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Androgen receptor regulation of the *seladin-1/DHCR24* gene: altered expression in prostate cancer

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Prostate cancer (CaP) represents a major leading cause of morbidity and mortality in the Western world. Elevated cholesterol levels, resulting from altered cholesterol metabolism, have been found in CaP cells. *Seladin-1* (SELective Alzheimer Disease INdicator-1)/*DHCR24* is a recently described gene involved in cholesterol biosynthesis. Here, we demonstrated the androgen regulation of seladin-1/DHCR24 expression, due to the presence of androgen responsive element sequences in its promoter region. In metastatic androgen receptor-negative CaP cells seladin-1/DHCR24 expression and cholesterol amount were reduced compared to androgen receptor-positive cells. In tumor samples from 61 patients who underwent radical prostatectomy the expression of seladin-1/DHCR24 was significantly higher with respect to normal tissues. In addition, in cancer tissues mRNA levels were positively related to T stage. In tumor specimens from 23 patients who received androgen ablation treatment for 3 months before surgery seladin-1/DHCR24 expression was significantly lower with respect to patients treated by surgery only. In conclusion, our study demonstrated for the first time the androgen regulation of the *seladin-1/DHCR24* gene and the presence of a higher level of expression in CaP tissues, compared to the normal prostate. These findings, together with the results previously obtained in metastatic disease, suggest an involvement of this gene in CaP.

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KEYWORDS: androgen responsive element; cholesterol; prostate cancer; seladin-1/DHCR24

Prostate cancer (CaP) is a major leading cause of male cancer related death, second only to lung cancer, and represents 10% of all cancer deaths in men in the United States. In this country, one in six men will be diagnosed with CaP during their life time.[1] In the past years there has been a strong increase in the proportion of patients diagnosed with CaP confined to the gland, as a consequence of widespread detection strategies based on the measurements of the level of prostate-specific antigen (PSA) in the blood.[2] After radical prostatectomy, the increase of serum PSA is a marker of biochemical recurrence. As PSA is an androgen-regulated gene, biochemical recurrence of CaP by PSA relapse is in itself evidence of the androgen receptor (AR) dependence of the tumor. The key role of androgen signalling has been further highlighted by the chromosomal rearrangements recently identified in the majority of CaP.[3] Following these unbalanced rearrangements, the coding sequences of ERG, a member of ETS transcription factors family, are leaded under the regulatory sequences of the androgen-regulated gene *TMPRSS2*.[3] The newly formed fusion gene thus becomes a candidate oncogene in CaP.[4] Recently, Hendriksen *et al*[5] observed that in primary CaP specimens reduced expression levels of AR-related genes, including s*eladin-1* (for SELective Alzheimer Disease INdicator-1; also known as *DHCR24* for 3-β-hydroxysterol-δ-24-reductase), correlated with an increased risk to develop metastases. DHCR24 is the enzyme that converts desmosterol into cholesterol.[6] The role of seladin-1/DHCR24 in cholesterol biosynthesis has been

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recently linked to membrane cholesterol content and lipid rafts regulation.[7] Lipid rafts are cholesterol-rich membrane compartments, that are able to selectively retain or exclude specific cohorts of proteins affecting intracellular signalling. Elevated cholesterol levels in CaP cell membranes have been linked with lipid rafts deregulation and consequent activation of signalling pathways that promote growth advantage.[8] An additional property of seladin-1/DHCR24 is to inhibit the activation of caspase 3, a key modulator of apoptosis.[9] However, another study indicated that this multifaced protein may have a more complex role in cell apoptosis. In fact, following oncogenic and oxidative stress, seladin-1/DHCR24 was found to bind P53 N-terminal domain and to displace E3 ubiquitin ligase Mdm2 from P53, thus resulting in P53 accumulation.[10] Altered levels of seladin-1/DHCR24 expression have been associated to a more aggressive phenotype in melanomas,[11] adrenal cortex tumors[12] and pituitary adenomas.[13] It has been hypothesized that the androgen-responsiveness of seladin-1/DHCR24 may be due to the presence of an androgen responsive element (ARE) found in the gene promoter region,[14] even though its functional activity has never been proven. In the present study we first focused on the functional activity of seladin-1/DHCR24 ARE sequences by a luciferase reporter gene assay in order to prove that these sequences mediate the androgen-responsiveness of seladin-1/DHCR24 in prostate cells. With regard to this issue, we then investigated the expression levels of this gene and the modifications of cell cholesterol content in AR-positive and AR-negative CaP cell lines. Finally, we determined the amount of seladin-1/DHCR24 mRNA and protein in tumor prostate specimens from radical prostatectomy in order to evaluate whether the expression of this gene may be implicated in CaP.

MATERIALS AND METHODS

Specimens, Clinical Data and Prostate Cancer Cell Lines

A total of 61CaP samples were obtained from 61 patients undergoing radical prostatectomy at the University of Florence and University of Rome 'La Sapienza' after informed consent of patients and prior institutional review boards approval. After complete staging procedure the option of radical prostatectomy was offered to all patients who met universally accepted criteria.[15] Biochemical recurrence was defined as a PSA level >0.5 ng/ml at any follow-up visit. Fresh CaP tissue was harvested according to Bova *et al*.[16] Frozen sections were used to confirm that the tumor samples contained at least 70% of cancer cells. For control, we used normal prostate tissue excised from the same prostates. Histologic review of radical prostatectomy specimens was performed by the same pathologist. Pathologic stage was assigned according to the 2002 TNM classification.[17] Tumor grade was assessed by the Gleason system on surgical samples. Fresh prostate tissue was harvested according to standardized procedures. The frozen tissue blocks were sectioned and collected for RNA extraction. Additional 24 CaP samples from patients, who received androgen ablation treatment before surgery based on the decision of the urologist, were included in the study in order to determine the expression of the *seladin-1/DHCR24* gene.

In addition, CaP cell lines were included in the study. We performed analyses by using the AR-negative cell lines PC3, DU145 and the AR-positive cell lines LnCap, DU145-AR[18] and PC3-AR[19] both stably transfected with hAR.

Quantitative Real-Time RT-PCR for Seladin-1/DHCR24

Total RNA was isolated from paired tumor and normal tissues with Trizol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. Total RNA was also obtained from the CaP cell lines. The concentration was determined spectrophotometrically with Nanodrop® ND-1000. Total RNA (0.5 μg) was reverse transcribed into cDNA by using TaqMan reverse transcription reagents with random examers (Applied Biosystems Inc., Foster City, CA, USA). The profile of the reverse transcription reaction was 10 min at 25°C, 30 min at 48°C and 5 min at 95°C. The absolute quantification of seladin-1/DHCR24 RNA was performed by real-time RT-PCR. TaqMan primers, probe and thermal cycling conditions were as previously described.[12] Primers and probe for PSA (Unigene ID Hs.171995) were Assay-On-Demand gene expression products (assay no. Hs00426859_g1) purchased from Applied Biosystems. The human GAPDH (Unigene ID Hs.479728) mRNA was chosen as the reference gene (Hs02758991_g1; Applied Biosystems). PCR mixture (25 μl final volume) consisted of $1\times$ final concentration of Assay-On-Demand mix, $1\times$ final concentration of Universal PCR Master Mix (Applied Biosystems) and 20 ng cDNA. Each measurement was carried out in triplicate. The mRNA quantitation was based on the comparative Ct method according to the manufacturer's instructions (Applied Biosystems) and data were normalized to GAPDH expression.

Plasmids and Transient Transfection Assays

The human seladin-1/DHCR24 promoter sequence from -4384 to -2892 containing the ARE element was amplified by PCR from human genomic DNA using the left primer with the *Kpn*I restriction enzyme site and the right primer with the *Nhe*I site: 5′-ggtacctcttggtcaatctgcattcg-3′ and 5′-gctagcctggagtcaaagcagcttcc-3′. The 1483 bp PCR product was subcloned into the pCRII TOPO vector (Invitrogen, SRL, Milan, Italy), sequenced and cut out by digestion with *Kpn*I and *Nhe*I.[20] The excided sequence was successively cloned into the *Kpn*I/*Nhe*I site of the tataluciferase vector[21] containing a minimal TATA promoter upstream of a luciferase reporter gene, to obtain selaprom-tataluc plasmid. CHO cell line (American Type Tissue Collection, Manassas, VA, USA) and human benign prostate hyperplasia (BPH) cells[22] were plated in 24-well plates and were transfected with 400 ng of reporter (selaprom-tataluc plasmid), 80 ng of AR (pSVAR0 expression plasmid),[23] and 10 ng of the pGL 4.75 Renilla luciferase normalization vector (Promega Corp., Madison, WI, USA) by using Lipofectamin 2000

(Invitrogen). Cells were treated with ethanol vehicle or R1881 (10 nM, 24 h) (NEN; PerkinElmer, Waltham, MA, USA). Luciferase reporter assays were performed by using the Dual-Glo Luciferase Reporter Assay System (Promega Corp.). Firefly luciferase light output was normalized by Renilla luciferase output after subtraction of background light output by Victor 3 (PerkinElmer).

Immunohistochemistry for Seladin-1/DHCR24

Sections (4-μm thick) were obtained from formalin-fixed and paraffin-embedded radical prostatectomy specimens. The sections were deparaffinized in Bio-Clear (Bio-Optica, Milan, Italy) and hydrated with graded ethanol. To block endogenous peroxidase activity, slides were treated with 3.0% hydrogen peroxidase in distilled water for 10 min. Antigen retrieval was routinely performed by immersing the sections in EDTA buffer pH 8.0 in thermostatate bath (DAKO A/S, Glostrup, Denmark) at 98°C for 30 min. Then the tissue sections were stained with a polyclonal antibody against seladin-1/DHCR24[9] at 1:50 dilution and incubated for 2 h at room temperature. Primary antibody bound to antigen was detected with a standard streptavidin–biotin–peroxidase technique (LabVision, Fremont, CA, USA) and visualized with 3,3′-diaminobenzidine (BioGenex, San Ramon, CA, USA) as chromogen. Nuclei were counterstained with Mayer's hematoxylin. Sections of strongly positive adrenal cortical adenoma were used as positive controls whereas negative controls were performed by replacing the primary antibody with a nonimmune serum at the same concentration.

Cholesterol Measurement

The amount of cholesterol was determined by gas chromatography–mass spectrometry (GC/MS) using stigmasterol as internal standard. Cells were harvested and homogenized as previously described.[24] The protein concentration was determined by using the Bradford method. Proteins (5 μg) were used for cholesterol determination. After the addition of stigmasterol (1000 ng), sterols were saponified in 1 N NaOH in MeOH at 40°C for 60 min and successively extracted with *n*-hexane. After evaporation, sterols were derivatized in *N*,*O*-bis(trimethylsilyl)-trifluoroacetamide (BSTFA) with 10% trimethyl-chlorosilane at 70°C for 30 min. BSTFA solution (2 μl) was automatically injected for analysis. Calibration curve of cholesterol (range 50–2000 ng) with 1000 ng of stigmasterol as internal standard was used for cholesterol quantification. Each point was obtained by adding the appropriate quantities of cholesterol and internal standard and performing the same procedure of the samples. The experimental conditions for the samples analysis were as previously reported.[24]

Statistical Analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences software (SPSS, Evanston, IL, USA). Statistical analysis of cell lines data was performed by *t*-test. The clinical and pathologic variables were explored for the associations with seladin-1/DHCR24 levels. Owing to nonparametric distribution, Mann–Whitney *U*-test and Wilcoxon's rank sum text for unpaired data were performed and correlation between two variables was ascertained by Spearman's correlation test. *P*-value <0.05 was considered statistically significant. Results were expressed as median or mean ± s.e. unless otherwise stated.

RESULTS

Androgen-Responsiveness of ARE Sequences in Seladin-1/DHCR24 Promoter Region

We first focused on the functional activity of seladin-1/DHCR24 ARE sequences by a luciferase reporter gene system.

When CHO cells were transiently transfected with selaprom-tataluc plasmid and pSVAR0 expression plasmid, and treated with the AR-agonist R1881 (10 nM), the luciferase activity of the cells showed a threefold increase compared to the basal level. The ARE-dependent transactivation by R1881 determined a marked increase (fivefold) of luciferase activity also in BPH cells (Figure 1).

Seladin-1/DHCR24 Expression in Prostate Cells and Correlation with Cell Cholesterol Amount

In order to investigate the androgen responsiveness of seladin-1/DHCR24 in prostate cell models, we measured the expression level of this gene by quantitative real-time RT-PCR (qRT-PCR) in the AR-positive CaP cell line DU145-AR[18] and in BPH cells[22] expressing endogenous AR. Treatment with R1881 (10 nM, 24 h) induced a 5- and

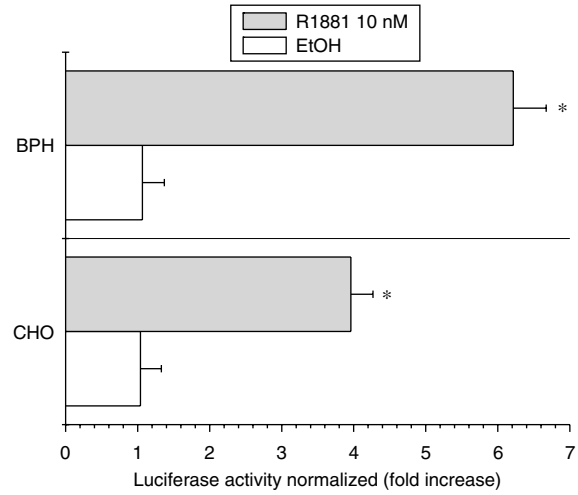

Figure 1 Functional analysis of seladin-1/DHCR24 ARE. CHO cells and human benign prostate hyperplasia (BPH) cells were transfected with selaprom-tataluc construct, pSVAR0 expression plasmid and pGL 4.75. Cells were treated with ethanol vehicle (EtOH) or R1881 (10 nM, 24 h). Firefly luciferase light output was normalized by Renilla luciferase output. Measurements, reported as fold increase *vs* vehicle, represent the mean ± s.e. of three independent experiments. Statistical analysis was performed by *t*-test; $^*P<0.05$ *vs* vehicle.

2.5-fold increase of seladin-1/DHCR24 expression *vs* untreated cells, respectively (Figure 2a). To further evaluate the involvement of AR in modulating seladin-1/DHCR24 expression and to assess the presence of a correlation with cell cholesterol content, we measured both the gene expression level by qRT-PCR and the cell cholesterol amount by GC/MS in CaP cell lines. We used AR-negative, ie DU145-Mock, PC3-wt and PC3-Neo, and AR-positive CaP cells, ie LNCaP, DU145-AR and PC3-AR. The highly invasive AR-negative cells were characterized by a lower expression of seladin-1/DHCR24 with respect to the AR-positive CaP cells (Figure 2b). Moreover, this different expression was paralleled by a modification of cholesterol content. In fact, in DU145-Mock total cholesterol was significantly lower compared to LNCaP cells (9.58 ± 0.75 *vs* 33.06 ± 3.78 ng, mean ± s.e., $P < 0.01$, *t*-test). These cell lines were selected as representative of cells not expressing or naturally expressing AR. Another cell line not expressing AR (ie PC3) was used for cholesterol measurement, and in this case the comparison was made between wild-type cells and cells in which AR expression was induced exogenously (PC3-AR). In PC3-AR cells the amount of cell cholesterol was higher than in PC3-Neo (7.69 ± 0.10 *vs* 6.31 ± 0.13 ng, mean ± s.e., $P < 0.01$, *t*-test).

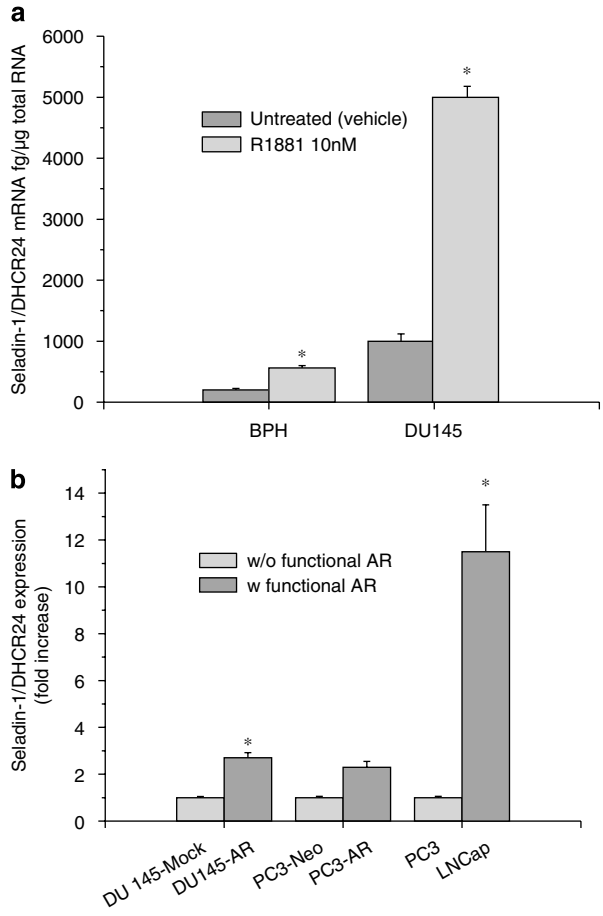

Figure 2 AR regulation of seladin-1/DHCR24 expression in prostate cells. (**a**) Expression of seladin-1/DHCR24 in DU145-AR and BPH cells in basal conditions and after exposure to R1881. (**b**) Expression of seladin-1/DHCR24 in different human CaP cell lines. The amount of mRNA was measured in DU145-Mock, PC3, PC3-Neo cell lines (without functional AR, w/o AR) and in DU145-AR, PC3-AR, LNCaP cells (with functional AR, w AR) and was reported as fold increase of AR-positive *vs* AR-negative cells, considered as 1. Statistical analysis was performed by *t*-test; $^*P < 0.05$ *vs* control cells.

Expression of Seladin-1/DHCR24 mRNA and Immunohystochemical Analysis in Human Normal and Tumor Prostate Tissues

Until now, no data about the expression of seladin-1/DHCR24 in CaP tissues *vs* paired normal tissues are available. The amount of mRNA for seladin-1/DHCR24 was investigated in tumor specimens derived from patients who underwent radical prostatectomy for clinically localized CaP. By using qRT-PCR we evaluated seladin-1/DHCR24 expression in a cohort of 61 CaP specimens collected consecutively at surgery (Table 1). The amount of seladin-1/DHCR24 mRNA was found to be significantly higher in prostate tumors compared to the adjacent normal tissue. The median expression level in the tumor tissue was 155 fg/μg total RNA as opposed to 57.6 fg/μg total RNA ($P = 0.021$) in the normal specimens. In order to confirm that higher mRNA expression corresponded to increased protein levels and to verify the

Table 1 Clinical and pathologic demographics of 61 men with clinically localized CaP treated by radical prostatectomy

Patients data	Surgery ($n = 61$)	Biochemical relapse
Follow-up ($n = 50$)	3–97 months (mean: 48.50 months)	(38%) $n = 19$
Age ($n = 54$)	51–78 years (mean: 54.62 years)	
Gleason score $\leqslant 7$	$n = 46$ (75%)	(24%)
Gleason score > 7	$n = 15$ (25%)	(60%)
pT2	$n = 33$ (54%)	(25%)
pT3-pT4	$n = 28$ (46%)	(43%)
Lymph node	$n = 61$	
Positive	$n = 8$ (13.1%)	(75%)
Negative	$n = 53$ (86.9%)	(26.4%)
Surgical margins	$n = 34$	
Positive	$n = 6$ (18%)	(33.3%)
Negative	$n = 28$ (82%)	(32.1%)
Bladder invasion	$n = 34$	
Positive	$n = 3$ (8.8%)	(100%)
Negative	$n = 31$ (91.1%)	(25.8%)

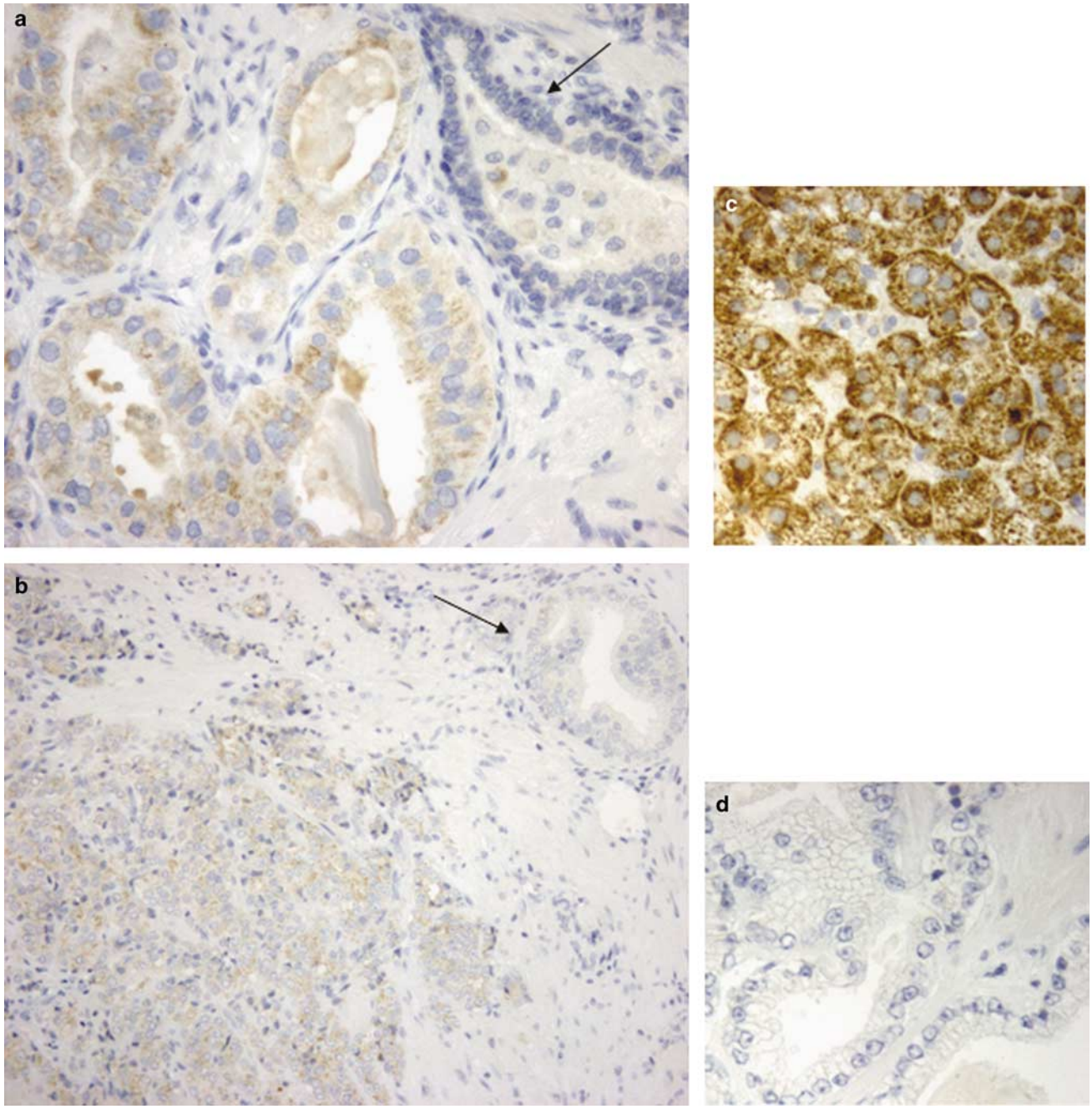

Figure 3 Immunohistochemistry for seladin-1/DHCR24 performed on formalin-fixed and paraffin-embedded CaP specimens at two different original magnifications (**a**) × 100 and (**b**) × 50. The arrows indicate areas of normal prostate tissue. (**c**) Section of human adrenal cortical adenoma, which strongly expresses seladin-1/DHCR24 protein (× 150). (**d**) CaP section stained with a nonimmune serum, used as a negative control.

localization of the protein in CaP sections, immunohystochemistry for seladin-1/DHCR24 was performed on 10 human prostate tissue specimens from our cohort of patients. A representative experiment is shown in Figure 3. Seladin-1/DHCR24 staining was detected predominantly in the cytoplasm and was expressed only at weak levels within the areas of normal prostate tissue (see arrows in Figure 3a and b), whereas a markedly stronger signal was detected in the cancerous glandular epithelia (Figure 3a and b). Sections of human adrenal cortical adenoma, which expresses high levels of seladin-1/DHCR24,[12] served as the positive control (Figure 3c). Control experiments by using nonimmune serum gave a negligible signal (Figure 3d). In addition, qRT-PCR showed that the expression of seladin-1/DHCR24 in prostate tumor samples is directly related to the mRNA levels of PSA ($R = 0.641$, $P = 0.0001$), in agreement with the androgen-responsiveness of the gene, as observed in CaP cell lines (Figure 4).

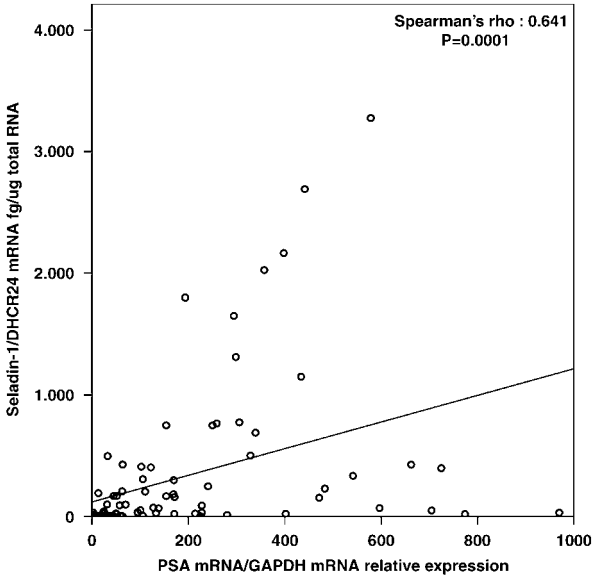

Figure 4 Correlation between the amount of seladin-1/DHCR24 and PSA expression in patients treated by surgery. Statistical analysis was performed by using Spearman's correlation test ($R = 0.641$; $P = 0.0001$).

With regard to the clinical parameters of the patients, higher levels of seladin-1/DHCR24 expression were significantly associated with a higher T stage ($P = 0.04$; Table 2). A direct correlation between seladin-1/DHCR24 expression and bladder invasion ($P = 0.006$) was also found, although the meaning of this finding is limited by the fact that only 3 out of the 34 analyzed histological samples fell into this category. It has to be said that in patients with locally advanced disease surgery is considered a possible treatment only in very selected cases[25,26] and therefore the availability of surgical samples is a very rare event.

Seladin-1/DHCR24 Expression in CaP After Androgen Ablation Treatment

To gain further insight on the androgen-mediated regulation of the *seladin-1/DHCR24* gene, we examined the levels of mRNA in CaP specimens of patients treated with androgen deprivation for 3 months before radical prostatectomy ($n = 24$). The amount of seladin-1/DHCR24 expression was significantly lower in this group of patients compared to the 61 patients, who underwent prostatectomy without prior treatment ($P = 0.005$; Figure 5).

DISCUSSION

Seladin-1/DHCR24 is broadly expressed in most peripheral tissues, including the prostate,[9] and are important in cholesterol biosynthesis by catalyzing the conversion of desmosterol into cholesterol.[6] Altered levels of expression of this gene have been detected in different neoplastic diseases[11–13] and reduced expression in CaP has been associated with an increased metastatic risk.[5] The androgen-responsiveness of seladin-1/DHCR24 has been hypothesized to be related to an ARE identified in the gene promoter region,[14] even though functional evidence was still lacking, so far. This study investigated the functional activity of seladin-1/DHCR24

Table 2 Relationship between seladin-1/DHCR24 expression and clinical features of patients treated by radical prostatectomy

Clinical features	Patients treated by surgery ($n = 61$)
PSA recurrence	$n = 58$
No	160.8
Yes	282.5
	$P = 0.73$
Stage	$n = 61$
pT2	65.8
pT3-T4	239.3
	P = 0.04
Lymph node	$n = 61$
No	168
Yes	230
	$P = 0.6$
Surgical margin	$n = 34$
No	169
Yes	1217.5
	$P = 0.07$
Bladder invasion	$n = 34$
No	170
Yes	2691
	P = 0.006
Gleason score	$n = 61$
<7	170
7	92.5
>7	28.2
	$P = 0.31$
PSA at diagnosis	$n = 38$
<9	207.5
$\geqslant 9$	239.3
	$P = 0.68$

PSA, prostate-specific antigen.
The numbers indicate seladin-1/DHCR24 expression (fg mRNA/μg total RNA, median value). Statistical analysis was performed by Mann–Whitney test and Wilcoxon's rank sum test; *n* represents the number of CaP patients falling into the indicated clinical features category. Significant values ($P < 0.05$) are in bold font.

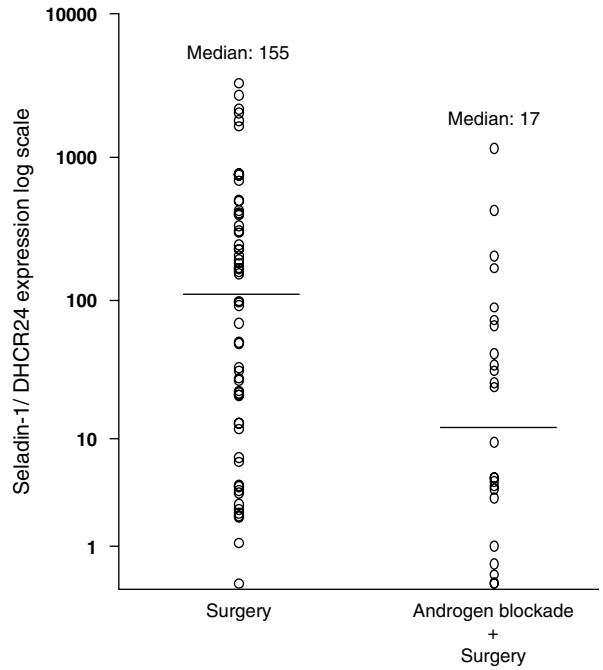

Figure 5 Seladin-1/DHCR24 expression (fg mRNA/μg total RNA, log scale) evaluated by qRT-PCR in CaP tissues from patients treated by surgery only or from patients treated by androgen ablation before surgery. Statistical analysis was performed by using Mann–Whitney test ($P = 0.005$).

ARE sequences by a luciferase reporter gene assay. The increase of luciferase activity in response to the AR agonist R1881 suggests the presence of a classical AR-mediated mechanism of transcriptional regulation of seladin-1/DHCR24 expression. This finding was confirmed by the increase of the amount of transcript elicited by R1881 in AR-positive CaP and BPH cells.

In order to investigate the relationship between seladin-1/DHCR24 expression and its enzymatic activity to produce cholesterol, the expression level of the gene was determined by qRT-PCR and the total cholesterol amount was measured by GC/MS. In AR-positive CaP cells the mRNA level of seladin-1/DHCR24 and the total cholesterol amount were higher compared to AR-negative cells. These results indicated that in CaP cell models the expression of seladin-1/DHCR24 paralleled the cholesterol content and that the presence of a functional AR was associated with higher levels of expression of the gene. It is now clear that the reexpression of the wild-type AR contributes to a more differentiated phenotype in CaP cell lines.[19,27] We observed increased levels of seladin-1/DHCR24 expression/activity following AR reexpression in AR-negative CaP cells. From this evidence we can speculate that both the expression and the function of seladin-1/DHCR24 are modulated by a newly restored androgen responsiveness. According to our findings, it has been demonstrated that a deregulated activation of the AR signalling during progression of CaP may occur also in a ligand-independent manner, through different mechanisms involving for instance AR activation by growth factors, receptor tyrosine kinases through the MAPK or the AKT pathways, or mutated coregulators.[28]

With regard to the enzymatic activity of seladin-1/DHCR24, involving the biosynthesis of cholesterol, it can be pointed out the fact that this gene may be involved in the organization of cholesterol-rich detergent-resistant membrane domains (DRMs), namely lipid rafts, as demonstrated by the observation that a reduced protein content has been found in DRMs of seladin-1/DHCR24-deficient mouse brains.[7] Interestingly, an involvement of lipid rafts in modulating cell signalling has been hypothesized. In particular, expansion of the cholesterol-rich membrane compartment, within certain limits, is likely to promote the formation of raft-resident signalling complexes that promote cell proliferation and survival in tumoral cells, including CaP.[29]

Whereas an association between decreased levels of seladin-1/DHCR24 and metastasis incidence has been found,[5] no evidence concerning the expression of this gene in CaP tissues *vs* adjacent normal tissues was available, so far. Therefore, we evaluated the amount of seladin-1/DHCR24 mRNA in specimens consecutively collected at surgery from a cohort of 61 patients diagnosed for a clinically localized CaP. The amount of transcript was significantly higher in tumoral tissues compared to the adjacent normal tissue. Accordingly, immunohystochemical analysis performed in 10 specimens showed higher protein expression in tumor glands *vs* adjacent normal areas. The analysis of the correlation between gene expression and clinical–pathological parameters revealed that increased gene expression was significantly associated with a higher T stage (T3–T4). This bimodal variation of seladin-1/DHCR24 expression (ie increased expression in CaP and reduced expression in metastatic disease) is in agreement with a similar behavior of a number of anrogen-responsive genes. In their work, Hendriksen *et al*[5] highlighted the fact that the expression of about 50% of the 200 androgen-responsive genes, which were considered, was upregulated in well-differentiated CaP, whereas the large majority of them was downregulated in metastatic disease, in which the AR pathway is disrupted. These results are in agreement with previous studies, in which gene expression profiling in CaP tissues was performed.[30,31]

Finally, in our study the androgen-mediated expression of seladin-1/DHCR24 was further confirmed in a group of patients subjected to androgen ablation treatment for 3 months before radical prostatectomy. In fact, in these specimens the expression levels of this gene were significantly lower than in patients treated only by surgery. Therefore, *seladin-1/DHCR24* may join a list of genes previously shown to be downregulated after androgen deprivation, such as *VEGF*[32] and *FGF8b*.[33]

In conclusion, in the present study we have shown for the first time that *seladin-1/DHCR24* is an androgen-dependent gene regulated by a specific ARE. In addition, the amount of expression in CaP tissues is higher than in adjacent normal tissues and appears to be associated with a higher T stage.

However, further studies designed for instance to overexpress or silence seladin-1/DHCR24 in *in vitro* models, as well as extensive *in vivo* studies, are necessary to fully elucidate the involvement of this protein, and therefore of cell lipid content, in modulating the intracellular pathways involved in CaP progression.

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