Virchows Arch (2013) 462:437–443
DOI 10.1007/s00428-013-1390-8

ORIGINAL ARTICLE

Multidrug resistance protein 4 (*MRP4*) expression in prostate cancer is associated with androgen signaling and decreases with tumor progression

Matteo Montani · Thomas Herrmanns · Michael Müntener · Peter Wild · Tullio Sulser · Glen Kristiansen

Received: 17 December 2012 / Revised: 12 February 2013 / Accepted: 25 February 2013 / Published online: 16 March 2013

Abstract Multidrug resistance protein 4 (MRP4) is a transmembrane transport protein found in many cell types and is involved in substrate-specific transport of endogenous and exogenous substrates. Recently, it has shown to be expressed in prostate cancer cell lines and to be among the most commonly upregulated transcripts in prostate cancer, although a comprehensive expression analysis is lacking so far. We aimed to investigate its expression by immunohistochemistry in a larger cohort of neoplastic and nonneoplastic prostate tissues (n=441) and to correlate its expression with clinicopathological parameters including PSA-free survival times and molecular correlates of androgen signaling (androgen receptor (AR), prostate-specific antigen (PSA), and forkhead box A (FoxA)). MRP4 is widely expressed in benign and neoplastic prostate epithelia, but its expression gradually decreases during tumor progression towards castrate-resistant disease. Concordantly, it correlated with conventional prognosticators of disease progression and—within the group of androgen-dependent tumors—with AR and FoxA expression. Moreover, lower levels of MRP4 expression were associated with shorter PSA relapse-free survival times in the androgen-dependent group. In benign tissues, we found zone-dependent differences of MRP4 expression, with the highest levels in the peripheral and central zones. Although MRP4 is known to be regulated in prostate cancer, this study is the first to demonstrate a gradual downregulation of MRP4 protein during malignant tumor progression and a prognostic value of this loss of expression.

Keywords Prostate cancer · MRP4 · Androgen receptor · FoxA · Immunohistochemistry

Introduction

Multidrug resistance protein 4 (MRP4/ABCC4) is a member of ATP-binding cassette transporters responsible for ATP-driven transmembranous transport of substrates. Its encoding gene is located on chromosome 13q32.1. To date, nine MRPs have been identified and appear to be able to transport a wide variety of endogenous and xenobiotic organic anions out of the cell. Each MRP has its own membrane location, tissue distribution, and substrate specificity [1–3]. Especially, MRP4 has a particular broad substrate specificity, encompassing cyclic nucleotides, ADP, eicosanoids, urate, steroid hormones, folate, and bile acids among endogenous substrates and several antiviral, antibiotic, cardiovascular, cytotoxic (methotrexate, 6-thioguanine, 6-mercaptopurine, topotecan) drugs (reviewed in [4]). MRP4 has been shown to be transcriptionally regulated by androgen receptor activation in prostate cancer cells [5, 6]. It was not only found to be expressed particularly in normal prostate glandular and renal tubular epithelium but has been found to be increased in prostate cancer in vivo and in prostate cancer cell lines, recently [6].

Electronic supplementary material The online version of this article (doi:10.1007/s00428-013-1390-8) contains supplementary material, which is available to authorized users.

M. Montani
Division of Clinical Pathology, Institute of Pathology, University of Bern, Bern, Switzerland

T. Herrmanns · M. Müntener · T. Sulser
Division of Urology, University Hospital Zurich, Zurich, Switzerland

P. Wild
Institute of Surgical Pathology, University Hospital Zurich, Zurich, Switzerland

G. Kristiansen (✉)
Institute of Pathology, University of Bonn, Sigmund-Freud-Str. 25, 53127 Bonn, Germany
e-mail: glen.kristiansen@ukb.uni-bonn.de

Moreover, MRP4 seems to be responsive not only to androgens or antiandrogens [6] but also to 17β-estradiol and also to the gender-specific pattern of growth hormone secretion [7, 8].

Recently, it has been suggested that MRP4 is potentially regulated by forkhead box A (FoxA) proteins (hepatocyte nuclear factor 3 (HNF3)) [9, 10], particularly FoxA1, a forkhead protein that is an important mediator of androgen and estrogen signaling [11–14] and is also centrally involved in the embryogenesis of the prostate [11, 15, 16] and moreover has prognostic relevance in primary prostate cancer [17].

In a previous study of our group, an array-based transcript analysis of normal prostate tissue and matched cancer tissue revealed MRP4 to be among the top up-regulated genes in prostate cancer tissue [18]. Since the clinical and prognostic significance of MRP4 protein expression in prostate cancer is still unknown, we analyzed a larger cohort (n=441) of prostate cancer cases and benign prostate hyperplasia (BPH) in order to correlate MRP4 expression with clinicopathological parameters including prostate-specific antigen (PSA) relapse-free survival times and moreover to molecular correlates of steroid hormone signaling including androgen receptor (AR), estrogen receptor (ER)α/β, and FoxA1.

Materials and methods

Patients

Four hundred forty-one patients (n=441) were enclosed in this study, who underwent radical prostatectomy, transurethral resection, or resection of organ metastasis between 1993 and 2006 at the University Hospital of Zurich due to either prostate cancer or BPH and of whom clinical data were obtained. The specimens were separated into radical prostatectomy specimens (RPE) (n=332), castrate-resistant prostate cancer (CRPC) (n=26), lymph node metastases (n=14), parenchymal metastases (n=25), and nonneoplastic prostate tissue (n=187), a part of the latter matching to the cancer patients (n=142). Nonneoplastic tissue consisted of hyperplastic transitional zone (n=18), central zone (n=6), peripheral zone (n=108), and atrophic peripheral zone (n=55). Patient's age ranged between 46 and 89 years (median 67 years). The distribution of the Gleason scores (GS) in the prostate cancer cohort (n=397) was as follows: GS 2–6: 47 (11.8 %); GS 7: 184 (46.3 %); and GS 8–10: 125 (31.5 %), whereas no information was available from 41 (10.3 %) patients. Stage distribution was as follows: 180 (45.3 %) fall into the T2a–c category, 97 (24.4 %) into the T3a–b, and 12 (3.0 %) into the T4 category, respectively. No data about tumor stage were available in 108 (27.2 %) patients. Two hundred twenty-five (56.7 %) had no lymph node metastasis (pN0 category), whereas 15 (3.8 %) were nodal positive (pN1). Lymph node status was not available in 157 (39.5 %). Relapse-free survival (RFS) was available in 258 patients and ranged from 0 to 182 months (mean = 70 months). Totally, 101 patients (25.4 %) experienced a PSA relapse during follow-up, defined as a rising PSA level exceeding 0.1 ng/ml, having reached a nadir after surgery. The median follow-up time (of all patients) was 62.5 months.

Tissue microarray construction and immunohistochemistry

A tissue microarray (TMA) was constructed as previously described [19]. This study was approved by the Cantonal Ethics Committee of Zurich (StV 25–2007).

The TMA blocks were freshly cut (3 μm) and mounted on superfrost slides (Menzel Gläser, Braunschweig, Germany). Immunohistochemical procedures were conducted by the Ventana Benchmark automated staining system (Ventana Medical Systems, Tucson, AZ, USA) using Ventana reagents.

MRP4 was detected by the polyclonal goat antibody anti-MRP4 (Everest Biotech; dilution 1:1,500, Bond diluent). A polyclonal goat antibody, anti-HNF3α/β (C-20) (Santa Cruz Biotechnology; dilution 1:400 in a Bond diluent) was used to detect FoxA expression. AR detection was done by using a monoclonal mouse antibody, antiandrogen receptor, clone F39.4.1 (BioGenex, San Ramon, CA; dilution 1:500 in a Bond diluent). A monoclonal rabbit antibody (antiestrogen receptor clone SP1; Ventana; prediluted in a Ventana diluent) was used to label ERα. In order to detect ERβ expression, a monoclonal mouse antibody, antihuman estrogen receptor beta 2, clone 57/3 (AbD Serotec Ltd.) has been used in a 1:200 dilution (BSA/TRIS). PSA was detected by a polyclonal rabbit antibody, antihuman prostate-specific antigen (DAKO A/S, Glostrup, Denmark; dilution 1:4,000 in a Ventana diluent). Ki-67 was visualized by a monoclonal mouse antibody, anti-Ki-67, clone MIB-1 (DAKO A/S, Glostrup, Denmark; dilution 1:20 in a Ventana diluent). Primary antibodies were detected by the UltraVIEW DAB detection kit using the benchmark CC1m heat-induced epitope retrieval. The signal was further enhanced with the amplification kit. Slides were counterstained with hematoxylin, dehydrated, and mounted.

Evaluation of immunohistochemical stainings

Evaluation of the stained TMAs has been done by two clinical pathologists (MM and GK) simultaneously on a multiheaded microscope. For MRP4, evaluation of staining intensity has been done with a four-tiered system: 0 (negative), 1+ (weak), 2+ (moderate), and 3+ (strong) for membranous positivity on every TMA spot (Fig. 1). A cytoplasmic staining was not evaluated. Mean expression levels (as shown in Fig. 2) were calculated as the sum of all individual expression levels in the different groups divided by the number of cases in this group. For AR, ERα/β, and Mib-1, nuclear staining intensity was

scored by a four-tiered system again. There was no detectable membranous staining. Quantification was made upon the strongest staining in an area of at least 25 % of the representative cells. Tissue loss in more than 75 % of a TMA spot resulted in exclusion of this case for further consideration.

Statistics

For correlation significance test, two-sided Spearman's ρ (rho) test and for descriptive statistics (cross tables), two-sided Fisher's exact test and χ^2 (chi-square) test have been used, respectively. Univariate survival analyses were conducted according to Kaplan–Meier (log rank test). These statistics were calculated with PASW18 (SPSS, Chicago, IL).

Results

Immunohistochemistry

Expression of MRP4 in benign prostate tissues

First, we investigated nonneoplastic tissues (n=187) for MRP4 expression and found it rather heterogeneous with zone-dependent differences: high levels (score 2+ or 3+) of membranous MRP4 expression in 13 (76.5 %) were seen in 17 tissues from the transitional zone, whereas five (83.5 %) of six central zone and 107 (99.1 %) of 108 peripheral zone samples showed a strong positivity. Interestingly, none of the atrophic samples (n=56) from the peripheral zone showed a strong positivity (Table 1; Online Resource 1).

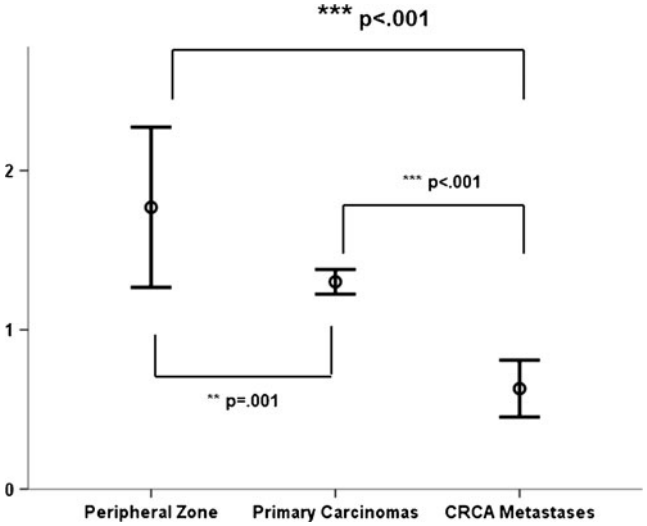

Fig. 2 Mean expression levels of MRP4 in different categories, such as normal tissue from the peripheral zone, primary carcinomas, and castrate-resistant and metastasizing disease, respectively (p<0.001). *Error bars* mark 95 % confidence intervals (n=410)

Expression of MRP4 in prostate cancer, metastasis, and CRPC

The predominantly membranous staining pattern of MRP4 is retained in prostate cancer. As compared to the high prevalence

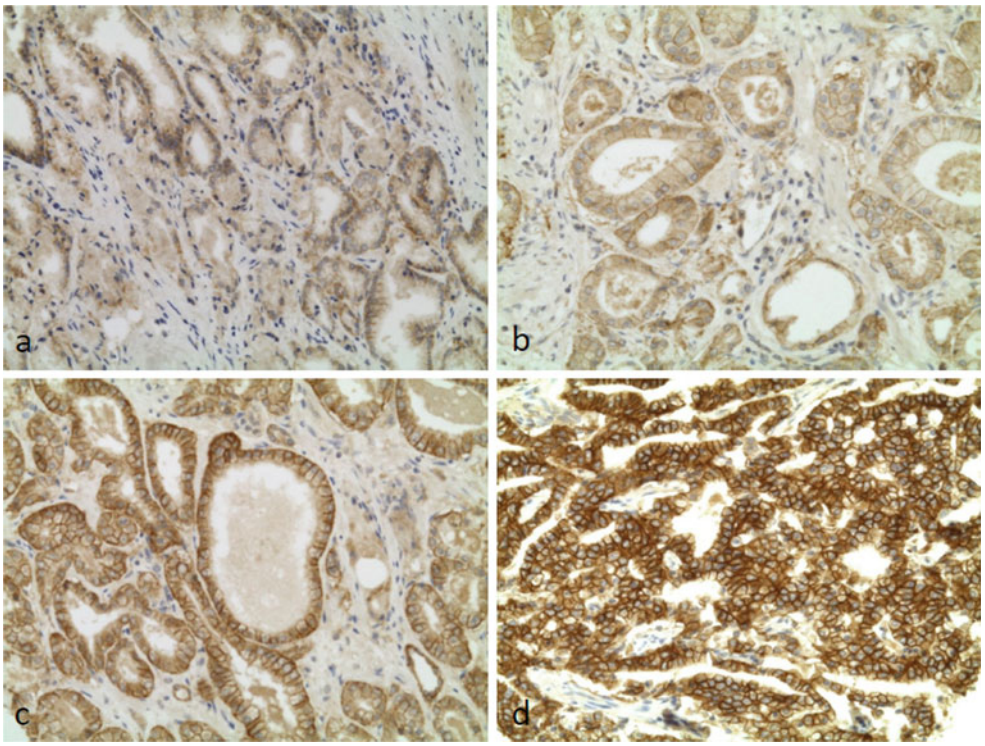

Fig. 1 Different immunohistochemical staining intensities for membranous MRP4 expression in prostate carcinomas. **a** Virtually no membranous positivity (score 0). **b** Weak and discontinuous positivity (score 1). **c** Strong but variable positivity (score 2). **d** Strong continuous positivity (score 3)

Table 1 Distribution of strong and weak membranous MRP4 positivity in nonneoplastic prostate tissue of transitional, central and peripheral zone, and atrophic peripheral zone

	MRP4		Total, *n* (%)
	Weak, *n* (%)	Strong, *n* (%)	
Transitional zone	4 (23.5)	13 (76.5)	17 (100)
Central zone	1 (16.7)	5 (83.3)	6 (100)
Peripheral zone	1 (0.9)	107 (99.1)	108 (100)
Atrophic peripheral zone	56 (100)	0 (0)	56 (100)
Total	62 (33.2)	125 (66.8)	187 (100)

Two-sided X^2 test (Pearson) $p<0.001$, $n=187$

of strong MRP4 expression in normal peripheral zone glands (105; 95.5 %) a decreasing rate of strong MRP4 positive carcinomas was observed towards advanced cancer: 138 (42.9 %) of 332 primary carcinomas and only seven (12.5 %) of 56 CRCA/metastatic carcinomas were strongly positive ($p<0.001$) (Table 2). Furthermore, we found the mean expression levels of MRP4 to significantly differ in those groups: 1.77 in nonneoplastic tissue, 1.30 in primary carcinomas, and 0.63 in advanced disease ($p<0.001$) (Fig. 2).

Correlation of MRP4 expression with clinicopathological and molecular parameters

In RPE samples, a strong and significant inverse correlation with Gleason score and tumor stage was found (Table 3). Of the 258 patients with available RFS time, a total of 101 (39.1 %) experienced a PSA relapse during follow-up. Thirty-nine (32.0 %) patients relapsed in the group with strong MRP4 expression and 62 (45.6 %) in the group with weak or no MRP4 expression. The 5-, 10-, and 15-year RFS rate within a confidence interval of 95 % in the MRP4 strongly expressing group was 74.1 % (65–81 %), 64.1 % (53–73 %), and 48.3 % (25–69 %); and in the MRP4 weakly expressing group was 67.8 % (59–75 %), 49.1 % (38–59 %), and 16.7 % (2–45 %), respectively. RFS times were significantly longer in cases with strong MRP4 expression (log rank Mantel–Cox $p=0.035$) (Fig. 3) yet failed significance in a multivariate analysis (Cox regression; data not shown). No significant correlation was found between MRP4 expression and preoperative serum PSA levels. MRP4 expression correlated significantly with tumoral epithelial and stromal AR expression ($p=0.002$ and $p=0.004$ resp.), FoxA1 ($p=0.002$), and PSA ($p<0.001$) expression and the proliferation rate ($p=0.002$) (Table 3). No significant correlation of epithelial and stromal ERα and β with MRP4 expression was detected (data not shown).

Furthermore, we analyzed the distribution of strongly MRP4 expression primary carcinomas among strongly and weakly AR expressing tumors as well as among carcinomas with strongly or weakly AR-positive stroma and found 78 (35.5 %) of the AR weakly expressing tumors to have a strong MRP4 expression, whereas 52 (55.9 %) of the tumors with strong FoxA expression have a strong MRP4 expression ($p=0.001$). Seventy-eight (35 %) of the tumors with weakly AR-positive stroma to have a strong MRP4 expression, whereas 55 (57.3 %) of the cases with strong stromal AR expression have a strong MRP4 expression.

Discussion

This is the first study to analyze MRP4 in a larger, clinically characterized prostate cancer cohort and the first description of its prognostic value in prostate cancer. So far, MRP4 is largely known to play a role in nonneoplastic conditions and has been linked to bile secretion and many other physiological roles. However, there is an increasing body of evidence that MRP4 plays a role in tumors as well [6, 20–22]. Furthermore, it is well documented that androgens induce and antiandrogens inhibit its expression [5, 6, 8]. Interestingly, MRP4 shows a gender- and organ-specific expression in the kidney, reaching higher levels in females due to estrogen stimulation [8]. Therefore, we investigated a large series of patients either suffering from prostate cancer or benign prostatic hyperplasia in order to assess expression of MRP4, hormone receptors, FoxA, and PSA with respect to their correlation to prognosis, prognostic relevant factors, and distribution among different histological

Table 2 Distribution of strong and weak membranous MRP4 positivity in normal peripheral prostate, primary carcinomas, and castrate-resistant and metastasizing disease

	MRP4		Total, *n* (%)
	Weak, *n* (%)	Strong, *n* (%)	
Normal peripheral prostate tissue	5 (4.5)	105 (95.5)	110 (100.0)
Primary carcinomas/RPEs	184 (57.1)	138 (42.9)	332 (100.0)
Castrate-resistant carcinomas and metastases	49 (87.5)	7 (12.5)	56 (100.0)
Total	238 (48.8)	250 (51.2)	488 (100.0)

Two-sided X^2 test (Pearson), $p<0.001$, $n=488$

Table 3 Nonparametric correlations between MRP4 and markers of androgen signaling in primary prostate carcinomas

	MRP4	AR tumor	AR stroma	FoxA	PSA tumor	Proliferation (Mib-1)	Gleason score	pT stage	Serum PSA
MRP4		0.172	0.160	0.170	0.246	0.171	−0.197	−0.130	0.019
		$p=0.002^a$	$p=0.004^a$	$p=0.002^a$	$p<0.001^a$	$p=0.002^a$	$p<0.001^a$	$p=0.027$	$p=0.785$
AR tumor	0.172		0.236	0.527	−0.066	0.244	−0.031	0.033	−0.129
	$p=0.002^a$		$p<0.001^a$	$p<0.001^a$	$p=0.251$	$p<0.001^a$	$p=0.576$	$p=0.580$	$p=0.039$
AR stroma	0.160	0.236		0.126	−0.012	−0.074	−0.306	−0.155	−0.168
	$p=0.004^a$	$p<0.001^a$		$p=0.024^b$	$p=0.835$	$p=0.175$	$p<0.001^a$	$p=0.008^a$	$p=0.006^a$
FoxA	0.170	0.527	0.126		−0.129	0.087	0.168	0.084	0.026
	$p=0.002^a$	$p<0.001^a$	$p=0.024^b$		$p=0.024^b$	$p=0.110$	$p=0.002^a$	$p=0.155^b$	$p=0.672$
PSA tumor	0.246	−0.066	−0.012	−0.129		−0.048	−0.253	−0.167	0.010
	$p<0.001^a$	$p=0.251$	$p=0.835$	$p=0.024^b$		$p=0.394$	$p<0.001^a$	$p=0.005^a$	$p=0.875$
Proliferation (Mib-1)	0.171	0.244	−0.074	0.087	−0.048		0.018	−0.065	−0.125
	$p=0.002^a$	$p<0.001^a$	$p=0.175$	$p=0.110$	$p=0.394$		$p=0.716$	$p=0.234$	$p=0.029$
Gleason score	−0.197	−0.031	−0.306	0.168	−0.253	0.018		0.408	0.405
	$p<0.001^a$	$p=0.576$	$p<0.001^a$	$p=0.002^a$	$p<0.001^a$	$p=0.716$		$p<0.001^a$	$p<0.001^a$
pT stage	−0.130	0.033	−0.155	0.084	−0.167	−0.065	0.408		0.348
	$p=0.027$	$p=0.580$	$p=0.008^a$	$p=0.155^b$	$p=0.005^a$	$p=0.234$	$p<0.001^a$		$p<0.001^a$
Serum PSA	0.027	−0.129	−0.168	0.026	0.010	−0.125	0.405	0.348	
	$p=0.669$	$p=0.039$	$p=0.006^a$	$p=0.672$	$p=0.875$	$p=0.029^b$	$p<0.001^a$	$p<0.001^a$	

Proliferation is the ratio of nuclear Mib-1-positive tumor cells. Serum PSA is obtained preoperatively. $n=250–407$

AR nuclear androgen receptor expression, *FoxA* nuclear FoxA expression in tumor cells, *PSA tumor* cytoplasmic PSA expression in tumor cells

[a] Correlation is significant at the 0.01 level (two-tailed)

[b] Correlation is significant at the 0.05 level (two-tailed). Spearman's ρ test

tumor types. A previous study investigated 84 radical prostatectomy specimens for MRP4 expression and found it expressed in normal and malignant prostate tissues with a loss in tumors treated with antiandrogens [6]. In our study, we confirm a strong expression of MRP4 in 42.9 % of primary tumors, whereas a considerably lower rate of strong expression (12.5 %) was observed in advanced (i.e., castrate-resistant and metastasized carcinomas) disease. However, in our cohort, the frequency of high expression and mean expression levels of MRP4 turned out to be highest in nonneoplastic tissues. Therefore, we unraveled several different types of nonneoplastic tissues and found that MRP4 is strongly expressed in the peripheral and central zone of the prostate, but not in the transitional zone (which was collected from resections due to BPH).

This was almost perfectly consistent with the AR expression in epithelial and stromal cells of these groups, suggesting that in the presence of normal androgen levels, the AR-dependent levels of MRP4 are proportional to the AR expression. Thus, the levels parallel in androgen-dependent primary tumors, too. These findings contrast to the findings of Ho et al. [6] where high MRP4 levels are described in androgen-dependent cancers, whereas normal tissue had lower levels of expression. This is probably best explained by the fact that, in this study, hyperplastic prostate tissue (from the transition zone) has been used as nonneoplastic controls. Furthermore, the strong correlation we found between MRP4 expression on the one hand and AR expression in the tumor cells and in the surrounding stromal cells on the other highly suggest an

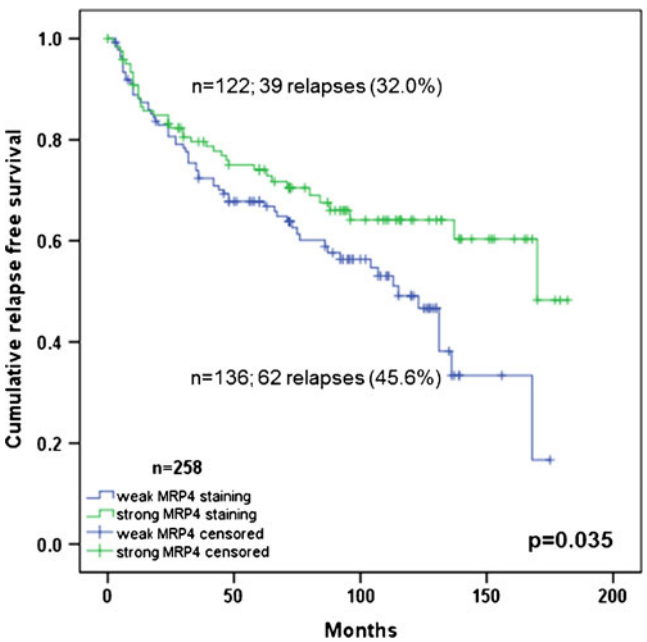

Fig. 3 Kaplan–Meier curve of biochemical PSA relapse-free survival of patients after radical prostatectomy due to primary prostate carcinomas with strong or weak MRP4 expression ($p=0.035$)

androgen dependency of MRP4 expression. This is reflected in the strong correlation of MRP4 and PSA expression we found in tumors as well in normal tissues. Interestingly, atrophic glands from the peripheral zone lose their MRP4 expression despite the presence of AR expression.

We also found a marked decreased MRP4 expression in CRPC and metastases despite a significantly elevated AR expression in these patients. It can be speculated that this can be explained by the effects of antiandrogenic therapy in these patients, which leads to a diminished androgen-induced AR-dependent transcription of MRP4. This was again paralleled by the reduced PSA expression in these tumors (Online Resource 2). The higher levels of AR expression in this group of tumors are long acknowledged and may be explained by sensitization of the tumor for the low levels of androgens upon ablation therapy (reviewed in [23]). Despite this "hypersensitivity" to androgens, androgen and AR-dependent transcription of MRP4 and PSA remain low in CRCA and metastases in our cohort. It has been repeatedly shown that MRP4 expression is upregulated by androgens and downregulated by antiandrogens [5, 6, 8]. However, as many contradictory results in previous studies show, the expression of AR (as well as its distribution) before and after antiandrogenic therapy remains a highly confusing topic, and its relationship to circulating androgen levels is poorly understood. But as recently reviewed [23], several studies confirmed an association between higher stage, higher grade, and poorer outcome in patients of whom the prostate carcinomas showed either high tumoral or low stromal AR expression (or both), which was reproducible in our cohort. Stromal and epithelial AR expression is linked to different androgen-dependent actions having a different prognostic relevance on one hand, and AR protein expression is not directly linkable to its biological sensitivity since it has been repeatedly shown that, in hormone refractory disease, AR is up to fourfold more sensitive than in primary carcinomas or benign hyperplasia (reviewed in [23]) on the other. Thus, correlations of AR expression with other proteins somewhat limit the validity of dependency of this proteins on androgen.

MRP4 correlated closely with FoxA expression in the subgroup of primary tumors. This might well reflect that the promoter region of MRP4 has a FoxA binding site (discussed in [8]), and thus, MRP4 is predominantly expressed in the presence of FoxA. However, we recently found that FoxA is an adverse prognostic factor, and its expression increases towards advanced prostate cancer [17], whereas, as shown in the present study, the expression of MRP4 decreases. This could be explained by the fact that FoxA acts as a coactivator of androgen receptor [11, 24] and is recruited in the absence of androgens on one hand and, upon therapeutic androgen depletion in advanced cancers, a lower MRP4 expression is expectable one the other. Furthermore, androgen dependence of MRP4 expression could be suggested by significant correlation between MRP4 and PSA expression. The favorable prognostic value of high MRP4 expression levels is again reflected by its inverse correlation with tumor stage and Gleason grade and by the association of low MRP4 expression with earlier biochemical PSA relapse.

Conclusions

In summary, this study shows a decreasing MRP4 expression with tumor progression to castrate-resistant disease, an inverse correlation with Gleason scores, and a correlation with PSA and AR expression. The strong correlation between MRP4 and AR expression highly suggests an androgen dependency of MRP4, which ought to be clarified in functional studies.

We also found a marked decreased MRP4 expression in CRPC and metastases despite a significantly elevated AR expression which can be explained by the effects of antiandrogenic therapy in these patients, leading to a diminished androgen induced transcription of MRP4. This was again paralleled by the reduced PSA expression in these tumors. Moreover, a weak MRP4 expression is associated with a shorter PSA relapse-free survival in hormone-naive prostate cancer cases.

Conflict of interest Any financial or conflicting interests in the publication of this article are disclosed by the authors.

References

1. Deeley RG, Westlake C, Cole SP (2006) Transmembrane transport of endo- and xenobiotics by mammalian ATP-binding cassette multidrug resistance proteins. Physiol Rev 86(3):849–899. doi:10.1152/physrev.00035.2005
2. Borst P, de Wolf C, van de Wetering K (2007) Multidrug resistance-associated proteins 3, 4, and 5. Pflugers Arch 453(5):661–673. doi:10.1007/s00424-006-0054-9
3. Kruh GD, Belinsky MG, Gallo JM, Lee K (2007) Physiological and pharmacological functions of Mrp2, Mrp3 and Mrp4 as determined from recent studies on gene-disrupted mice. Cancer Metastasis Rev 26(1):5–14. doi:10.1007/s10555-007-9039-1
4. Russel FG, Koenderink JB, Masereeuw R (2008) Multidrug resistance protein 4 (MRP4/ABCC4): a versatile efflux transporter for drugs and signalling molecules. Trends Pharmacol Sci 29(4):200–207. doi:10.1016/j.tips.2008.01.006
5. Cai C, Omwancha J, Hsieh CL, Shemshedini L (2007) Androgen induces expression of the multidrug resistance protein gene MRP4 in prostate cancer cells. Prostate Cancer Prostatic Dis 10(1):39–45. doi:10.1038/sj.pcan.4500912
6. Ho LL, Kench JG, Handelsman DJ, Scheffer GL, Stricker PD, Grygiel JG, Sutherland RL, Henshall SM, Allen JD, Horvath LG (2008) Androgen regulation of multidrug resistance-associated protein 4 (MRP4/ABCC4) in prostate cancer. Prostate 68(13):1421–1429. doi:10.1002/pros.20809

7. Chen C, Klaassen CD (2004) Rat multidrug resistance protein 4 (Mrp4, Abcc4): molecular cloning, organ distribution, postnatal renal expression, and chemical inducibility. Biochem Biophys Res Commun 317(1):46–53. doi:10.1016/j.bbrc.2004.03.014S0006291X04004887
8. Maher JM, Cheng X, Tanaka Y, Scheffer GL, Klaassen CD (2006) Hormonal regulation of renal multidrug resistance-associated proteins 3 and 4 (Mrp3 and Mrp4) in mice. Biochem Pharmacol 71(10):1470–1478. doi:10.1016/j.bcp.2006.02.005
9. Bochkis IM, Rubins NE, White P, Furth EE, Friedman JR, Kaestner KH (2008) Hepatocyte-specific ablation of Foxa2 alters bile acid homeostasis and results in endoplasmic reticulum stress. Nat Med 14(8):828–836. doi:10.1038/nm.1853
10. Le Lay J, Kaestner KH (2010) The Fox genes in the liver: from organogenesis to functional integration. Physiol Rev 90(1):1–22. doi:10.1152/physrev.00018.2009
11. Gao N, Zhang J, Rao MA, Case TC, Mirosevich J, Wang Y, Jin R, Gupta A, Rennie PS, Matusik RJ (2003) The role of hepatocyte nuclear factor-3 alpha (Forkhead Box A1) and androgen receptor in transcriptional regulation of prostatic genes. Mol Endocrinol 17(8):1484–1507. doi:10.1210/me.2003-0020
12. Yu X, Gupta A, Wang Y, Suzuki K, Mirosevich J, Orgebin-Crist MC, Matusik RJ (2005) Foxa1 and Foxa2 interact with the androgen receptor to regulate prostate and epididymal genes differentially. Ann N Y Acad Sci 1061:77–93. doi:10.1196/annals.1336.009
13. Laganiere J, Deblois G, Lefebvre C, Bataille AR, Robert F, Giguere V (2005) From the cover: location analysis of estrogen receptor alpha target promoters reveals that FOXA1 defines a domain of the estrogen response. Proc Natl Acad Sci U S A 102(33):11651–11656. doi:10.1073/pnas.0505575102
14. Nakshatri H, Badve S (2009) FOXA1 in breast cancer. Expert Rev Mol Med 11:e8. doi:10.1017/S1462399409001008
15. Kaestner KH, Knochel W, Martinez DE (2000) Unified nomenclature for the winged helix/forkhead transcription factors. Genes Dev 14(2):142–146
16. Carlsson P, Mahlapuu M (2002) Forkhead transcription factors: key players in development and metabolism. Dev Biol 250(1):1–23
17. Gerhardt J, Montani M, Wild P, Beer M, Huber F, Hermanns T, Muntener M, Kristiansen G (2012) FOXA1 promotes tumor progression in prostate cancer and represents a novel hallmark of castration-resistant prostate cancer. Am J Pathol 180(2):848–861. doi:10.1016/j.ajpath.2011.10.021
18. Kristiansen G, Pilarsky C, Wissmann C, Kaiser S, Bruemmendorf T, Roepcke S, Dahl E, Hinzmann B, Specht T, Pervan J, Stephan C, Loening S, Dietel M, Rosenthal A (2005) Expression profiling of microdissected matched prostate cancer samples reveals CD166/MEMD and CD24 as new prognostic markers for patient survival. J Pathol 205(3):359–376. doi:10.1002/path.1676
19. Tischler V, Fritzsche FR, Wild PJ, Stephan C, Seifert HH, Riener MO, Hermanns T, Mortezavi A, Gerhardt J, Schraml P, Jung K, Moch H, Soltermann A, Kristiansen G (2010) Periostin is up-regulated in high grade and high stage prostate cancer. BMC Cancer 10:273. doi:10.1186/1471-2407-10-273
20. Peng XX, Shi Z, Damaraju VL, Huang XC, Kruh GD, Wu HC, Zhou Y, Tiwari A, Fu L, Cass CE, Chen ZS (2008) Up-regulation of MRP4 and down-regulation of influx transporters in human leukemic cells with acquired resistance to 6-mercaptopurine. Leuk Res 32(5):799–809. doi:10.1016/j.leukres.2007.09.015
21. Sekine S, Ogawa R, Ojima H, Kanai Y (2011) Expression of SLCO1B3 is associated with intratumoral cholestasis and CTNNB1 mutations in hepatocellular carcinoma. Cancer Sci 102(9):1742–1747. doi:10.1111/j.1349-7006.2011.01990.x
22. Copsel S, Garcia C, Diez F, Vermeulem M, Baldi A, Bianciotti LG, Russel FG, Shayo C, Davio C (2011) Multidrug resistance protein 4 (MRP4/ABCC4) regulates cAMP cellular levels and controls human leukemia cell proliferation and differentiation. J Biol Chem 286(9):6979–6988. doi:10.1074/jbc.M110.166868
23. Mohler JL (2008) A role for the androgen-receptor in clinically localized and advanced prostate cancer. Best Pract Res Clin Endocrinol Metab 22(2):357–372. doi:10.1016/j.beem.2008.01.009
24. Gao N, Ishii K, Mirosevich J, Kuwajima S, Oppenheimer SR, Roberts RL, Jiang M, Yu X, Shappell SB, Caprioli RM, Stoffel M, Hayward SW, Matusik RJ (2005) Forkhead box A1 regulates prostate ductal morphogenesis and promotes epithelial cell maturation. Development 132(15):3431–3443. doi:10.1242/dev.01917