells, with substantially less in A103 cells and no detectable expression in PrEC cells. The expression in LNCaP cells was induced by DHT similarly to PSA, a hallmark androgen-induced gene. These results parallel our microarray findings and further confirm that, at the mRNA level, MRP4 expression is indeed positively regulated by DHT.

Real-time quantitative PCR was utilized to measure the magnitude of the DHT effect on MRP4 expression. One day of DHT treatment induced ETV1 expression by about 2.5-fold, and this induction increased to 4.5-fold with 2 days of DHT and decreased to about twofold after 3 days (Figure 1b).

To determine if DHT can increase MRP4 protein expression levels in LNCaP cells, immunofluorescence and Western blot experiments were performed. MRP4 is a transmembrane protein. In light of this, anti-MRP4 antibodies were used in probing endogenous MRP4 protein expression in intact LNCaP cells. Immunofluorescence results showed membrane staining for MRP4 (Figure 1c). Moreover, for those LNCaP cells incubated with DHT, more intense MRP4 straining was observed (Figure 1d). In addition to demonstrating its cell-surface localization, these results further affirmed MRP4 protein expression in PC cells. To measure more quantitatively the androgen induction of endogenous MRP4 protein, Western blot analysis was carried out using membrane fractions from LNCaP cells. Our results provide strong evidence that MRP4 protein levels are upregulated by DHT (Figure 1d). Together, these results reveal for the first time MRP4 upregulation by androgens in LNCaP PC cells.

Previous results from our lab have shown that the protooncoprotein c-Jun can coactivate AR-regulated gene expression.[14,17] In these earlier studies, we demonstrated that AR transcriptional activity can be enhanced by c-Jun(Ala63/73), a mutant protein that is deficient in activator protein-1 transactivation.[14,17] Interestingly, M37 cells, LNCaP cells that stably express this c-Jun mutant

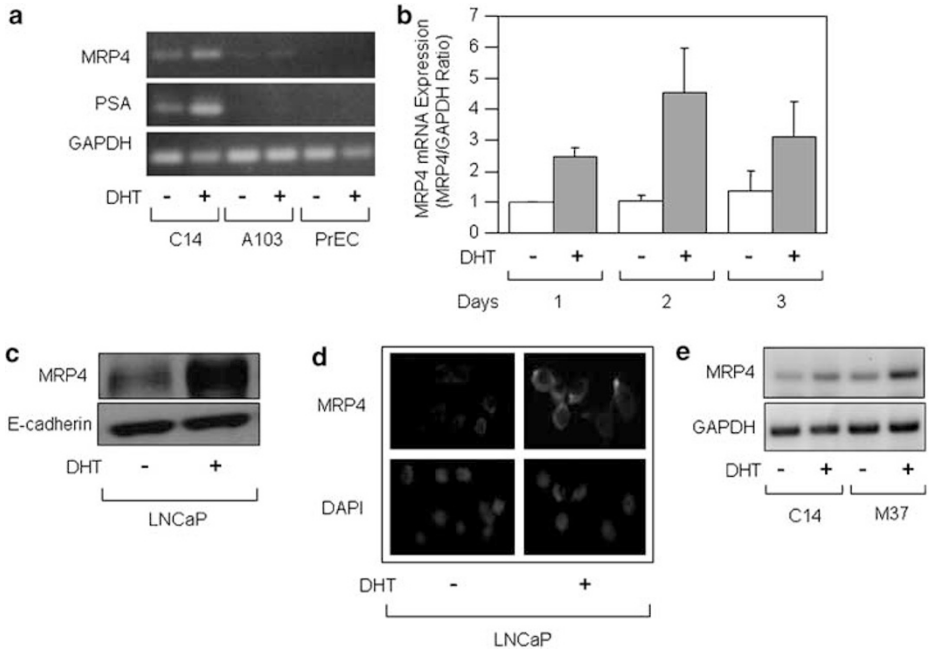

Figure 1 MRP4 mRNA and protein expression levels are enhanced by androgens in hormone-dependent LNCaP cells. Cells were treated with ethanol (−) or 100 nM DHT (+) and then subjected to several different analyses. (**a**) Semiquantitative RT-PCR was used to measure DHT-induced expression of MRP4 and PSA in C14 (LNCaP), A103 (PC-3) and PrEC cells. (**b**) Real-time quantitative PCR was used to measure MRP4 expression in response to 1, 2 or 3 days of DHT treatment. (**c**) Western blotting was used to measure DHT-induced expression of MRP4 in LNCaP cells. E-cadherin was used as a control for protein amount. (**d**) Immunocytochemistry was used to measure DHT-induced expression of MRP4 in LNCaP cells. 4′,6-Diamidino-2-phenylindole was used to stain cell nuclei. (**e**) Semiquantitative RT-PCR was used to measure DHT-induced expression of MRP4 in C14 and M37 (LNCaP cells expressing c-Jun(Ala63/73)).[14] Note that GAPDH was used as a control for mRNA amount in (**a**), (**b**) and (**d**).

protein, exhibit elevated DHT-induced expression of MRP4 as compared to C14 control cells, which are stably transfected with an empty vector (Figure 1e). This finding suggests that c-Jun coactivation can lead to enhanced expression of MRP4.

MRP4 is expressed in hormone-refractory PC cells

Localized PC is dependent on androgens, but after androgen blockade therapy, androgen-independent tumors usually develop.[11,12] C81 cells have been shown to mimic hormone-refractory PC.[18] As shown in Figure 2a, DHT induction of MRP4 mRNA expression is similar in androgen-independent C81 cells and the androgen-dependent parental C33 cells. Western blot analysis confirmed MRP4 protein expression in C81 cells, demonstrating a DHT induction of MRP4 protein in both C33 and C81 cells (Figure 2b). In agreement with RT-PCR (see Figure 1a), MRP4 protein expression in PC-3 cells was weak. These results demonstrate that DHT induces expression of MRP4 in both androgen-dependent and androgen-independent PC cells.

LNCaP cells express multiple members of MRP family

MRP4 is a member of a protein family that also includes MRP1, MRP2, MRP3 and MRP5,[3] all of which were measured by RT-PCR for expression in prostate cells. MRP1 and MRP5 are strongly expressed in LNCaP cells (C14), and more weakly expressed in PC-3 (A103) and PrEC cells (Figure 3). MRP2 is not detectably expressed in any of the cell lines and MRP3 is only expressed in PC-3 cells (Figure 3). Interestingly, the expressions of MRP5, a close MRP4 structural and functional homolog, and, to a less extent, MRP1 are downregulated by DHT in LNCaP cells (Figure 3). These data show that LNCaP cells express multiple members of the MRP family and that MRP4 is the only member that is uniquely expressed in LNCaP cells.

MRP4 depletion makes LNCaP cells more sensitive to methotrexate

MRP4 is a membrane pump involved in the efflux of nucleotide analogs. To elucidate MRP4 function in PC cells, experiments were designed to reduce endogenous MRP4 levels in LNCaP cells. Using MRP4 siRNA oligonucleotides, significant MRP4 mRNA and protein knockdowns were achieved, as compared to nonspecific control siRNA (Figure 4a and b). As shown in Figure 4a, the siRNA inhibitory effect on MRP4 mRNA expression was evident even after 4 days of DHT treatment. To test whether MRP4-depleted LNCaP cells are sensitized to chemotherapeutic drugs, LNCaP cells transfected with MRP4 siRNA oligos were treated with either methotrexate or etoposide. As shown in Figure 4c, siRNA-mediated reduction of MRP4 renders LNCaP cells significantly more sensitive to methotrexate-induced cell death, in both the absence and presence of DHT. On the other hand, siRNA transfection did not significantly alter the sensitivity of LNCaP cells to etoposide-induced death, at various concentrations of etoposide (Figure 4d and data not shown). Collectively, these results show that MRP4 knockdown sensitizes LNCaP cells to the cytotoxic effects of methotrexate but not etoposide.

MRP4 is expressed in prostate adenocarcinoma

To establish whether MRP4 is expressed in prostate tumors, MRP4 mRNA expression was analyzed by RT-PCR in normal, benign prostatic hyperplasia (BPH) and malignant prostatic cancer (MPC) tissues. Intriguingly, MRP4 mRNA expression was only detected in C1 and C2 MPC tissues (Figure 5), which also express significant levels of AR. The absence of measurable MRP4 expression in normal tissue is consistent with its absence in the normal PrEC cells (see Figure 1a). The expression of several other genes was measured to better characterize these tissues. PSA expression is high in both MPC and several BPH tissues. Notably, the expression pattern of

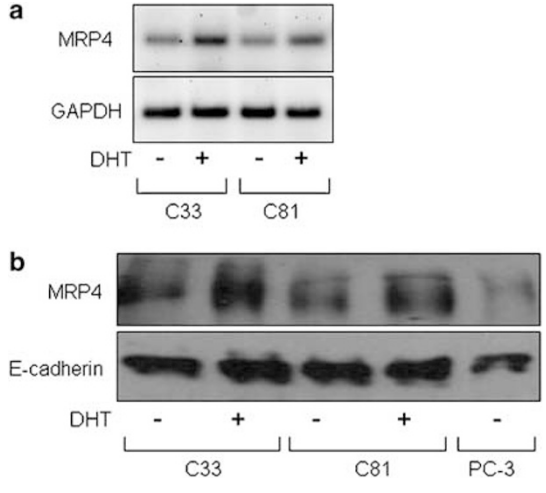

Figure 2 Androgen-independent LNCaP cells express MRP4. Cells were treated with ethanol (−) or 100 nM DHT (+) and then subjected to either (**a**) semiquantitative RT-PCR or (**b**) Western blotting to measure expression of MRP4 in C33 (androgen-dependent LNCaP parental cells), C81 (androgen-independent LNCaP cells) or PC-3 cells. Note that GAPDH (**a**) or E-cadeherin (**b**) was used as controls for RNA or protein amount, respectively.

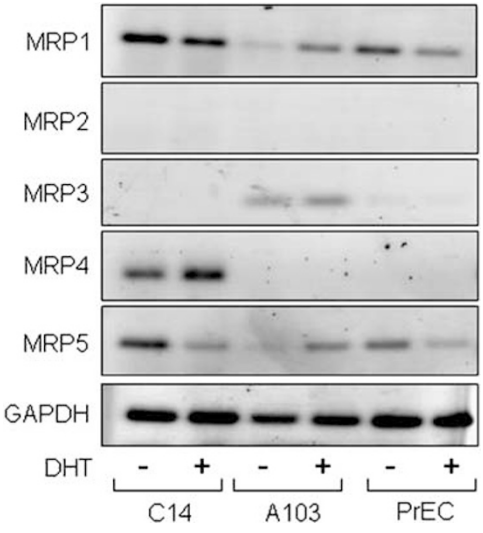

Figure 3 Multiple MRP family members are expressed in LNCaP cells. Semiquantitative RT-PCR was used to measure the expression of MRP1, MRP2, MRP3, MRP4 and MRP5 in C14, A103 and PrEC cells in the presence of either ethanol (−) or 100 nM DHT (+).

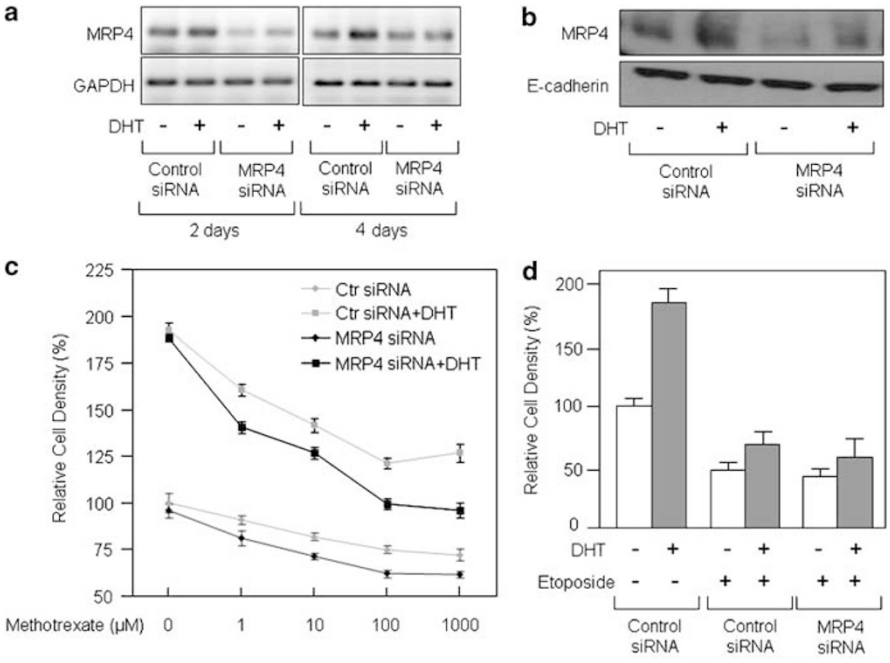

Figure 4 Disruption of MRP4 expression sensitizes LNCaP cells methotrexate-induced cytotoxicity. LNCaP cells were transfected with either MRP4 siRNA or an unrelated control siRNA and MRP4 expression was measured by semiquantitative RT-PCR (**a**) and Western blotting (**b**) after 2 (**a** and **b**) or 4 days (**a**) of DHT treatment. Note that GAPDH and E-cadherin were used as controls for RNA or protein amount, respectively. (**c**) LNCaP cells transfected with either MRP4 siRNA or control siRNA were treated with ethanol (−) or 100 nM DHT (+) and (**c**) different concentrations of methotrexate or (**d**) 30 μM etoposide. Note that cell density is given relative to the number of cells in the presence of transfected control siRNA without DHT or methotrexate (**c**) or (**d**), and this number was set to 100%. In both (**c**) and (**d**), data points represent averages of three independent experiments plus standard deviations.

MRP4 parallels that of EZH2, a gene marker for PC.[19] E-cadherin expression is significantly reduced in the BPH and MPC tissues as compared to normal, consistent with the literature.[20] All these data confirm that our tissue samples represent different stages of PC and demonstrate that MRP4 is highly expressed in malignant PC.

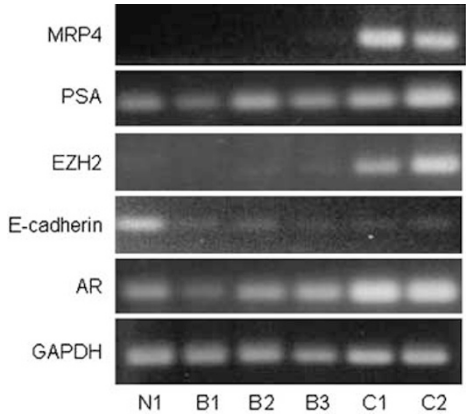

Figure 5 MRP4 mRNA is expressed only in prostate adenocarcinoma tissue. Total mRNA was isolated from prostate tissues (acquired from Cooperative Human Tissue Network) that are normal (N1), BPH (B1-B3) or malignant PC (C1, C2), and subjected to semiquantitative RT-PCR to measure the expression of MRP4, PSA, EZH2, E-cadherin and AR. GAPDH was used as a control for RNA amount.

Discussion

In this study, we report the identification of MRP4 as a novel androgen upregulated gene. Using the Affymetrix gene chip system, we have identified a number of known and novel androgen-regulated genes. From these microarray data, MRP4 was shown to be among the most highly androgen upregulated genes. Using semiquantitative RT-PCR, real-time quantitative PCR and Western blot experiments, we have validated the Affymetrix data on MRP4. As anticipated, increased mRNA expression levels were observed in DHT-treated LNCaP cells. In contrast, MRP4 mRNA is only weakly expressed in PC-3 cells and not detected in PrEC cells. These RT-PCR data were confirmed by both Western blot and immunocytochemistry analyses. Further, the immunocytochemistry experiments confirmed plasma membrane localization of MRP4 protein. Together, these results strongly argue that MRP4 is indeed an androgen upregulated gene.

Our findings above raise the question of how androgens and AR regulate MRP4 expression. To study the possibility that AR directly binds to the MRP4 promoter, we first analyzed the nucleotide sequence of MRP4 genomic DNA upstream of MRP4 exon 1. Utilizing an online DNA sequence analysis program (bimas.dcrt.gov/molbio/matrixs/), we identified by sequence three consensus androgen-responsive elements (AREs) that are found from 3–6 kilobase pairs (kb) upstream of exon 1. Surprisingly, chromatin immunoprecipitation assays failed to detect any AR binding to any of these three elements, in contrast to strong AR recruitment to the PSA promoter in the same experiment (data not shown). This result suggests that either AR is binding to other regions

of the MRP4 promoter or the AR effect is indirect. With regard to the former, sequence analysis of about 5 kb of genomic DNA failed to identify an other ARE-like sequences. As for an indirect AR effect, it is possible that AR activates a gene whose product is responsible for transactivation of MRP4. Interestingly, we have recently identified two novel androgen-regulated pathways in PC cells that may be involved in this process. The first pathway links androgens with elevated levels of cGMP (data not shown), which previously have been implicated in inducing MRP4 expression.[21] The other pathway demonstrates androgen induction of ETV1 (data not shown), a member of the Ets family of transcription factors whose activities have been associated with multidrug resistance-induced angiogenesis in hepatocarcinoma cell lines.[22] Future work can study the possible involvement of either pathway in MRP4 expression.

Another important question to arise from our expression data is what role does MRP4, a cell surface-expressed protein, play in PC. To begin addressing this question, we investigated the MRP4 mRNA expression profile in androgen-independent LNCaP cells and prostate adenocarcinoma tissues. Androgen induction of MRP4 expression was observed in androgen-independent LNCaP cells, as in androgen-dependent cells. This result mimics what we have observed for several known androgen-regulated genes, including PSA and TMPRSS2.[14] Regarding MRP4 expression in prostate tissues, detectable expression was only observed in malignant PC. These data suggest that high MRP4 expression offers PC cells, both androgen-dependent and -independent, some important function that is dispensable for normal prostate cells.

MRP4 belongs to a family of anionic ATP-dependent transporters that pump out of cells a wide array of anticancer drugs. In many types of cancers, several members of MRP family have been shown to induce drug resistance against chemotherapeutic drugs.[5] For example, MRP1 was identified based on its overexpression from an antracycline-resistant lung cancer cell line.[2] Another member, MRP2, has been shown to induce multidrug resistance in human hepatic cancer cells.[23] In ovarian carcinoma and human embryonic kidney 293 cells, MRP3 has been shown to confer resistance to diverse anticancer drugs including etoposide, vincristine and methotrexate.[24] In recent reports, MRP4 has been shown to induce resistance to topotecan,[25] the antiretroviral compounds, PMEA and AZTMP,[8] and methotrexate.[8] In contrast, viral expression of MRP4 in NIH3T3 cells does not induce resistance against a wide range of anticancer drugs including etoposide, vincristine, taxol and daunorubicin.[8]

To explore the role of MRP4 in LNCaP cells, endogenous levels of this protein were depleted using siRNA and cells were treated with etoposide, vincristine (data not shown) and methotrexate. In support of the previous data in other cells,[8] our results clearly demonstrate that decreased MRP4 expression does not alter the sensitivity of LNCaP cells to either etoposide or vincristine (data not shown). In contrast, sensitivity to methotrexate, a nucleotide-based chemotherapeutic drug, is significantly increased in cells with lower levels of MRP4. This finding supports an earlier report demonstrating MRP4-induced resistance to methotrexate in NIH3T3 cells.[8] It is important to emphasize here that LNCaP cells with depleted MRP4 levels still have high expression of MRP1 and MRP5 (data not shown), both of which confer resistance to methotrexate.[21] Thus, it is conceivable that the sensitivity of LNCaP cells to methotrexate can be greatly enhanced if the endogenous expressions of MRP1 and MRP5, as well as MRP4, are diminished. The expression of MRP4, MRP1 and MRP5 in LNCaP cells may imply functional redundancy by these proteins in PC cells. It is interesting, however, to note that our data show that in LNCaP cells androgens induce MRP4 expression, but inhibit the expression of MRP1 and MRP5, suggesting that these three MRP proteins may serve distinct functions. Whether this is reflective of what happens in prostate tumors remains to be seen.

Acknowledgements

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