Androgen Regulation of Multidrug Resistance-Associated Protein 4 (MRP4/ABCC4) in Prostate Cancer

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BACKGROUND. MRP4/ABCC4 is an ATP-binding cassette transporter expressed in normal prostate. This study aimed to define the pattern of MRP4/ABCC4 expression in normal and malignant prostate tissue and the relationship of MRP4/ABCC4 expression and function in response to androgen signaling.

METHODS. Eighty-four radical prostatectomy specimens from patients with localized prostate cancer (PC) (22 neoadjuvant androgen ablation, AA, 62 no AA), 42 non-cancer and 16 advanced PCs were assessed for MRP4/ABCC4 mRNA/protein expression. The effect of DHT and bicalutamide on LNCaP cells was assessed by immunoblotting. HEK293 cells (+/−MRP4/ABCC4) were assessed for the ability to efflux androgens and anti-androgens.

RESULTS. MRP4/ABCC4 mRNA/protein levels were higher in localized PC compared to non-cancer ($P = 0.006$). MRP4/ABCC4 levels were significantly decreased in PCs treated with AA compared to cancers exposed to normal testosterone levels ($P < 0.0001$). MRP4/ABCC4 expression in normal human tissues was limited to the prostate and the renal tubules. MRP4/ABCC4 protein levels increased in LNCaP cells after DHT which was partially blocked by bicalutamide. However, DHT did not alter the activation of the MRP4/ABCC4 promotor in luciferase reporter assays and testosterone, DHT, flutamide and hydroxy-flutamide were not substrates for MRP4/ABCC4.

DISCUSSION. Elevated MRP4/ABCC4 expression is found in malignant compared to benign prostate tissue while lower MRP4/ABCC4 expression is seen after AA. Furthermore, MRP4/ABCC4 is upregulated by androgen and downregulated by anti-androgen treatment in vitro potentially through an indirect mode of action. These data strongly suggest that MRP4/ABCC4 is an androgen-regulated gene important in the progression to PC and may be a potential drug target. *Prostate 68: 1421–1429, 2008.* © 2008 Wiley-Liss, Inc.

KEY WORDS: **prostate cancer; MRP4; androgen signaling**

No conflicts of interest are associated with this work.

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INTRODUCTION

Prostate cancer is the most commonly diagnosed cancer and a major cause of cancer death in men in Western countries [1,2]. Despite the prevalence of this disease, the precise mechanisms involved in prostate carcinogenesis and progression remain uncertain. The transition from normal to malignant prostate epithelial cells involves a number of genetic alterations in several different molecular pathways regulating normal cell proliferation and differentiation, in particular androgen receptor signaling [3]. As has been the case in other cancers such as breast [4], colon [5] and non-Hodgkin's lymphoma [6], a better understanding of the biology of prostate cancer is expected to lead to new treatments and biological markers of outcome.

ABC-type multidrug transporters mediate drug resistance by energy-dependent drug efflux from cells as well as influencing the pharmacokinetics of substrate drugs by directional transport across epithelial and endothelial membranes in the body. MRP4/ABCC4 is a member of the ABCC subclass that transports a variety of molecules, principally organic anions such as cyclic nucleotides cAMP and cGMP [7], prostaglandins [8,9], DHEAS and bile acids [7] but also many drugs including the CPT-11 metabolite SN-38 [10], topotecan [11], methotrexate [12] and anti-retrovirals, such as PMEA [12,13].

MRP4/ABCC4 was first implicated by our group in prostate carcinogenesis through transcript profiling experiments aimed at identifying prostate-specific genes upregulated in prostate cancer [14,15]. MRP4/ABCC4 mRNA was expressed more highly in prostate cancer compared to normal prostate tissue with a greater than threefold increase in expression. In addition, there was little expression in a normal body atlas which included 164 samples of 40 different normal tissue types suggesting MRP4/ABCC4 could be prostate specific [14,15]. MRP4/ABCC4 is known to transport estrogens and steroids [7,16], so it was hypothesized that it might also transport androgens and/or androgen receptor antagonists. Therefore, this study aimed to assess the expression of MRP4/ABCC4 mRNA and protein in benign and malignant prostate tissue and determine any relationships with androgen transport and action.

METHODS

Patient Cohort

Three cohorts of specimens were used in this study. Tissue microarrays were constructed from the archival formalin-fixed, paraffin-embedded tissue for all three cohorts as described previously [17]. Cohort 1: A cohort of archival formalin-fixed, paraffin-embedded specimens ($n = 84$) were collected from men treated with radical prostatectomy for localized prostate cancer at St Vincent's Hospital, Sydney (mean age 60 years, range 49–74 years). This cohort has a 12% relapse rate at a median follow-up of 43 months (9–71 months). Of those, 22 had received at least 3 months of androgen ablation therapy prior to surgery. Cohort 2: Paraffin-embedded tissue from 16 patients who underwent a transurethral resection of the prostate for prostate cancer in the setting of established bony metastases (Stage D2) were also assessed. Cohort 3: 14 types of normal body tissues, which were surplus to assessment in surgical specimens, were collected from 71 patients to form a normal body atlas. This project was approved by the St. Vincent's Campus Research Ethics Committee (H00/088).

In Situ Hybridization

MRP4/ABCC4 mRNA expression was assessed using in situ hybridization in a subset of the localized prostate cancer cohort ($n = 49$). A 425-base pair probe for PSA (nucleotides 819–1243) was used as a positive control for prostate tissue and a section of prostate processed with no probe as a negative control. A 424-base pair probe for MRP4/ABCC4 was derived from MRP4/ABCC4 cDNA (nucleotides 3786–4210). In situ hybridization was performed at a hybridization temperature of 60°C using the method described previously [18]. Expression was scored as positive or negative.

Immunohistochemistry

MRP4/ABCC4 was detected using the monoclonal antibody MI4–10 which does not cross-react with other MRPs [10]. Samples of kidney proximal and distal tubules (+/− antibody) and liver were included as positive and negative tissue controls, respectively [10]. Sections (4 μm) were dewaxed and rehydrated prior to unmasking in a waterbath for 20 min using pH 9.0 Target Retrieval Solution (DAKO, Carpinteria, CA). Endogenous peroxidases and biotin were blocked with 3% hydrogen peroxide and biotin blocking system (DAKO), respectively. Non-specific binding sites were blocked with protein block (DAKO) for 10 min. The sections were incubated with anti-MRP4/ABCC4 1:50 for 30 min at room temperature, followed by the secondary antibody, biotinylated rabbit anti-rat 1:200 (Vector Laboratories, Burlingame, CA). Chromogenic detection and counterstaining was performed as described previously [18]. Immunostaining was initially assessed by one investigator (L.G.H.) and subsequently reviewed independently by a histopathologist (J.G.K.). Both cytoplasmic and membranous (plasma membrane) staining patterns were observed

but only membranous expression was analyzed as MRP4/ABCC4 functions only as a membranous protein [12]. Membranous immunoreactivity was scored as a percentage of the total number of either carcinoma or benign cells. Non-cancer prostate tissue was defined as benign prostatic epithelium adjacent to cancer in the peripheral zone. Each element in the tissue microarrays was scored separately and the results averaged across all the cores present for an individual patient to determine their score for non-cancer and cancer. The inter-observer Spearman rank coefficients for MRP4/ABCC4 expression was 0.72. All major discrepancies in scoring were reviewed and a consensus reached.

Treatment and Immunoblotting of LNCaP Cells

LNCaP cells from ATCC were cultured in RPMI-1640 medium with 10% supplemented calf serum, 100 U/ml penicillin and 100 μg/ml streptomycin. Log phase cells were resuspended in similar medium containing serum that had been charcoal-stripped to remove androgen, plated at a density of 10^6 cells per well in 6-well plates and allowed to attach overnight. Thereafter, cells were maintained for 48 hr under one of five conditions, each in triplicate: medium alone, medium plus 1 μM 5α-dihydrotestosterone (DHT), medium plus 10 μg/ml bicalutamide, bicalutamide on day 1 followed by DHT on day 2, or DHT on day 1 followed by bicalutamide on day 2. Media plus additives were replenished each day. On the third day, the plates were placed on a bed of ice and cells harvested by scraping.

Cell pellets were lysed in buffer containing 0.5% SDS, 10 mM Tris pH 7.4, 10 mM KCl, 1.5 mM $MgCl_2$ and a protease inhibitor cocktail (Sigma, St. Louis, MI). Lysates were sonicated on ice to shear DNA. Protein concentrations were quantified by Bradford assay. Lysates were mixed with equal volumes of 2× Laemmli buffer, warmed to 37°C for 5 min and fractionated by SDS-PAGE on 8% gels. Proteins were transferred to PVDF membrane by electroblotting. Blots were cut into 3 parts according to molecular weight markers, probed respectively for MRP4/ABCC4 using monoclonal antibody M4I-10 [10] at 1:500, for prostate-specific antigen (PSA) as a positive control for androgen-induced activity using monoclonal ER-PR8 (DAKO) at 1:250, and for α-tubulin as a loading control using monoclonal YL1/2 (AbCam, Kilmartin) at 1:5000. Bound monoclonal antibodies were detected with horseradish peroxidase-linked secondary antibodies (Santa Cruz, Santa Cruz, CA) and a chemiluminescence substrate (Pierce, Milwaukee, WI), visualized and quantified on a Kodak 4000 MM imaging station. These experiments were performed in triplicate and confirmed by independent replication.

Androgen Accumulation Assay

HEK293 parent cell line and HEK293 cells transfected with an MRP4/ABCC4 expression vector [19] were used for this experiment. Log phase cells were plated at a density of 1×10^6 per well in 6-well plates and allowed to attach overnight; attachment was improved by inclusion of $MgCl_2$ at 20 mM. The following day the growth medium was replenished with medium containing either ^{3}H-substrate (0.5 μCi/ml), the same plus 50 μM dipyridamole (an inhibitor of ABCC proteins), or vehicle only (ethanol, final concentration <0.1%). Experiments were performed using testosterone, dihydrotestosterone, flutamide and hydroxyflutamide as the substrate of interest. Plates were returned to a humidified incubator for 60 min. The plates were then placed on ice, media aspirated, cells washed twice with ice-cold PBS + 1% FBS, and then solubilized with 0.5 ml Solvable (PerkinElmer, Boston, MA) by gentle shaking at room temperature for 2 hr. Tritiated substrate in the lysates was quantified by scintillation counting. All experiments were performed in triplicate and independently replicated.

MRP4/ABCC4 Promoter Activity Assays

The assays utilized the Promega Dual Luciferase system which includes a *Renilla* luciferase control for transfection efficiency (pRL-TK). The firefly luciferase reporter contained a human MRP4/ABCC4 promoter sequence excised from BAC clone RP11-789G22, running from the AlfI site 20 bp 5′ of the ATG to the *Xba*I site 2.5 kB upstream, inserted into pGL3 (Promega). The shorter (0.8 kb) promoter was the proximal *Xho*I fragment. The reporters were active in cell lines that express MRP4/ABCC4. Log phase LNCaP cells (ATCC) were plated at 10^5 cells per well in 24-well plates in RPMI-1640 plus 10% FBS that had been charcoal-stripped to remove androgens. Cells were allowed to attach overnight and then co-transfected using Lipofectamine 2000 (Invitrogen, Rockville, MA) with 0.16 μg of either pGL3, pGL3-MRP4/ABCC4(0.8 kb) or pGL3-MRP4/ABCC4(2.2 kb), plus 0.64 μg of pRL-TK, i.e. in a ratio of 1:4. Dihydrotestosterone was added as required 6 hr later to 1 μM final concentration. Luciferase activity was monitored 24 hr later, using an automated system for substrate injection and timed readings on a Wallach Victor Plate Reader I. All assays were performed in triplicate.

Statistical Analysis

Paired and unpaired non-parametric tests were used to assess differences between expression patterns—

Wilcoxon signed rank and Mann–Whitney *U* tests, respectively. Chi-squared test was used to assess the relationship between MRP4/ABCC4 expression and clinicopathological features. Kaplan–Meier analysis was used to assess the association with relapse-free survival. *t*-Tests were used to assess the differences between levels of protein seen on immunoblotting. A *P* value of <0.05 was required for significance. All reported *P* values are two-sided. Statistical analyses were performed using Statview 4.5 software (Abacus Systems, Berkeley, CA) and PRISM (GraphPad Software, San Diego, CA).

RESULTS

MRP4/ABCC4 mRNA Expression in Prostate Cancer

In order to validate our transcript profiling data [14,15], in situ hybridization was performed on a subset of this profiled prostate cancer cohort. The whole cohort was not used as the processing of the in situ hybridization resulted in significant tissue loss thus quantitative data was only available on 49 cases. Expression was observed in the epithelium of prostate tissue. Half of the cancers (57%) expressed MRP4/ABCC4 mRNA and there was a statistically significant decrease in the expression of MRP4/ABCC4 mRNA in adjacent non-cancer tissue ($P < 0.001$) (Table I) (Fig. 1A,B).

MRP4/ABCC4 Protein Expression in Prostate Cancer

All types of prostate epithelium demonstrated at least some membranous and cytoplasmic expression of MRP4/ABCC4 protein. The percentage of cells with membranous MRP4/ABCC4 expression was highest in localized prostate cancers from men with normal testosterone levels (Fig. 2B) when compared with a significant decrease in paired non-cancer prostate tissue ($P = 0.006$) (Table II, Fig. 2A). Prostate cancer and non-cancer prostate tissue from men who were treated with androgen ablation therapy pre-operatively had a significantly lower level of MRP4/ABCC4 expression compared to uncastrated prostate cancer patients suggesting an effect of androgen on MRP4/ABCC4 levels (Table II, Fig. 2C,D). Furthermore, MRP4/ABCC4 expression was also lower in advanced prostate cancer (Table II). There was no correlation between MRP4/ABCC4 expression and known clinicopathological prognostic factors such as Gleason score ($P = 0.4$), Gleason grade ($P = 0.1$) and pathological stage ($P = 0.3$). There was also no statistical association with biochemical relapse-free survival (HR 2.2, 95% CI 0.3–18.6, $P = 0.5$) although it was interesting to note that all the relapses so far have occurred in the group with >50% MRP4/ABCC4 expression. Analysis of the normal tissue expression, demonstrated very limited MRP4/ABCC4 protein expression except in prostate, kidney (distal and proximal tubules) and scattered in Leydig cells of the testis (Table III).

Androgen Regulation of MRP4/ABCC4 Expression in Vitro

Given the human data suggesting androgen-regulation of MRP4/ABCC4, the effect of testosterone on MRP4/ABCC4 expression in vitro was assessed. LNCaP cells, which contain a functional but mutated androgen receptor, were used as the model system. MRP4/ABCC4 expression was upregulated with DHT treatment ($P = 0.0016$) but decreased with the anti-androgen treatment, bicalutamide ($P = 0.026$) (Fig. 3A,B). Sequential treatment with bicalutamide followed by DHT prevented upregulation of MRP4/ABCC4 while bicalutamide could also decrease MRP4/ABCC4 after DHT treatment (Fig. 3A,B). PSA expression as a marker of androgen action was appropriately upregulated following androgen stimulation and this effect was totally abrogated in the presence of bicalutamide.

An MRP4/ABCC4 promoter luciferase reporter construct transfected into LNCaP cells was active but its activity was not altered by adding DHT (Fig. 4). The reporters used included the proximal 0.8 kb 5′ of the ATG and the 2.2 kb region 5′ of the ATG in MRP4/ABCC4 exon 1. Neither reporter demonstrated activation of the promotor by DHT despite clear effects on protein expression as evidenced by the immunoblotting results (Fig. 4). Analysis of the MRP4/ABCC4 genomic sequence revealed putative androgen receptor binding sites in isolated positions quite distal to the transcription start site the nearest ones being

TABLE I. MRP4/ABCC4 mRNA Expression in Benign and Malignant Prostate Tissue

	Number of cases positive for MRP4/ABCC4 mRNA	Difference between PC and non-cancer prostate tissue
Localized PC (n = 49)	28/49 (57%)	$P < 0.0001$
Non-cancer prostate tissue (n = 43)	8/43 (17%)	

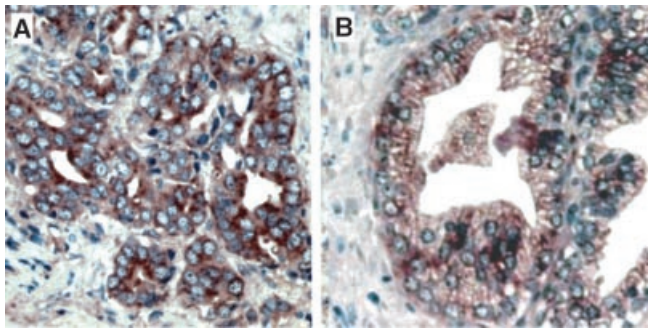

Fig. 1. MRP4/ABCC4 mRNA expression in benign and malignant prostate tissue. Photomicrographs of MRP4/ABCC4 mRNA expression in (**A**) prostate cancer and (**B**) non-cancer prostate tissue by in situ hybridization (200×).

at −3156 and +2753 relative to the start of exon 1 consistent with previous data showing no androgen receptor binding to the MRP4/ABCC4 promotor [20]. This argues against direct regulation of the MRP4/ABCC4 promoter even though androgen response elements are known to be able to act as distal enhancers.

Androgens as a Substrate for MRP4/ABCC4

In order to further investigate the role of MRP4/ABCC4 in prostate carcinogenesis, experiments were designed to assess whether androgens or anti-androgens are substrates for MRP4/ABCC4 as androgen signaling is the major pathway driving prostate carcinogenesis. HEK293 cells +/− MRP4/ABCC4 expression were treated with androgen (testosterone or the active metabolite, dihydrotestosterone) or anti-androgen (flutamide or the active metabolite, hydroxyflutamide). Figure 5 demonstrates that all four substrates increased their own cellular uptake into HEK293 cells but that MRP4/ABCC4 overexpression or blockade with dipyrimidole does not alter the accumulation of testosterone, dihydrotestosterone, flutamide or hydroxyflutamide. These findings suggest that modulation of the intracellular levels of these compounds is not mediated by MRP4/ABCC4.

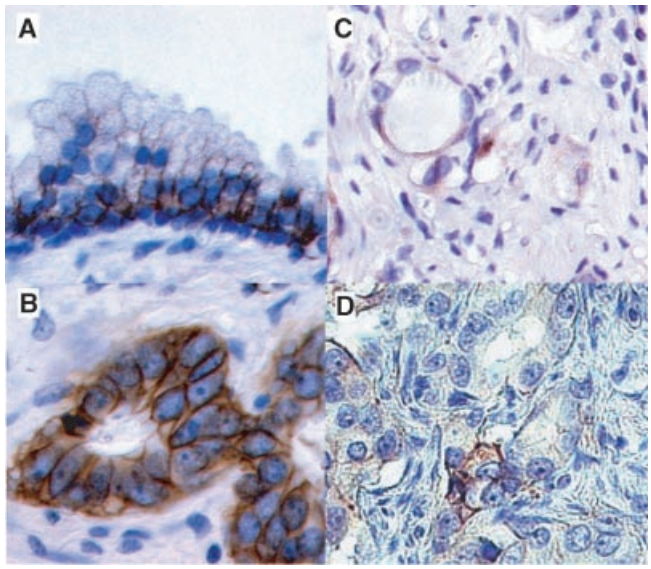

Fig. 2. MRP4/ABCC4 protein expression in benign and malignant prostate tissue. **A**: Membranous MRP4/ABCC4 staining in <10% non-cancer prostate cells (400×). **B**: Membranous MRP4/ABCC4 staining in >95% of PC cells in a patient with normal testosterone levels (400×). **C**: Membranous MRP4/ABCC4 staining in <10% PC cells from a patient treated with neoadjuvant androgen ablation therapy (400×). **D**: Membranous MRP4/ABCC4 staining in <10% of PC cells in a patient with normal testosterone levels (400×).

DISCUSSION

The immunohistochemical analysis presented herein confirms that the prostate is one of the major sites of MRP4/ABCC4 expression in the body and is the first study to demonstrate that elevated expression is associated with progression to prostate cancer. Although a previous study demonstrated androgen-induction of MRP4/ABCC4 in vitro [20], our study shows that anti-androgen treatment decreases the level of MRP4/ABCC4 expression both during 48 hr culture in vitro and after long term androgen deprivation in human prostate cancers. This correlation between the clinical and in vitro data has not been demonstrated previously and provides further evidence for a potential role for MRP4/ABCC4 in prostate carcinogenesis.

Furthermore, we have demonstrated that androgens and anti-androgens, the presumed substrates, are not transported by MRP4/ABCC4 although they enhance its activity. Although classically sex steroids and their analogues were thought to enter cells by passive diffusion across lipophilic membranes, recent studies have shown that protein-bound cellular uptake is an important operative mechanism [21]. It is also notable that the anti-androgens flutamide and its major active metabolite hydroxyflutamide increased their own cellular uptake. It remains possible that the sulfated conjugates are substrates, as is the case for estradiol and dehydroepiandrosetrone 3-sulfate (DHEAS) [16], but the latter are inactive forms which can be desulfated into active steroids [22]. Numerous studies have failed to find an association between circulating DHEAS levels and prostate cancer risk [23], while in vitro studies have also shown no effect of DHEAS treatment on the rat prostate [24]. Whether this provides any basis for the cancer promoting effects of DHEAS is uncertain.

Although protein expression of MRP4/ABCC4 was increased by DHT treatment, there was no effect on MRP4/ABCC4 promoter activity consistent with the

TABLE II. Protein Expression of MRP4/ABCC4 in Benign and Malignant Prostate Tissue

Pathology	Mean % of cells with membranous expression ± SEM	*P*-value compared to localized PC
Non-cancer prostate tissue[a] (n = 42)	56 ± 3	0.006
Localized PC[a] (n = 62)	74 ± 3	—
NHT PC[b] (n = 22)	39 ± 7	<0.0001
NHT non-cancer prostate tissue[b] (n = 20)	10 ± 5	<0.0001
D2[c] (n = 15)	41 ± 10	0.004

[a]Prostate tissue from men with normal testosterone levels.
[b]Prostate tissue from men with castrate levels of testosterone after 3 months of neoadjuvant hormonal therapy (NHT).
[c]Primary prostate cancer tissue from men with bone metastases.

lack of functional androgen response elements found there. Androgen effects can occur in the absence of androgen response elements in the gene of interest through involving an intermediary factor that is androgen responsive such as the NF-κB/IκB pathway in male human endothelial cells [25]. This study demonstrated activation of the VCAM-1 promoter by DHT through NF-κB activation [25]. Further investigation is needed to establish how DHT upregulates MRP4/ABCC4 expression.

Hormonal regulation of MRP4/ABCC4 has previously been observed in mouse kidneys [26]. It was noted that females have higher levels of MRP4/ABCC4 mRNA in the kidney than males, that the difference was abolished by gonadectomy or treatment of females with androgen. These data suggest that androgen represses MRP4/ABCC4 expression in the kidney, in contradistinction to our data showing androgen-

TABLE III. Normal Tissue Expression of MRP4/ABCC4 by Immunohistochemistry

Tissue	Number of cases	MRP4/ABCC4 expression
Colon	10	–
Lung	6	–
Salivary glands	5	–
Spleen	5	–
Ovary	5	–
Small intestine	6	–
Skin	4	–
Kidney	6	+++ apical membrane tubular expression
Breast	2	–
Skeletal muscle	2	–
Prostate	10	++ epithelium
Stomach	4	–
Testis	3	+ Leydig cells
Liver	3	–

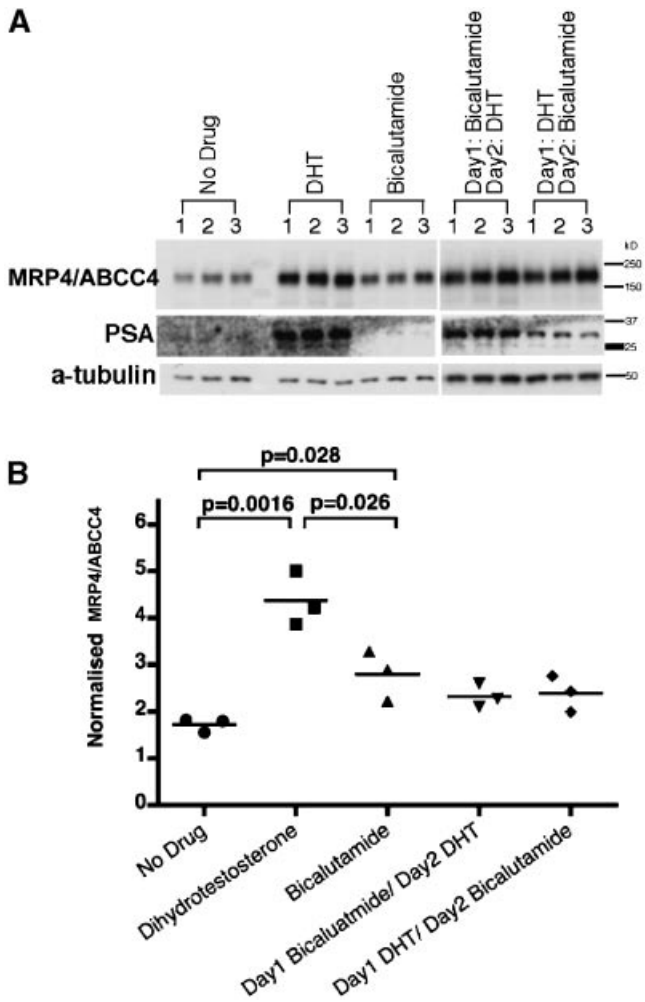

Fig. 3. Androgen-responsive LNCaP cells were treated with androgen (dihydrotestosterone) and an anti-androgen (bicalutamide) to assess the effect of androgen on MRP4/ABCC4 expression. **A**: Immunoblot showing expression of MRP4/ABCC4 and PSA normalized for α-tubulin expression without treatment, with dihydrotestosterone treatment, with bicalutamide treatment and bicalutamide/dihydrotestosterone treatment. **B**: Quantification of the immunoblot MRP4/ABCC4 bands from the image in (A) normalised to α-tubulin. Results of *t*-tests assessing triplicate observations.

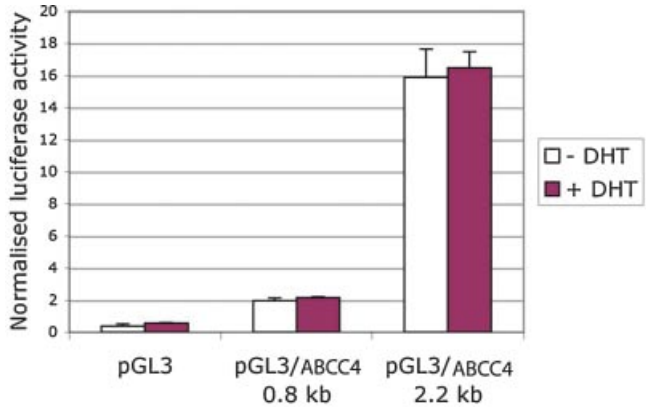

Fig. 4. Failure of androgen to directly activate the MRP4/ABCC4 promoter in LNCaP cells. DHT (1 μM) was added to the cells 6 hr post-transfection with either an MRP4/ABCC4 promoter reporter or vector-only control. Luciferase activity was assayed 24 hr later. Chart shows mean ± SE of triplicate observations.

stimulation of MRP4/ABCC4 expression in the malignant prostate cell line.

The immunohistochemical data from the tissue microarrays indicates quite a restricted pattern of expression for MRP4/ABCC4, the principal sites being kidney and prostate. This is in agreement with previous analyses of MRP4/ABCC4 mRNA in tissues of humans [27], mice [28], and rats [29]. However, we detected no expression in liver or lung, in contrast to some other reports [29,30]. Localized expression of MRP4/ABCC4 has been detected in brain endothelia, the choroid plexus [11] and blood platelets [31]. The combination of limited MRP4/ABCC4 expression in normal tissues and high expression in prostate cancers is favourable when considering MRP4/ABCC4 as a potential therapeutic target in this disease.

Unlike other family members, MRP4/ABCC4 has been identified in only a few cancers to date. Cell line data demonstrate low expression in lung, kidney, bladder and colon cancer [27]. MRP4/ABCC4 expression in pancreatic cancer and childhood ALL was

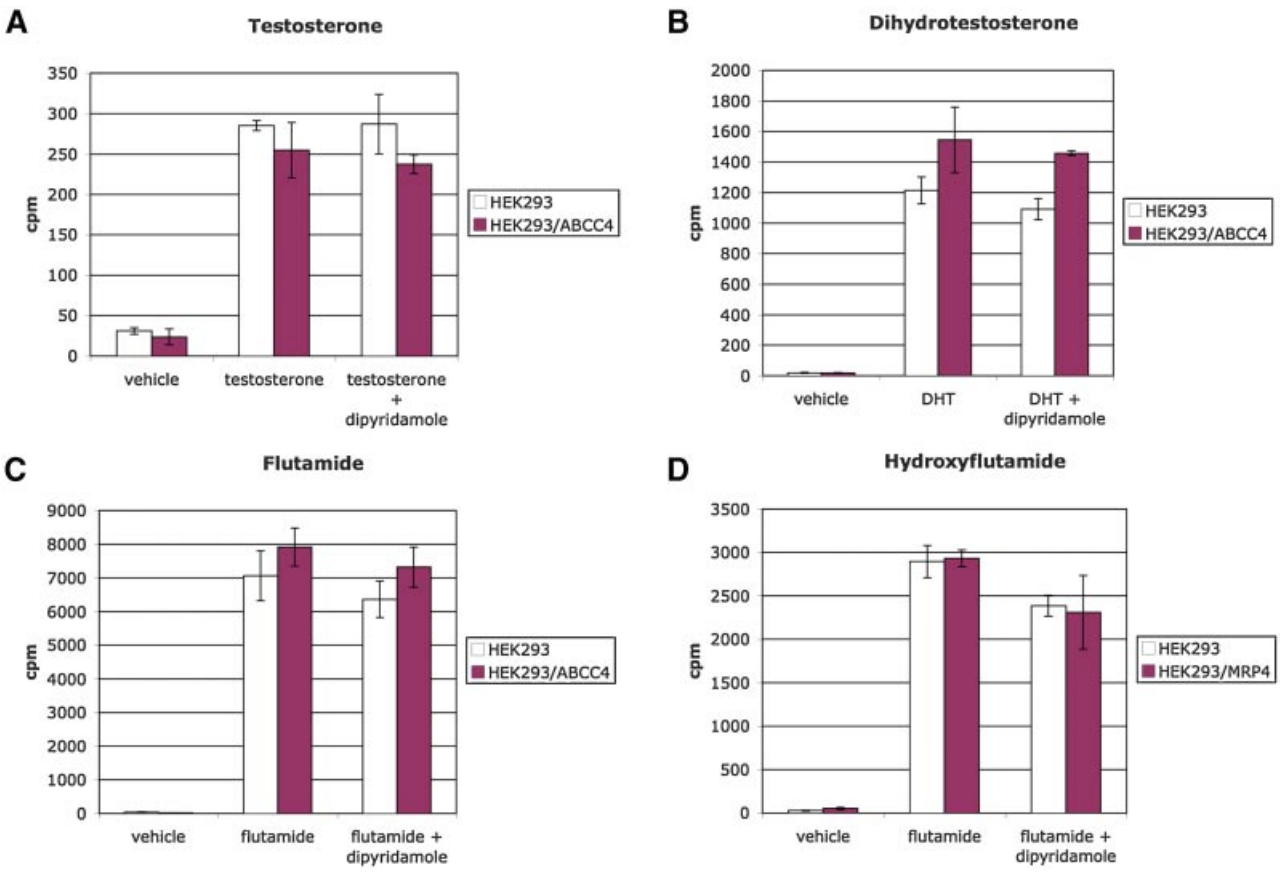

Fig. 5. Identification of potential androgen/anti-androgen substrates of MRP4/ABCC4. Accumulation of androgens and anti-androgens were assessed in HEK293/MRP4/ABCC4 transfectants vs. the parental cell line. Panels compare accumulation of (**A**) testosterone, (**B**) dihydrotestosterone, (**C**) flutamide and (**D**) hydroxyflutamide in HEK293 cells and the MRP4/ABCC4 transfectant with the same cells treated with dipyridamole, an inhibitor of ABCC transporter functions. Graphs show mean ± SD of triplicate experiments.

unrelated to treatment outcomes [32,33]. The only other human cancer cohort study of MRP4/ABCC4 expression did however reveal a relationship with both high N-MYC expression and a poor prognosis in children with neuroblastoma [10]. MRP4/ABCC4 does not confer resistance to drugs routinely used in the treatment of neuroblastoma so another mechanism is needed to explain its prognostic value in this context. The MRP4/ABCC4-knockout mice show enhanced central nervous system accumulation of chemotherapy drugs, in particular Topotecan, but no tumorigenic phenotype [11].

MRP4/ABCC4 does not transport chemotherapy drugs used in the routine treatment of prostate cancer, i.e. Docetaxel, Mitoxantrone, active androgens or anti-androgens, and is thus unlikely to be a factor in drug resistance. However, as noted above, the same is true in the context of neuroblastoma where MRP4/ABCC4 expression is a strong predictor of poor survival but does not transport any of the chemotherapeutics used to treat the disease [9]. Our cohort did not have sufficient follow-up to ascertain if a similar relationship exists in prostate cancer although the preliminary data suggested that this is an avenue worth pursuing. It is well established that MRP4/ABCC4 functions as a prostaglandin (PG) efflux transporter for PGE1 and PGE2 [8,9]. Furthermore, this function can be inhibited by non-steroidal anti-inflammatory drugs [9]. PGE2 has been implicated in prostate carcinogenesis as it is elevated in prostate cancer [34] and activates cell proliferation in prostate intraepithelial neoplasia cells, a prostate cancer precursor, through activation of Interleukin-6/GP130/STAT-3 signaling [35]. In keeping with this finding, treating TRAMP mice with non-steroidal anti-inflammatory drugs results in regression of mouse prostate intraepithelial neoplasia [36]. Thus, MRP4/ABCC4 may be important in the progression to prostate cancer as the androgen-mediated increase in MRP4/ABCC4 may cause an efflux of PGE1 and PGE2 from prostate cells and result in inflammation known to promote prostate carcinogenesis.

In conclusion, MRP4/ABCC4 is an androgen-responsive protein whose expression is increased in the progression to prostate cancer but decreased with androgen withdrawal. Furthermore, both mRNA and protein data support limited expression in the human normal body atlas with kidney and prostate as the major sites. These results warrant further investigation of the mechanism of androgen regulation of MRP4/ABCC4, its potential as a predictive biomarker of treatment outcome and its potential as a target for therapeutic modulation in prostate cancer, in particular, in trials of NSAID and COX-2 inhibitors in this disease.

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