

Somatostatin effects on the proteome of the LNCaP cell-line

ZHAOXU LIU^{1,5}, SOFIA BENGTTSSON², MORTEN KROGH³, MARCELA MARQUEZ¹,
STEN NILSSON¹, PETER JAMES², AYODELE ALIAYA⁴ and ANDERS R. HOLMBERG¹

¹Department of Oncology and Pathology, Karolinska Institute, SE-171 76 Stockholm; ²Department of Protein Technology,

³Complex Systems Division, Department of Theoretical Physics, Lund University, Lund SE-22100, Sweden;

⁴Department of Biological and Medical Research, King Faisal Specialist Hospital, 11211 Riyadh, Saudi Arabia;

⁵Aging and Health Center, School of Nursing/Qilu Hospital, Shandong University, 250012 Jinan, P.R. China

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Abstract. Some clinical results indicate that somatostatin (sms) might be useful in the treatment of advanced prostate cancer (HRPC). Because of its transient *in vivo* half-life only more stable derivatives of sms are of interest in this context. Recent studies have shown that natural sms can be conjugated to a carbohydrate (smsdx) with preservation of sms-like effects on the prostatic tumor cell proteome. The present study identifies some of the affected proteins in an effort to elucidate pathways and proteins that might be of importance for the potential usefulness of sms treatment in HRPC. After incubating the LNCaP cell-line with sms14/smsdx, comparative proteomics was used for analysing and identifying affected proteins. Protein expression patterns were analysed with two-dimensional polyacrylamide gel electrophoresis and mass spectrometry. Catalytic mitochondrial and mitochondrial-associated proteins were significantly affected (fold change ~ 2 or higher) and they were in general up-regulated. Apoptosis-related proteins were both up-regulated (VDAC1, VDAC2) and down-regulated (PRDX2, TCTP). The fold change was >2 for PRDX2 and <2 for the others. There was a strong agreement between sms and smsdx on the up- and down-regulation of proteins. Sms/smsdx triggered up-regulation of catalytic mitochondrial proteins and seemed to affect apoptosis-related proteins. This could indicate important pathways on which smsdx might be able to curb the progression of HRPC. The results encourage a pending clinical phase II study.

Introduction

Prostate cancer is a major health problem in the Western world being the second leading cause of cancer related death

in men (1). The prognosis of early-stage well-differentiated prostate cancer is favourable however some patients have extra-prostatic invasion at the time of diagnosis and some of these have metastatic disease (2). In early-stage localised disease, radical prostatectomy or radiation therapy, or a combination of both modalities, are considered curative. In metastatic disease androgen-ablation therapy is standard treatment, however the disease progresses eventually and becomes unresponsive to hormonal treatment (hormone refractory prostatic cancer, HRPC). At this stage few treatment options exist and new modalities are urgently needed. Recently, a taxan derivative has shown some treatment efficacy in HRPC (3). Additionally, recent results with sms derivatives combined with dexamethasone or in combination with estrogens seem promising (4).

Somatostatin (sms) is important in the regulation and physiological control of various organs including the prostate. Sms can inhibit tumor growth (growth arrest), be cytotoxic (induction of apoptosis) and inhibit tumor angiogenesis (5,6). The sms analog Octreotide inhibits the growth of MCF-7 by activating wt p53 and Bax and triggers rapid apoptosis in this cell-line (7). Sms receptors (SSTRs) might be potential targets for prostate cancer treatment. However, optimal dose/dose interval and the optimal sms derivative still need to be determined.

Recently an sms derivative (smsdx) based on natural sms14 having affinity to all five SSTR subtypes, was developed (8,9). The long half-life and the pan affinity of smsdx distinguish it from all other sms derivatives in clinical use which have shorter half-lives and limited SSTR affinity (affinity only to SSTR2 and 5). Smsdx has completed a clinical phase I study revealing low toxicity and high tolerability (10).

LNCaP is a human prostate cancer cell-line and often used in various *in vitro* studies. It has been established from supra-clavicular lymph node metastases (11). These cells express androgen receptors (ARs) and exhibit androgen-sensitive cell growth. Although there is a physiologically equivalent level of androgenic activity in the regular culture condition, LNCaP cells gradually lose their androgen requirement for growth upon passage, mimicking tumor progression in patients (12-14).

In a recent study, it was demonstrated that smsdx affect the proteome of LNCaP in an sms14-like way i.e. sms14 effects are preserved in the construct (15). In the present study,

Correspondence to: Dr Anders R. Holmberg, CCK R8/3, Urologic Oncology Group, Karolinska University Hospital, Karolinska Institute, SE-171 76 Stockholm, Sweden
E-mail: arh@telia.com

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alterations in protein expression patterns of LNCaP after incubation with sms/smsdx were analysed. Two-dimensional gel electrophoresis (2DE) in combination with mass spectrometry was used for the identification of differentially affected proteins. The character of some of the proteins and their possible role in how sms might be able to curb the progression of HRPC were elucidated.

Materials and methods

Cell line culture. LNCaP human prostate cancer cell line (American Type Culture Collection, Rockville, MD, USA) was cultured in RPMI-1640 containing 10% fetal bovine serum (FBS), 2 mM glutamine and 100 IU/ml penicillin-100 µg/ml streptomycin at 37°C in a humidified atmosphere of 5% CO₂. The medium was changed twice a week and the cells were trypsinized and subcultivated once a week. Smsdx was prepared as described previously (9). Sms was from Ferring, Kiel, Germany. The cell culture was treated with smsdx or with sms for 3 days, 1 nM per day, as described by Brevini *et al* (16). Controls were untreated cells (negative) and cells treated with sms (positive).

Sample preparation for 2D-PAGE. The cellular extraction from LNCaP and the preparation of the total cell lysate were performed as previously described by Franzen *et al* (17). Protein determination was made using Pierce BCA protein assay reagent (Rockford, IL, USA). Protein (500 µg) was mixed with rehydration buffer [8 M urea, 2% CHAPS, 0.002% bromophenol blue, 18.2 mM DTT, 0.5% pharmalyte (pH 3-10 NL)], left at room temperature for 15 min and centrifuged for 10 min before being applied to a 24-cm immobilized pH gradient strip (pH 3-10 NL) for overnight rehydration.

2D-PAGE and image analysis. First-dimension isoelectric focusing was carried out on GE Healthcare IPG-phor according to the manufacturer's instructions, with a total focusing time of around 70 kVh. The strips were equilibrated in 15 ml equilibration solution [6 M urea, 50 mM Tris-HCl (pH 8.8), 30% (w/v) glycerol, 2% (w/v) SDS, 0.002% bromophenol blue] reduced with 65 mM DTT for 15 min, followed by 15-min equilibration in equilibration solution with 135 mM iodoacetamide added. The IPG-strips were then loaded and run on a 12.5% SDS-PAGE gel (25°C) with 10 W/gel until the bromophenol blue dye front had run off the base of the gel. The gels were stained in colloidal coomassie (LabDesign Boule Nordic AB) overnight and then destained in water according to the manufacturer's instructions. The gels were scanned using GE Healthcare ImageScanner. Spot detection and matching was performed in ImageMaster 2D Platinum (GE Healthcare) with three manual landmarks set.

Statistical analysis. Three gels were run for each of the three groups; control, smsdx, and sms. The master gel belonged to the smsdx group. The 1083 spots on the master gel were matched with spots on the other 8 gels, when possible. If no matching spot was found on a gel, it was designated as a missing value. A spot can be missing either due to an experimental error or due to low abundance. The two treated groups, sms and smsdx, were compared separately with the

control group using a Mann-Whitney test. Since the control group and a treated group contains three measurements each, the lowest possible two-sided Mann-Whitney p-value is 0.1, which is achieved exactly when all 3 values in one group are lower or higher than all 3 values in the other group. This can only happen when there are no missing values. The number of spots without any missing values in the smsdx and control group was 468. Out of these, 137 had a p-value of 0.1, leading to a false discovery rate of 0.29. To include the rest of the spots, missing values were set to zero in the Mann-Whitney test. So, the spots with a p-value of 0.1 were those with the three highest measurements in one group, and all measurements in the other group were either lower or missing. The number of spots in the control vs smsdx comparison with at least one missing value was 615. Out of these, 100 had a p-value of 0.1, leading to a false discovery rate of 0.62. The total number of spots with a p-value of 0.1 for the control vs. smsdx comparison was hence 237 out of 1083, leading to a false discovery rate of 0.46. For the control vs. the sms group, the corresponding numbers were: 140 out of 436 (FDR = 0.31) without missing values, 83 out of 647 (FDR = 0.78) with missing values, and 223 out of 1083 (FDR = 0.49) in total.

Mass spectrometry analysis. Proteins were identified with a vMALDI-LTQ instrument (Thermo Electron, San José, CA). 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 plate containing the combined extracts was evaporated to dryness. Each sample was prepared by constituting the dried peptides in 2.5 µl of matrix solution [2.5 mg/ml of α-cyano-4-hydroxy-cinnamic acid (Sigma) in 50% acetonitrile containing 0.05% TFA]. Sample (2.0 µl) was spotted on a clean MALDI target slide surface and allowed to dry. The samples were analysed with a vMALDI-LTQ (Thermo Electron). The analysis was performed using Xcalibur 1.4 software in data dependent mode. A survey scan (MS) was followed by MS/MS scans on the 5 most abundant ions. This string of 6 scan events was repeated six times for each sample spot. Dynamic Exclusion™ ensured that in total 30 different peptides were selected and fragmented for each sample. The MS spectra were collected in the 900-2,000 Da mass range while the mass range for the MS/MS spectra was automatically selected by the system based on a Q value of 0.25. The standard collision energy of 38 was set for all analyses. A time limit of 5 min/sample was selected, whether or not the 30 MS/MS spectra could be acquired. Database searches were performed 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.

Results

Functional classification of proteins. The proteins were classified using the Swiss-Prot website, the Genome Browser website, and the National Center for Biotechnology Information

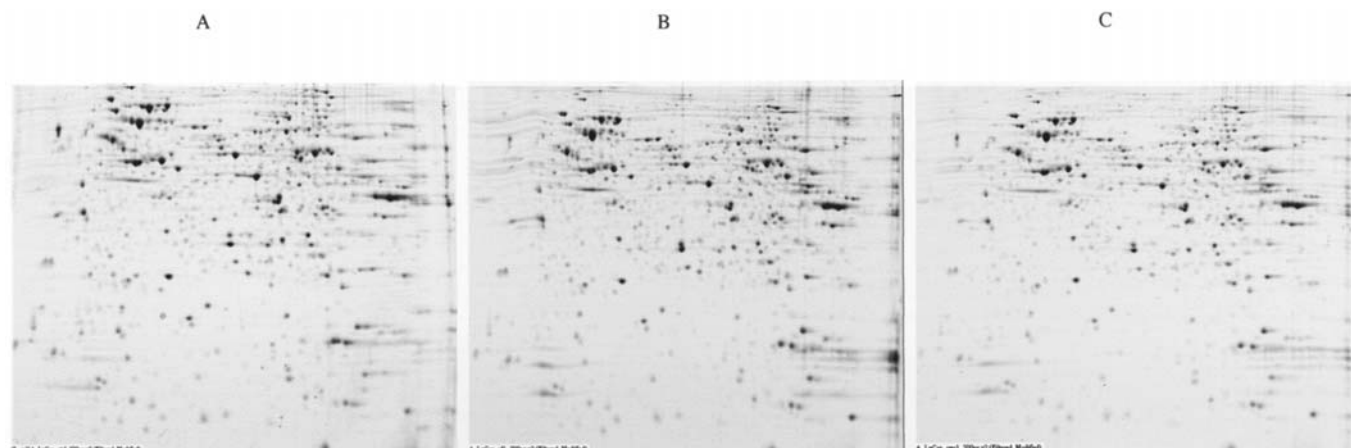


Figure 1. Representative examples of 2-DE gels derived from control, smsdx and sms treated prostate cancer cell line LNCaP. Whole-cell lysate was subjected to 2-DE using IPG strips pH 3-10 in the first and 12.5% SDS polyacrylamide gel in the second dimension.

(NCBI) Locuslink website. They were categorized on the basis of their known biochemical functions including metabolism, signalling transduction, maintenance of cell structure, cell-cell interactions, transport/trafficking, transcriptional regulation and cell cycle regulation

2D gel electrophoresis analysis. Fig. 1 shows representative 2D gels of control, sms and smsdx samples. Three replicates were run for each group, control, sms and smsdx. The sms and smsdx were compared separately with the control group using a Mann-Whitney test. A total of 172 spots were identified using MALDI-MS/MS, 140 different proteins were identified and consequently the same protein was sometimes found in multiple spots.

Sms/smsdx affected proteins. Proteins affected by sms/smsdx incubation with a fold change ≥ 1.6 and a Mann-Whitney p-value of 0.1 when discarding missing values are listed in Table I. Several of these were catalytic mitochondrial proteins and they were usually up-regulated, e.g. ACO2, PNPTT1, ACADS, only isocitrate dehydrogenase (NAD) subunit α was down-regulated. One protein, PRDX2, was apoptosis related and was down-regulated. Other proteins affected by sms/smsdx are listed in Table II (fold change < 1.6). For significantly affected proteins, there was a clear agreement in the up- and down-regulation between sms and smsdx (Tables I and II).

Discussion

Somatostatin has an important role in biological processes including neurotransmission, exocrine/endocrine secretion, and cell proliferation. It acts via a family of five GPCRs (G-protein-coupled receptors), i.e. SSTR1-SSTR5. Its anti-proliferative action results either from inhibition of trophic or growth factor secretion or from interference with the cell cycle progression (18). These properties make sms derivatives interesting drug candidates for the treatment of cancers expressing SSTRs especially those where efficacious treatment options are missing as in the case of HRPC. In this study, several mitochondrial proteins and apoptosis/cancer related proteins were identified. There was a strong correlation

between sms and smsdx concerning the up- and down-regulation of the proteins. Additionally, the regulation was in general as could be expected (up or down depending on function). The mitochondria plays a key role in many cellular functions including energy production, fatty acid metabolism, pyrimidine biosynthesis, calcium homeostasis, and cell signalling. It serves as a control point in regulating the intrinsic and extrinsic apoptotic pathway in response to DNA damage. Apoptotic cell death can be induced by a number of triggers released from the mitochondrial outer membrane (e.g. cytochrome c, Smac/Diablo, and apoptosis-inducing factor, AIF, caspase-dependent/independent pathways). The release can be blocked by overexpression of Bcl-2 (19). Mitochondria is important in the control of cell survival and death (20) and is an important target for anticancer chemotherapy (21). Several of the proteins involved in the MMP event (mitochondrial membrane permeabilization proteins), e.g. VDAC, IDH3A, and NADP, were identified. MMP is a critical event in the process leading to chemotherapy induced apoptosis. The permeabilization is controlled by the permeability transition pore complex (PTPC). Bax can associate with the voltage dependant anion channel (VDAC), which is also located in the outer mitochondrial membrane. The VDAC-Bax complex promotes pore formation, outer MMP, and the release of AMPs to activate caspases and induce apoptosis. Bcl-2 family proteins also regulate the release of apoptogenic cytochrome c by the mitochondrial channel VDAC (22). VDAC1 has been shown to co-immunoprecipitate with the anti-apoptotic protein Bcl and suggested to be involved in forming the mitochondrial pore which releases cytochrome c during apoptosis (23). From the present results it seems reasonable to assume that sms/smsdx can affect the mitochondria of LNCaP in a way that eventually could trigger mitochondrial-mediated apoptosis.

The regulation of peroxiredoxins (PRDX) is of importance for apoptosis and cell proliferation (24,25). PRDXs can be regulated by changes in phosphorylation, redox and possibly by oligomerization states. It appears that high levels of PRDXs inhibit apoptosis and induce cell proliferation (26). PRDX2 prevents cytochrome c release from mitochondria and inhibits lipid peroxidation. Suppression of PRDX2 is

Table I. Proteins with strong differential expression (>1.6 in fold change) between smsdx vs. control and sms vs. control.

Protein name	Short name	UniProt ID	Fold change smsdx vs. ctrl	Fold change sms vs. ctrl	Trends sms/smsdx vs. ctrl
Apoptosis					
Voltage-dependent anion channel 1	VDAC1	P21796	1.65	1.72	sms/smsdx > ctrl
DNA binding					
TATA-binding protein associated factor 2N	RBP56	Q92804	3.71	2.62	sms/smsdx > ctrl
Heterogeneous nuclear ribonucleoprotein D0	HNRPD	Q14103	2.24	1.79	sms/smsdx > ctrl
Electron transport					
Protein disulfide-isomerase	PDIA1	P07237	Inf	Inf	unique to sms/smsdx
Ubiquinol-cytochrome c reductase iron-sulfur subunit, mitochondrial	UCRI	P47985	Inf	Inf	unique to sms/smsdx
Metabolism					
Short chain 3-hydroxyacyl-CoA dehydrogenase, mitochondrial	HCDH	Q16836	1.66	1.68	sms/smsdx > ctrl
Glycerol-3 phosphate dehydrogenase, mitochondrial	GPDM	P43304	1.62	1.75	sms/smsdx > ctrl
Acetyl-CoA acetyltransferase cytosolic	THIC	Q9BWD1	Inf	Inf	unique to sms/smsdx
Isocitrate dehydrogenase (NAD) subunit α mitochondrial	IDH3A	P50213	-1.78	-1.72	ctrl > sms/smsdx
Aconitate hydratase, mitochondrial	ACON	Q99798	2.50	2.17	sms/smsdx > ctrl
Proliferation					
Peroxiredoxin 2	PRDX2	P32119	-2.06	-2.14	ctrl > sms/smsdx
Heterogeneous nuclear ribonucleoproteins C1/C2	HNRPC	P07910	2.56	2.65	sms/smsdx > ctrl
Protein binding					
Early endosome antigen 1	EEA1	Q15075	Inf	Inf	unique to sms/smsdx
Lamin A/C	LMNA	P02545	Inf	Inf	unique to sms/smsdx
Transcription					
Chromobox protein homolog 3	CBX3	Q13185	Inf	Inf	unique to sms/smsdx
Transcription intermediary factor 1- β	TIF1B	Q13263	Inf	Inf	unique to sms/smsdx
Transport					
Voltage-dependent anion-selective channel protein 2, mitochondrial	VDAC2	P45880	1.84	1.79	sms/smsdx > ctrl

No missing values and the lowest possible two-sided Mann-Whitney p-value ($=0.1$). Protein accession numbers from SwissProt/TrEMBL are given and 'trend' indicates the direction of the change in expression level. The proteins are grouped according to molecular function. Smsdx and sms appear to have a similar effect demonstrated by the concordance in protein expression levels. Ctrl, control.

associated with enhancement of radiation sensitivity (27). In the present study, sms/smsdx down-regulated PRDX2.

The heat-shock protein HSPB1 was affected by sms/smsdx. However, the regulation was different between sms and smsdx. Sms down-regulated while smsdx up-regulated (fold change <1.6). In advanced cancer, HSP27 expression is associated with poor clinical outcome. HSP70, like HSP27, protects cells from stress-induced caspase-dependent apoptosis, both upstream and downstream of the apoptosis associated mitochondrial events (28). Evidence for signalling and pathways leading to changes in the cancer cells apoptotic threshold is scarce. If anticancer drugs are administered at suboptimal concentrations, i.e. to trigger apoptosis, therapy resistance may actually be enhanced through up-regulation of anti-apoptotic factors such as HSP27 (29). Consequently, the optimal dosage

of sms derivatives needs to be explored and determined to elucidate its clinical usefulness. The discrepancy seen between sms and smsdx may be related with the appropriate dose, i.e. smsdx might be needed in a higher concentration to have a similar effect on HSPB1 as sms. The chaperone protein, GRP78, was up-regulated by both sms and smsdx.

GRP78 is expressed on the cell surface of prostate cancer cells and appears to mediate the signal transduction of β 2-macroglobulin (30,31). It correlates with the development of androgen-independent disease and shorter overall survival in prostate cancer patients. Arap *et al* (32) have recently identified GRP78 as a potential molecular target that may prove useful for translation into clinical applications.

Stathmin, a family of microtubule-destabilizing proteins, was down-regulated by sms/smsdx. It plays a critical role

Table II. Proteins with no missing values and a two-sided Mann-Whitney p-value of 0.1 when comparing smsdx vs. control and sms vs. control.

Protein name	Short name	UniProt ID	Fold change smsdx vs. ctrl	Fold change sms vs. ctrl	Trends sms/smsdx vs. ctrl
Antioxidant activity					
Superoxide dismutase 1, soluble	SODC	P00441	-1.3781	-1.4002	ctrl > sms/smsdx
Peroxiredoxin 6	PRDX6	P30041	-1.2558	-1.2730	ctrl > sms/smsdx
ATP binding					
Stress-70 protein	GRP75	P38646	-1.3487	1.3941	sms/smsdx > ctrl
Glutamate dehydrogenase 1	DHE3	P00367	-1.3958	1.4294	sms/smsdx > ctrl
Glutathione synthetase	GSHB	P48637	-1.5522	-1.6268	ctrl > sms/smsdx
Nucleoside diphosphate kinase A	NDKA	P15531	-1.3200	-1.4463	ctrl > sms/smsdx
DNA binding					
TATA-binding protein associated factor 2N	RBP56	Q92804	1.6136	1.4939	sms/smsdx > ctrl
Heterogeneous nuclear ribonucleoprotein K	HNRPK	P61978	1.2153	1.3092	sms/smsdx > ctrl
Heterogeneous nuclear ribonucleoprotein D0	HNRPD	Q14103	1.4724	1.3167	sms/smsdx > ctrl
Paraspeckle protein 1	Q8WXF1	Q8WXF1	1.3522	1.3365	sms/smsdx > ctrl
ER activity					
SAPK substrate protein 1	SAKS1	Q04323	-2.0809	-1.3321	ctrl > sms/smsdx
FAD binding					
Dihydrolipoyl dehydrogenase	DLDH	P09622	1.6057	1.5586	sms/smsdx > ctrl
Acyl CoA dehydrogenase, medium-chain specific	ACADM	P11310	1.5840	1.4986	sms/smsdx > ctrl
Electron transfer flavoprotein α -subunit, mitochondrial	ETFA	P13804	1.4264	1.4131	sms/smsdx > ctrl
GTP binding					
Tubulin β chain	TBB2C	P68371	-1.2300	-1.4093	ctrl > sms/smsdx
Metabolism					
Inorganic pyrophosphatase	IPYR	Q15181	-1.3124	-1.3867	ctrl > sms/smsdx
Esterase D/formylglutathione hydrolase	ESTD	Q9BVJ2	-1.2460	-1.2094	ctrl > sms/smsdx
Triosephosphate isomerase	TPIS	P60174	-1.2045	-1.2217	ctrl > sms/smsdx
Triosephosphate isomerase	TPIS	P60174	-1.3283	-1.2866	ctrl > sms/smsdx
Adenine phosphoribosyltransferase	APT	P07741	-1.2496	-1.1947	ctrl > sms/smsdx
Aconitate hydratase	ACON	Q99798	1.3559	1.3665	sms/smsdx > ctrl
D-3-phosphoglycerate dehydrogenase	SERA	O43175	-1.3422	-1.4193	ctrl > sms/smsdx
D-3-phosphoglycerate dehydrogenase	SERA	O43175	-1.2660	-1.1457	ctrl > sms/smsdx
Lipoamide acyltransferase component of branched-chain α -keto acid dehydrogenase complex	ODB2	P11182	1.5934	1.6911	sms/smsdx > ctrl
Fructose-bisphosphate aldolase C	ALDOC	P09972	1.4677	1.3982	sms/smsdx > ctrl
3-hydroxyisobutyryl-Coenzyme A hydrolase	Q53GF2	Q53GF2	1.4179	1.2932	sms/smsdx > ctrl
Delta3,5-delta2,4-dienoyl-CoA isomerase	ECH1	Q13011	1.1811	1.2482	sms/smsdx > ctrl
3-hydroxyisobutyrate dehydrogenase, mitochondrial	3HIDH	P31937	1.2684	1.2447	sms/smsdx > ctrl
Proliferation					
78 kDa glucose-regulated protein	GRP78	P11021	1.3518	1.4180	sms/smsdx > ctrl
Translationally controlled tumor protein	TCTP	P13693	-1.4436	-1.5709	ctrl > sms/smsdx
Rho GDP-dissociation inhibitor 1	GDIR	P52565	-1.4795	-1.4039	ctrl > sms/smsdx
Protein binding					
Keratin, type II cytoskeletal 1	K2C1	P04264	1.4002	1.2694	sms/smsdx > ctrl
Heat-shock protein β -1	HSPB1	P04792	1.7470	-1.3354	smsdx > ctrl > sms
RNA binding					
Lupus La protein	LA	P05455	-1.5795	-1.3085	ctrl > sms/smsdx
Heterogeneous nuclear ribonucleoprotein L	HNRPL	P14866	1.2634	1.2494	sms/smsdx > ctrl
Heterogeneous nuclear ribonucleoprotein L	HNRPL	P14866	1.3782	1.3079	sms/smsdx > ctrl
Heterogeneous nuclear ribonucleoprotein H	HNRPH	P31943	1.2937	1.2375	sms/smsdx > ctrl

Table II. Continued.

Protein name	Short name	UniProt ID	Fold change smsdx vs. ctrl	Fold change sms vs. ctrl	Trends sms/smsdx vs. ctrl
RNA binding					
Cleavage stimulation factor, 50 kDa subunit	CSTF1	Q05048	1.1899	1.2589	sms/smsdx > ctrl
Proteasome subunit α type 1	PSA1	P25786	-1.3587	-1.4821	ctrl > sms/smsdx
RNA splicing					
Far upstream element binding protein 2	FUBP2	Q92945	1.3225	1.2989	sms/smsdx > ctrl
Far upstream element binding protein 2	FUBP2	Q92945	1.7236	1.4466	sms/smsdx > ctrl
Signal transduction					
Protein DJ-1 protein	PARK7	Q99497	-1.4112	-1.5998	ctrl > sms/smsdx
Stathmin	STMN1	P16949	-1.3243	-1.4821	ctrl > sms/smsdx
Structural molecular activity					
Lamin B1	LMNB1	P20700	1.4199	1.7539	sms/smsdx > ctrl
Translation					
Eukaryotic translation initiation factor 5A-1	IF5A1	P63241	-1.3835	-1.6624	ctrl > sms/smsdx

Listed proteins have a fold change <1.6 and 'trend' indicate the direction of the change. Protein accession numbers from SwissProt/TrEMBL are given and the proteins are grouped according to molecular function. Smsdx and sms appear to have a similar effect demonstrated by the concordance in protein expression levels. Ctrl, control.

in the regulation of mitosis (33-35). High levels of stathmin expression were described in a variety of human malignancies, including prostate carcinoma (36). Prostate cancer is believed to provide one of the best models for the development of therapeutics that target stathmin (34). The level of expression of stathmin was shown to correlate with the malignant behaviour of prostate cancer cells. In fact, it was proposed that the level of expression of stathmin may serve as an important prognostic marker in prostate cancer. Mistry and Atweh (34) developed replication-deficient bicistronic adenoviral vectors that co-express green fluorescent protein and ribozymes that target stathmin mRNA. The therapeutic potential of these recombinant adenoviruses was tested in an experimental androgen-independent LNCaP prostate cancer model. Adenovirus-mediated transfer of anti-stathmin ribozymes resulted in efficient transduction and marked inhibition of stathmin expression in these cells.

The translationally controlled tumor protein (TCTP) was down-regulated by sms/smsdx. It is expressed in LNCaP and plays a role in controlling the apoptotic process (37). TCTP represents a novel anti-apoptotic protein involved in cell survival and apoptosis regulation (38) and may play a prominent role in the elongation cycle of translation (39). Accordingly, decreasing the expression of TCTP in cancer cells might be a way to decrease the efficiency of protein synthesis, and down-regulate cell proliferation. TCTP is also overexpressed in chemoresistant melanoma cell lines (40). This role in the process of tumor reversion might offer a lead to the identification of drugs that are able to diminish TCTP expression (41).

DJ-1 plays a direct role in the control of apoptosis in human prostate cells (42) and it was down-regulated by sms/smsdx. The underexpression of DJ-1 results in decreased

phosphorylation of PKB/Akt, while its overexpression leads to hyperphosphorylation of PKB/Akt and increased cell survival. DJ-1 expression correlates negatively with PTEN immunoreactivity and positively with PKB/Akt hyperphosphorylation. DJ-1 is thus a key negative regulator of PTEN that may be a useful prognostic marker for cancer (43). Treatment of cells from the human lung cancer cell line NCI-H157 with paclitaxel and MEK inhibitor U0126 leads to a decrease in DJ-1 protein expression (44).

In conclusion, this analysis shows that sms and smsdx regulate a number of important proteins in the LNCaP prostatic cancer cell line. Several proteins were strongly regulated. The regulation was clearly concordant between sms and smsdx. Additionally, it was in general in agreement with the growth inhibitory actions of sms. The results support further clinical evaluation of smsdx and a phase II study on advanced prostate cancer patients is pending.

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