Comparison of protein expression in two prostate cancer cell-lines, LNCaP and DU145, after treatment with somatostatin

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Abstract. The mechanisms underlying prostate cancer progression are poorly understood. Proteins responsive to androgens may be involved in the development and progression of prostate cancer and the ultimate failure of androgen-ablation therapy. Therapy with somatostatin (sms) analogues could be a possible therapeutic alternative to chemotherapy in hormone refractory prostate cancer patients. We used two prostate cancer cell-lines, LNCaP (androgen-dependent) and DU145 (androgen-independent), to compare the protein expressions. Both cell lines were treated with sms and its derivative smsdx. Smsdx is a glycosylated poly sms with high stability suitable for clinical use. A comparison study of protein expression was analyzed by means of two-dimensional gel electrophoresis (2DE) followed by mass spectrometric analysis. Marked quantitative differences were observed in the protein expression profiles in sms/smsdx treated LNCaP and DU145 cells compared to the control cells. One third of the detected proteins were differentially expressed (PRDXs, hnRNPs, HSPs, RKIP). Concordance in protein expression patterns was observed between smsdx and sms treated cells with strong agreement between the up- and down-regulation of proteins. Fifty-eight (isoforms of 49 proteins) protein spots were identified and found differentially expressed at 2-fold change between LNCaP and DU145 cells. Thirty-one proteins in LNCaP have higher expressions than in DU145. Twenty-seven proteins in DU145 have higher expressions than in LNCaP. Most of the differentially expressed proteins (2-fold) between LNCaP and DU145 cells were affected by sms/smsdx treatment (1.2- to 2.6-fold change). Sms/smsdx affects the mitochondria of prostate cancer cells in a way that eventually triggers mitochondrial-mediated apoptosis. Regulation of certain proteins (e.g., RKIP, VDACs) by sms/smsdx suggests that sms/smsdx exerts its effects on prostate cancer cells via MAPK pathway and by regulating the activities of phosphotyrosine phosphatases.

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Introduction

Prostate cancer is the most common malignancy and the second leading cause of cancer-related mortality in men in the Western world (1). Although androgen ablation is the most effective therapy for patients with advanced prostate cancer, progression to androgen independence (AIPC) and hormone refractory (HRPC) eventually occurs (2). Androgen-independent prostate cancer carries a bleak prognosis with short survival time. AIPC metastasizes preferentially to the skeleton which is associated with considerable morbidity such as pain and fractures.

Several pathways provide insights into the mechanism of androgen action and schemes by which cancer cells subvert normal growth control and escape treatment attempts. Understanding the pathways that lead to AIPC will eventually lead to the development of new therapies. In our previous studies (3,4), smsdx (glycosylated poly sms with high stability suitable for clinical use) was found to be able to trigger up-regulation of catalytic mitochondrial proteins and seemed to affect apoptotic-related proteins in androgen-dependent (AD) prostate cancer LNCaP cells. One hypothesis could be that there are groups of proteins crucially involved in the progression and transition to HRPC. Given the importance of proteins regulated by sms/smsdx in the disease development and progression, characterization of the affected proteins may provide candidates for biomarkers and elucidate the regulatory mechanisms of sms/smsdx effects.

The aim of this study was to compare the protein expression in androgen-dependent and androgen-independent prostate cancer cells after sms and smsdx treatment. Through proteomic analysis, we could explore potential candidate biomarkers and elucidate sms/smsdx effect pathways in prostate cancer.

Materials and methods

Cell culture. LNCaP and DU145 human prostate cancer cell lines (American Type Culture Collection, Rockville, MD,

Table I. The RT-PCR primers sequences and amplified genes.

Amplified gene	Primers	Sequence	Product (bp)
PRDX2	Sense	5'-GCCCACGCAGCTTTCAGTCA-3'	623
	Antisense	5'-AGCCAGCCTAATTGTGTTTG-3'	
TCTP	Sense	5'-GAGGGGAAGATGGTCAGTAGG-3'	278
	Antisense	5'-TGCTTGATTTGTTCTGCAGC-3'	
HSP27	Sense	5'-CCAGAGCAGAGTCAGCCAGCAT-3'	576
	Antisense	5'-CGAAGGTGACTGGGATGGTGA-3'	

USA) were cultured in RPMI-1640 containing 10% foetal bovine serum (FBS), 2 mM glutamine and 100 IU/ml penicillin-100 μg/ml streptomycin at 37˚C in a humidified atmosphere of 5% CO_2. The medium was changed two times a week and the cells were trypsinized and subcultivated once a week. Smsdx was prepared as described previously (5). Sms was from Ferring, Kiel, Germany. The cell culture was treated with smsdx or with sms for three days, 1 nM per day, as described by Brevini *et al* (6). Controls were untreated cells (negative) and cells treated with sms14 (positive).

Sample preparation and protein extraction and concentration. All the cultured cells were prepared according to a cell preparation method (7), with slight modification. Briefly, the cell monolayers were washed twice in phosphate-buffered saline (PBS) and then scraped off in ice-cold PBS including protease inhibitors (PIH), phenylmethylsulfonyl fluoride (PMSF) 0.2 mM and 0.83 mM benzamidine pelleted at 660 x g, 3 min (4˚C) and washed one time before final centrifugation at 2700 x g, 5 min. The wet weight of the cell pellet was recorded and the cells were stored at -80˚C until further processing. Each sample was then dissolved lysis buffer containing 8 M urea, 4% SDS, reducing agents and protease inhibitors. Protein determination was made using Pierce BCA protein assay reagent (Rockford, IL, USA).

Electrophoresis, scanning and image analysis. 2-DE was performed as previously described (7). Pharmalyte 3-10 for IPG strip 3-10 were used for isoelectric focusing, and 12.5% linear gradient SDS-polyacrylamide gels were used in the second dimension. Gels were stained with silver nitrate as described by Rabilloud *et al* (8) and scanned at 100-μm resolution using a Molecular Dynamics (Sunnyvale, CA, USA) laser densitometer. Both quantitative and qualitative 2-DE data sets were analyzed using PDQuest software, a 2-DE software analysis program (9) obtained from Pharmacia Biotech (Uppsala, Sweden).

Mass spectrometry analysis. Protein spots of LNCaP cells were selected for identification by mass spectrometry as described before (7). In short, proteins were identified with a vMALDI-LTQ instrument (Thermo Electron, San José, CA, USA). The spot picking, destaining, digestion, extraction, sample preparation and spotting on MALDI target plates were carried out using a spothandling workstation (ETTAN Spothandling workstation, GE Healthcare) and a standard protocol provided by GE Healthcare. The MS spectra were collected in the 900-2000 Da mass range while the mass range for the MS/MS spectra were automatically selected by the system based on a Q-value of 0.25.

Protein identification. Database searches were done using both the MASCOT and Sequest search algorithm against the human session of the IPI protein database (version 2.38). The two searches were compared in the in-house developed software Promiscuous MS/MS. A minimum of two peptides and a Mascot score of 45 were required for a protein to be accepted as identified.

RNA extraction and RT-PCR. Total LNCaP cellular RNA, after treatment with sms and smsdx in different concentration (0, 0.1, 1.0, 10, 50, 100 nM for 24 h) and incubation time (0, 1, 4, 8, 16, 24 h for 10 nM), was extracted using the TRIzol® (Invitrogen, Carlsbad, CA, USA), according to the manufacturer's instruction. cDNA was synthesized using random primers (N6) and MMLV reverse transcriptase. The PCR for PRDX2, TCTP, HSP27 mRNA were performed by using the following primer pairs (Table I).

A total of 32 PCR cycles at 94˚C for 30 sec, 53˚C for 40 sec and 72˚C for 60 sec for PRDX2; at 94˚C for 60 sec, 60˚C for 60 sec for TCTP; at 94˚C for 30 sec, 57˚C for 30 sec, and 72˚C for 40 sec for HSP27, and 72˚C for 60 sec for was carried out. ß-actin expression was used as a control for RNA loading and reverse transcription efficiency and amplified with its specific primers using 25 cycles. PCR products were resolved in 2% agarose gels, stained with ethidium bromide, and visualized in UV light.

Results

Creation of 2DE protein map of LNCaP. We successfully identified a set of 222 proteins (isoforms and variants) for 2DE map in LNCaP cell line. The highest and lowest protein molecular masses that we found were 111 kDa for 150 kDa oxygen-regulated protein (HYOU1) and 14.8 kDa for gastric-associated differentially-expressed protein YA61P (YA61). Proteins pI range from 4.59 to 10.52.

Variation in protein expression between LNCaP and DU145 cell line. After analysis using PDQuest software version 7.0,

Table II. Thirty-one lower expressed proteins (>2-fold) in DU145 than in LNCaP.

Protein name	Short name	Uniprot ID	DU145 smsdx	DU145 sms	LNCaP smsdx	LNCaP sms
Creatine kinase, B chain	CKB	P12277	2.0	1.2	-1.3	-
Septin 2	S EPT2	Q15019	2.0	2.0	-	1.6
Serine-threonine kinase receptor-associated protein	STRAP	Q9Y3F4	-1.2	-2.0	1.5	-
Ubiquinol-cytochrome c reductase iron-sulfur subunit	UQCRFS1	P47985	-1.5	-1.2	NA	-
Heterogeneous nuclear ribonucleoproteins C1/C2	HNRPC	P07910	1.5	-1.5	-	2.6
Leucine aminopeptidase	LAP3	Q6P0L6	1.2	-1.2	1.5	-
Malate dehydrogenase	MDH2	P40926	1.2	-1.2	-	2.1
Voltage-dependent anion channel 1	VDAC1	P21796	1.5	-1.5	1.7	1.7
Enabled protein homolog	ENAH	Q8N8S7	-2.0	1.5	1.8	1.4
78 kDa glucose-regulated protein	GRP78	P11021	1.2	-	1.3	1.3
Endoplasmin	HSP90B1	P14625	1.5	-	2.3	-
Far upstream element binding protein 1	FUBP1	Q96AE4	1.2	-	1.3	1.3
Fibroblast growth factor-5	FGF5	P12034	1.2	-	-	2.8
Glycerol-3-phosphate dehydrogenase	GPD2	P43304	1.2	-	1.6	1.7
Creatine kinase, ubiquitous	CKMT	P12532	-2.0	-	1.4	-
Heat-shock protein ß-1	HSPB1	P04792	-1.2	-	-	1.3
Mitochondrial inner membrane protein	IMMT	Q16891	-1.2	-	-	1.4
Protein disulfide-isomerase A3	PDIA3	P30101	-1.2	-	1.2	-
ATP synthase γ chain	ATP5C1	P36542	-	1.2	-	3.3
Far upstream element binding protein 2	KHSRP	Q92945	-	2.0	-	-
Flavin reductase	BLVRB	P30043	-	1.5	-1.4	-
Heat-shock protein ß-1	HSPB1	P04792	-	1.2	1.7	-1.3
Heterogeneous nuclear ribonucleoprotein D0	HNRPD	Q14103	-	1.5	-	1.8
Electron transfer flavoprotein α-subunit	ETFA	P13804	-	-2.0	-	1.4
Heterogeneous nuclear ribonucleoproteins C1/C2	HNRPC	P07910	-	-	2.9	-
Lupus La protein	SSB	P05455	-	-	-1.6	-1.3
Probable mitochondrial import receptor subunit TOM40 homolog	TOMM40	O96008	-	-	2.6	2.1
Prostatic binding protein	PEBP1	P30086	-	-	-1.3	-
Acetyl-CoA acetyltransferase, cytosolic	ACAT2	Q9BWD1	-	-	-	NA
Septin 11	S EPT11	Q9NVA2	-	-	-	1.2
Voltage-dependent anion channel 1	VDAC1	P21796	-	-	-	1.7

Some of listed differentially expressed proteins in DU145 and LNCaP can be regulated by sms and/or smsdx in up- and down-regulated manner.

we found 344 protein spots were differentially expressed at 2-fold change between LNCaP and DU145 cells. One hundred and twenty-five protein spots in DU145 cells were matched with the reference protein map of LNCaP cells. Fifty-eight protein spots were identified and found differentially expressed at 2-fold change between LNCaP and DU145 cells. These spots represented different isoforms of 49 proteins. In general, the distribution of isoforms obtained in the present study was as follows: HnRPH1 was represented by three isoforms, HSPB1 by three, hnRPC by two, KRT8 by two, PSIP1 by two, TAF15 by two, VDAC1 by two.

Differential protein expression between LNCaP and DU145 cell line. Fifty-eight different protein expressions (2-fold change) were found between LNCaP and DU145 cells. Thirty-one proteins in LNCaP have higher expressions than in DU145. Twenty-seven proteins in DU145 have higher expressions than in LNCaP (Tables II and III). For example,

Table III. Twenty-seven higher expressed proteins (>2-fold) in DU145 than in LNCaP.

Protein name	Short name	Uniprot ID	DU145 smsdx	DU145 sms	LNCaP smsdx	LNCaP sms
α enolase	ENO1	P06733	1.5	1.5	-	1.3
Heat shock cognate 71 kDa protein	HSPA8	P11142	1.2	2.0	-1.3	-
Lens epithelium-derived growth factor p75	PSIP1	Q8N4N4	2.0	1.5	2.1	3.4
Paraspeckle protein 1	PSPC1	Q8WXF1	1.2	1.2	1.4	1.3
Thioredoxin-dependent peroxide reductase	PRDX3	P30048	1.2	1.2	1.3	-
Cofilin, non-muscle isoform	CFL1	P23528	-1.2	-1.5	-	-
Lens epithelium-derived growth factor p75	PSIP1	Q8N4N4	-1.5	-1.2	-	3.0
Glyoxalase I	GLO1	Q04760	-1.5	-1.2	-	-1.4
Mitochondrial processing peptidase ß subunit	PMPCB	O75439	-2.0	-1.2	1.2	-
Heterogeneous nuclear ribonucleoprotein H	HNRPH1	P31943	1.2	-1.2	1.9	-
Lamin B1	LMNB1	P20700	-1.2	1.2	1.6	-
Dihydrolipoyl dehydrogenase	DLD	P09622	1.2	-	-	1.5
Keratin, type II cytoskeletal 8	KRT8	P05787	-1.2	-	4.0	2.1
Keratin, type II cytoskeletal 8	KRT8	P05787	-	-2.0	-	3.8
Peptidyl-prolyl cis-trans isomerase A	PPIA	P62937	-	-1.5	1.2	-
Peroxiredoxin 2	PRDX2	P32119	-	-1.2	-	-2.1
Polyribonucleotide nucleotidyltransferase 1	PNPT1	Q9BRU3	-	-1.5	2.8	1.8
Heat-shock protein ß-1	HSPB1	P04792	-	-	-	-1.5
Heterogeneous nuclear ribonucleoprotein H	HNRPH1	P31943	-	-	-1.4	-
Heterogeneous nuclear ribonucleoprotein H	HNRPH1	P31943	-	-	-	1.2
Heterogeneous nuclear ribonucleoproteins A2/B1	HNRPA2B1	P22626	-	-	-	2.7
Splicing factor, arginine/serine-rich 1	SFRS1	Q07955	-	-	-	-1.7
Stress-70 protein	HSPA9	P38646	-	-	1.8	-
TATA-binding protein associated factor 2N	TAF15	Q92804	-	-	1.6	1.5
TATA-binding protein associated factor 2N	TAF15	Q92804	-	-	-	2.6
UBA/UBX 33.3 kDa protein	SAKS1	Q04323	-	-	-2.1	-1.3
Voltage-dependent anion-selective channel protein 2	VDAC2	P45880	-	-	1.8	1.8

Some of listed differentially expressed proteins in DU145 and LNCaP can be regulated by sms and/or smsdx in up- and down-regulated manner.

prostatic binding protein (PEBP1/RKIP) has a higher level expression in androgen-dependent LNCaP cells than in androgen-independent DU145 cells.

Sms/smsdx affected proteins. After treatment with sms and smsdx, we found strong agreement with the protein expression regulation in DU145 cells (Table IV). Most of the differentially expressed proteins (>2-fold) between LNCaP and DU145 cells were affected by sms/smsdx treatment (1.2- to 2.6-fold change) (Tables II and III). Fig. 1 shows down-regulation of protein expression of PRDX2 (A) and TCTP (B) (2-fold) after treatment with 1 nM sms/smsdx for three days in the prostate cancer cell line LNCaP.

RT-PCR results. Of the identified differentially expressed proteins between LNCaP and DU145 cells, PRXII, TCTP, HSP27 were tested on the mRNA expression in LNCaP after treatment with sms and smsdx in different concentration and incubation time. Fig. 2 shows up- and mRNA down-expression of PRDX2 (A) and TCTP (B), respectively, after incubation with smsdx in different concentrations and incubation time in LNCaP cell line. PRDX2 was found to be up regulated in a dose-dependent manner. TCTP mRNA was down-regulated in the current study. HSP27 mRNA was also down-regulated (data not shown).

Discussion

Prostate cancer continues to be the most common malignancy diagnosed in men in the western countries. In Europe, there were about 190,000 new cases in 2004 (10), 301, 500 incident cases, and 87,400 deaths in 2006 (11). The transition of prostate cancer from androgen-dependent (ADPC) to androgen-independent (AIPC) and hormone refractory

Table IV. Twenty-two proteins regulated by sms/smsdx (~2-fold) in DU145.

Protein name	Short name	Uniprot ID	smsdx	sms
60 kDa heat shock protein	HSPD1	P10809	2.0	2.0
Creatine kinase, B chain	CKB	P12277	2.0	1.2
Lens epithelium-derived growth factor p75	PSIP1	Q8N4N4	2.0	1.5
Septin 2	SEPTII	Q15019	2.0	2.0
δ3,5-δ2,4-dienoyl-CoA isomerase	ECH1	Q13011	2.0	-
Actin, cytoplasmic 1	ACTB	P60709	1.2	-2.0
ATP synthase α chain	ATP5A1	P25705	1.2	2.0
Far upstream element binding protein 2	KHSRP	Q92945	1.2	2.0
Fibroblast growth factor-5	FGF5	P12034	1.2	2.0
Heat shock cognate 71 kDa protein	HSPA8	P11142	1.2	2.0
Ran-specific GTPase-activating protein	RANBP1	P43487	1.2	2.0
Creatine kinase, ubiquitous	CKMT1A	P12532	-2.0	-1.2
Enabled protein homolog	ENAH	Q8N8S7	-2.0	-2.0
Eukaryotic initiation factor 5A	EIF5A	P63241	-2.0	-1.5
Far upstream element binding protein 2	KHSRP	Q92945	-2.0	-2.0
Heterogeneous nuclear ribonucleoprotein H	HNRPH1	P31943	-2.0	-1.5
Mitochondrial processing peptidase ß subunit	PMPCB	O75439	-2.0	-1.2
Translationally controlled tumor protein	TCTP	P13693	-2.0	-
Chromatin assembly factor 1 subunit C	RBBP4	Q09028	-1.5	-2.0
Serine-threonine kinase receptor-associated protein	STRAP	Q9Y3F4	-1.2	-2.0
Gastric-associated differentially-expressed protein YA61P	YA61	Q9NZ23	-	2.0
Keratin, type II cytoskeletal 8	KRT8	P05787	-	-2.0

Listed proteins are regualted by both sms and smsdx in the same trend except actin, cytoplasmic 1 in an opposite manner.

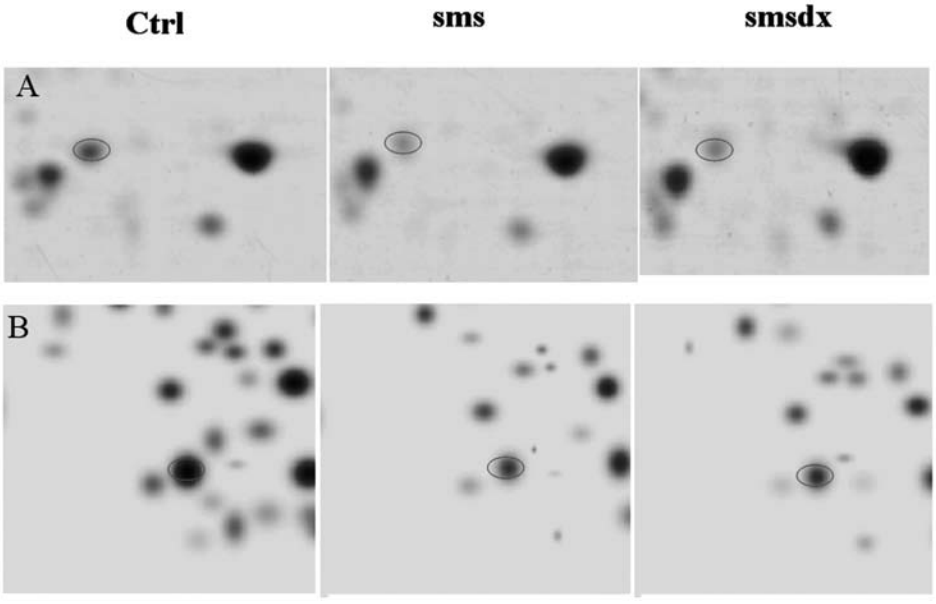

Figure 1. The identified protein expression of PRDX2 (A) and TCTP (B) (2-fold down-regulation) after treated with 1 nM sms/smsdx for three days in the prostate cancer cell line LNCaP.

(HRPC) making anti-androgen therapy ineffective remains a significant clinical problem. Chemotherapy has only limited efficacy in AIPC and HRPC (12). During androgen-dependent progression, prostate cancer cells depend on the androgen receptor as the primary mediator of growth and survival. Certain growth factors, such as insulin-like growth-factor-1

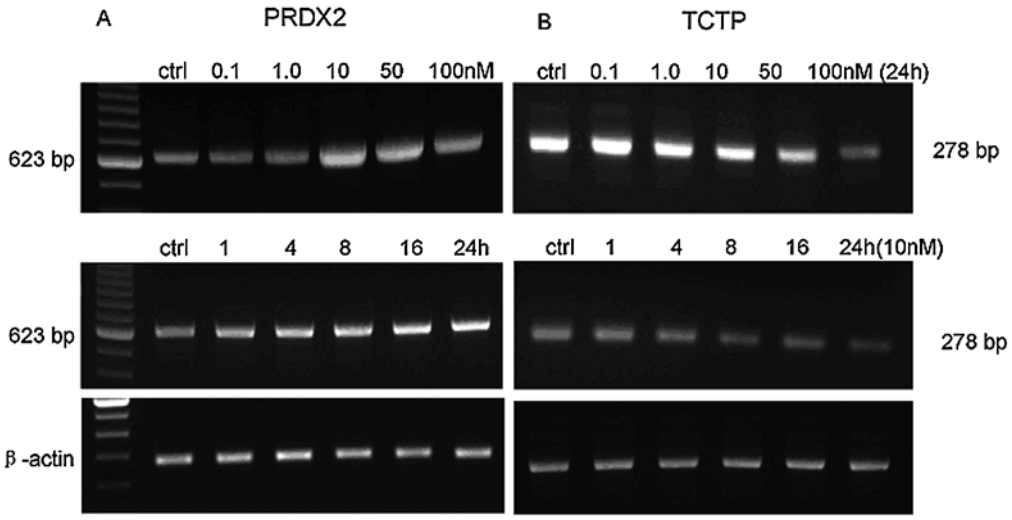

Figure 2. PRDX2 and TCTP mRNA expression in LNCaP cell line after incubation with smsdx. Up-regulation of PRDX2 (A) and down-regulation of TCTP (B) mRNA in smsdx treated LNCaP cell line with different concentrations (0, 0.1, 1.0, 10, 50, 100 nM for 24 h) (upper lane) and incubation time (0, 1, 4, 8, 16, 24 h for 10 nM) (middle lane); control, lower lane.

(IGF-1), can activate the androgen receptor (AR) and can therefore induce AR target genes in the absence of androgen. IGF-1 can induce a 5-fold rise in PSA secretion in LNCaP cells (13). The use of sms analogues as monotherapy in patients with HRPC has produced only modest clinical responses. However, Koutsilieris *et al* recently developed a therapeutic 'combination concept' i.e. combining sms with other drugs, aiming at suppressing the bioavailability of IGF-1 and downstream biological effectors. A potential mechanism for the efficacy of this combination regimen involves the abrogation of the protective effect of IGF-I on prostate cancer cells (14,15). Activation of phosphotyrosine phosphatases (PTPs) by somatostatin receptors (SSTRs) represents one of the main intracellular mechanisms involved in the antiproliferative effect of sms analogues (16). The mitogen activated protein (MAP) kinase cascade represents one of the major regulators of cell growth by hormones and growth factors. Effects of sms on the MAP kinase cascade are regulated through SSTR1 (17). Kosari *et al* found that SSTR1 is associated with systemic progression in prostate cancer after comparisons of gene expression profiles obtained from the epithelial cells of non-neoplastic tissue, primary tumor, and metastatic tumor samples, SSTR1 was found to be the most prominent candidate prognostic biomarker in aggressive prostate cancer (18). The universal affinity of smsdx may be of importance when treating HRPC that predominantly expresses this SSTR1 subtype.

Marked quantitative differences were observed in the protein expression profiles in sms/smsdx treated LNCaP and DU145 cells compared to the control cells. One third of the detected proteins were differentially expressed (PRDXs, hnRNPs, HSPs, TCTP, RKIP). Concordance in protein expression patterns were observed between smsdx and sms treated cells with strong agreement between the up- and down-regulation of proteins indicating preservation of sms effects of smsdx. Some proteins differentially expressed in androgen-dependent and androgen-independent cells can be regulated by the treatment of sms and smsdx. These proteins possibly elucidate some of the molecular mechanism of prostate cancer progression from androgen responsive to androgen refractory. The results support androgen regulatory activity on the LNCaP and DU145 cells at the level of protein.

In peroxiredoxin (PRDX) family, PRDX1, 2, 3 and 6 were identified in prostate cancer cell lines LNCaP and DU145. PRDX2 and PRDX6 were down-regulated by sms/smsdx in LNCaP and DU145 cells. PRDX3 was up-regulated by sms/smsdx in these two cell lines. PRDX2 is known to protect cells from oxidative damage and to confer resistance to oxidative damage to cancer cells. PRDX2 had higher expression in highly metastatic prostate cancer cells (19). However, the role of PRDX2 in tumor progression is poorly understood. In this study, PRDX2 protein has a higher expression in DU145 cells compared with LNCaP cells. PRDX2 mRNA was significantly up-regulated by sms and smsdx treatment in LNCaP cells. PRDX2 protein expression was down-regulated by sms (4). The different manners of regulation by sms/smsdx in PRDXs are thought to be related to phosphorylation, overoxidation and proteolysis of PRDXs. The roles of PRDXs in regulating levels of hydrogen peroxide, an intracellular signaling molecule common to many cytokine-induced signal-transduction pathways, possibly lead to different expressions in LNCaP and DU145 cells.

The heterogeneous nuclear ribonucleoproteins (hnRNPs) have central roles in DNA repair, telomere biogenesis, cell signalling and in regulating gene expression at both transcriptional and translational levels. Yang *et al* found that stable transfection of hnRNPA1 in the LNCaP cells suppressed AR-mediated cell growth and the expression of prostate-specific antigen, and this suppressive effect was abolished by the addition of ARA54-small interfering RNA (20). In the current study, hnRNPK, hnRNPH1, hnRNPD, hnRNPC, hnRNPA2B1 and hnRNPL were identified. hnRNPH1, hnRNPA2B1 have higher expression and hnRNPD, hnRNPC lower expression up to 2-fold change in DU145 cells compared to LNCaP cells. hnRNPH was down-regulated by sms/smsdx in DU145. hnRNPD was unregulated by sms/smsdx in DU145 and

LNCaP cells. hnRNPA2B1 was up regulated by sms in LNCaP but not in DU145 cells. The hNRNPs were regulated by sms/smsdx in different manner, sometime up sometime down. Their differential expression of hnRNPs sheds light on the underlying transition mechanism from prostatic androgen-dependence to androgen-independence and the different aggressive and metastatic abilities between LNCaP and DU145 cells.

Heat shock proteins (HSPs) have strong cytoprotective effects and behave as molecular chaperones for other cellular proteins. Several of these proteins have been demonstrated to directly interact with components of the cell signalling pathways, for example, those of the tightly regulated caspase-dependent programmed cell death machinery, upstream, downstream and at the mitochondrial level (21). Among the different HSPs, HSP27 and HSP70 are the most strongly induced after stresses such as anticancer drugs, oxidative stress or irradiation. Both HSP27 and HSP70 are abundantly expressed in cancer cells and therefore have been suggested as important prognostic factors in malignant diseases (22). In the current study, some of HSPs were regulated by sms/smsdx. For example, HSP60/HSPD1 was up-regulated by both sms and smsdx in DU145. Since it is known that HSPs bind to and inactivate ARs, one could assume that increased concentration of HSP60 may block the binding of androgens to the receptor.

The translationally controlled tumor protein (TCTP) is an abundantly expressed protein. Arcuri *et al* found expression of TCTP in the human prostate and in prostate cancer cells (23), and suggested the involvement of the protein in key-processes such as apoptosis, cellular differentiation, and in the control of sperm functions. TCTP was found to antagonize apoptosis by inserting into the mitochondrial membrane and inhibiting Bax dimerization (24). So deregulation of TCTP by smsdx in the current study suggested its possible involvement in the sms analogue cytostatic effect via mitochondrial-mediated pathway.

Raf kinase inhibitory protein (RKIP) was found to regulate activation of MAPK, NF-κB and G protein coupled receptors (GPCRs) (25). RKIP plays an important role in cancer invasion and metastasis (26). Its levels were found to be reduced or absent in variants of established cell lines derived from metastatic prostate cancer (27). Overexpression of RKIP sensitizes tumor cells to chemotherapeutic drug-induced apoptosis (28). Fougner *et al* found a significant correlation between the adenoma RKIP level and the clinical effect of Octreotide in addition to the SSTR2a protein expression (29). Therefore, a synergistic inhibitory effect on the MEK-ERK pathway is a possible mechanism for the association between adenoma RKIP level and the clinical efficacy of sms analogues. In the present study, RKIP was found in a higher level of expression in androgen-dependent LNCaP cells, but lower expression in androgen-independent DU145 cells. Low RKIP levels in DU145 were suggested to be correlated with enhanced extracellular signal-regulated-kinase (ERK)/MAPK pathway activation. This suggested RKIP has a possible role in the transition of androgen dependency and is a potential target for prostate cancer.

In conclusion, the proteomic analysis has yielded quantitative and qualitative results elucidating the effect of androgen deprivation on the proteome of prostatic cancer AD and AI cell lines. From the present results it seems reasonable to assume that sms/smsdx affects the mitochondria of LNCaP and DU145 cells in a way that eventually triggers mitochondrial-mediated apoptosis. Induced expression of SSTRs and regulation of certain proteins by sms/smsdx in the present study suggest that sms/smsdx exerts its effects on prostate cancer cells via the MAPK pathway and by regulating the activities of PTPs.

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