CANCER GENOMICS & PROTEOMICS 3: 325-346 (2006)

The Proteome Profile of the Human Osteosarcome Saos2 Cell Line

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Abstract. *The human Saos2 cell line was one of the first generated cell lines and is known to be used in all sorts of biomedical research. Knowledge of its protein expression is limited and no comprehensive study on the proteome of this cell type has been reported to date. Proteomics technologies were applied in order to analyse the proteins of the Saos2 cell line. Total protein extracts were separated by two dimensional gel electrophoresis (2-DE) and analysed by MALDI-MS and MALDI-MS-MS following in-gel digestion with trypsin and, finally, protein identification was carried out by peptide mass fingerprint (PMF) and post source decay (PSD), respectively. Approximately 4,000 spots were excised from four 2-DE gels and were analysed, resulting in the identification of 349 different gene products. The majority of the identified proteins were enzymes, regulatory proteins and transporters, while leukocyte markers and oncogenes were also included. Our findings include 10 protooncogenes (STRAP, FUBP1, SRC8, NPM, PARK7, DJ-1, PSD10, OXRP, GAGD2 and TPD54) related to the chromosomally instable character observed in the Saos2 cell line. Considering these, the Saos2 2-DE database shapes the basis for future expressional studies at the protein level and it forms a useful tool in anticancer research.*

Proteomics technologies constitute powerful analytical tools, which, in combination with other high throughput screening techniques, are widely used for the analysis of biological specimens. Protein identification procedures are greatly enhanced by robotic systems and automated mass spectra acquisition devices which allow complete examination of biological samples, such as cell lines. Protein analysis by two-dimensional gel electrophoresis (2-DE) coupled to mass spectrometry offers nowadays the standard approach to the construction of comprehensive protein databases and clearly reveals the dynamics and the potential uses of the material under study (1). Such databases are mainly used to provide information concerning normal and disease states, can, thus, de involved in diagnosis and treatment procedures and could potentially lead to biomarker discovery (2, 3). The study of a cell line provides good information both about the nature of the malfunction, as well as probable causes creating it. Cell line platforms are the bare essential tools in understanding primal behaviors of disease states, such as cancer, as well as predicting probable cure, treatment approaches and helping design and develop anticancer drugs. In this regard, the proteome of the immature human T cell line CCRF-CEM (4) as well as the HeLa cell line (5) have been analyzed in detail.

In the present work proteomic technologies were applied to study the Saos2 cell line. This cell line has been derived from the primary osteogenic sarcoma of an 11-year-old Caucasian female available since 1973 and has been one of the first generated cell lines used for biomedical and anticancer research. One of the basic characteristics of the Saos2 cell line is the existence of mutations both in the p53 and Rb genes. As a result these mutations lead to a complete absence of p53 and Rb protein, both at mRNA and protein level (6). These two genes are involved in tumor suppression and cell cycle control. The p53 protein prevents genomic instability by receiving stress signals and transcriptionally inducing genes that cause cell cycle arrest, apoptosis or senescence (7, 8). On the other hand, the Rb protein is important in cell cycle control by mediating the repression of E2F transcription factor family members and by interacting with histone

Abbreviations: PMF, peptide mass fingerprint; PSD, post source decay; DMEM, Dulbecco's modified Eagle's medium; FCS, fetal calf serum.

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Key Words: Osteosarcoma, Saos2, proteomics, 2-DE protein database, mass spectrometry, MALDI-MS.

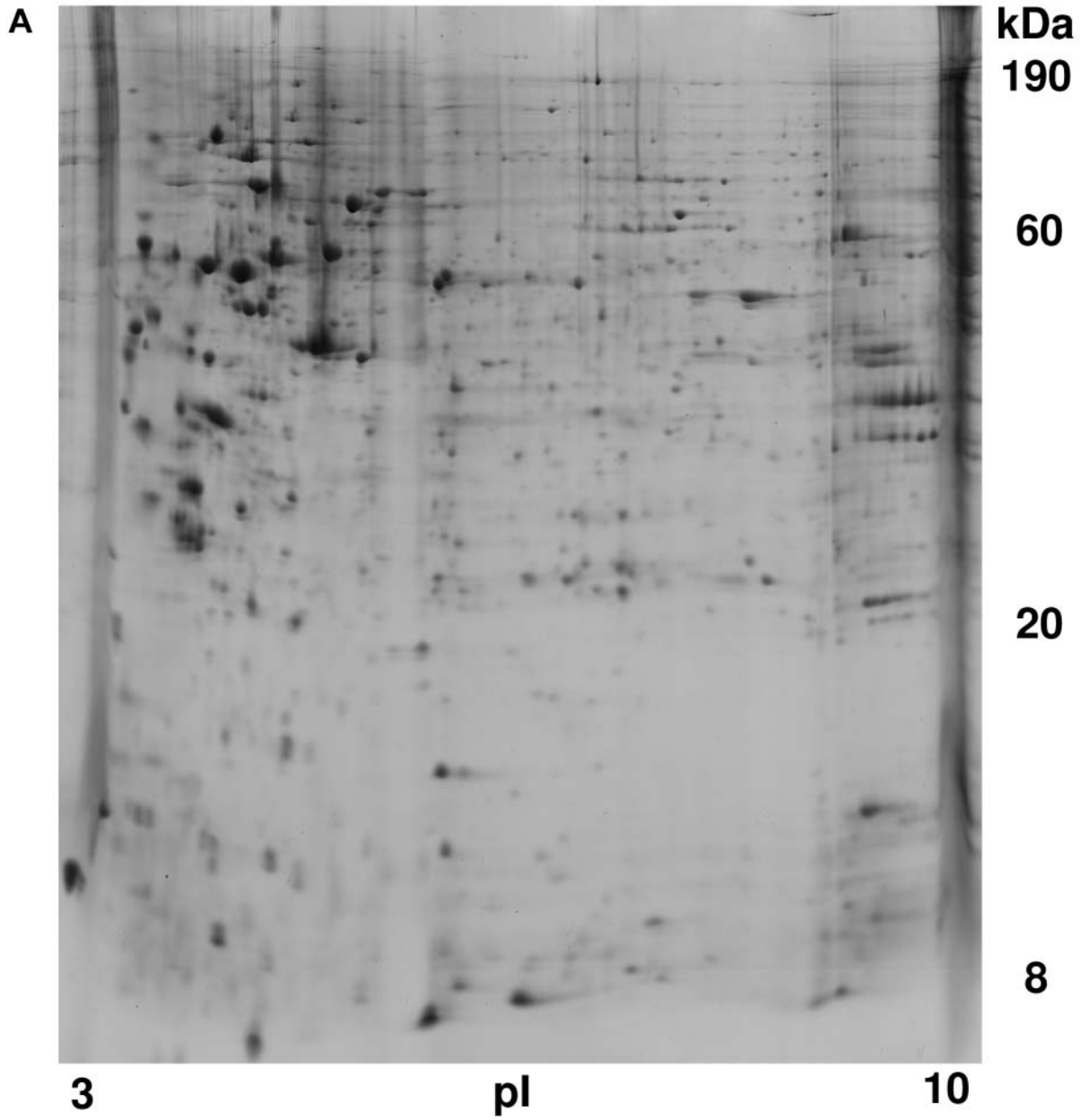

Figure 1. *Two dimensional gel electrophoresis of total protein extract from the Saos2 cell line. Proteins were extracted and separated on IPG strip, followed by a 12% SDS-polyacrylamide gel, as described in Materials and Methods. The gels were stained with Coomassie blue. A: separation on IPG strip pH 3-10 non-linear, B: separation on IPG strip pH 4-7 non-linear.*

deacetylases (9). As a result of these two mutations, Saos2 cells, exhibit intense chromosomal instability and a high rate of aneuploidy and structural chromosomal aberrations were observed mostly on chromosomes 1, 8, 17 and 20 (10, 11).

Biochemistry, bone formation, osteoporosis, atherosclerotic-cardiovascular disease and biomedical applications, are a few of the fields mentioned in the literature using Saos2 cells for the experimental purposes, because of their osteoblast properties (12-14). Saos2 cells have also been employed in drug development, molecular carcinogenesis and for the study of solid tumors (15-18). Furthermore, Saos2 cells have been used for the generation of stable inducible systems, in the study of various molecules, functional networks and metabolic pathways affecting different diseases (19).

This study reports the construction of the previously unreported protein database for Saos2, which consists of

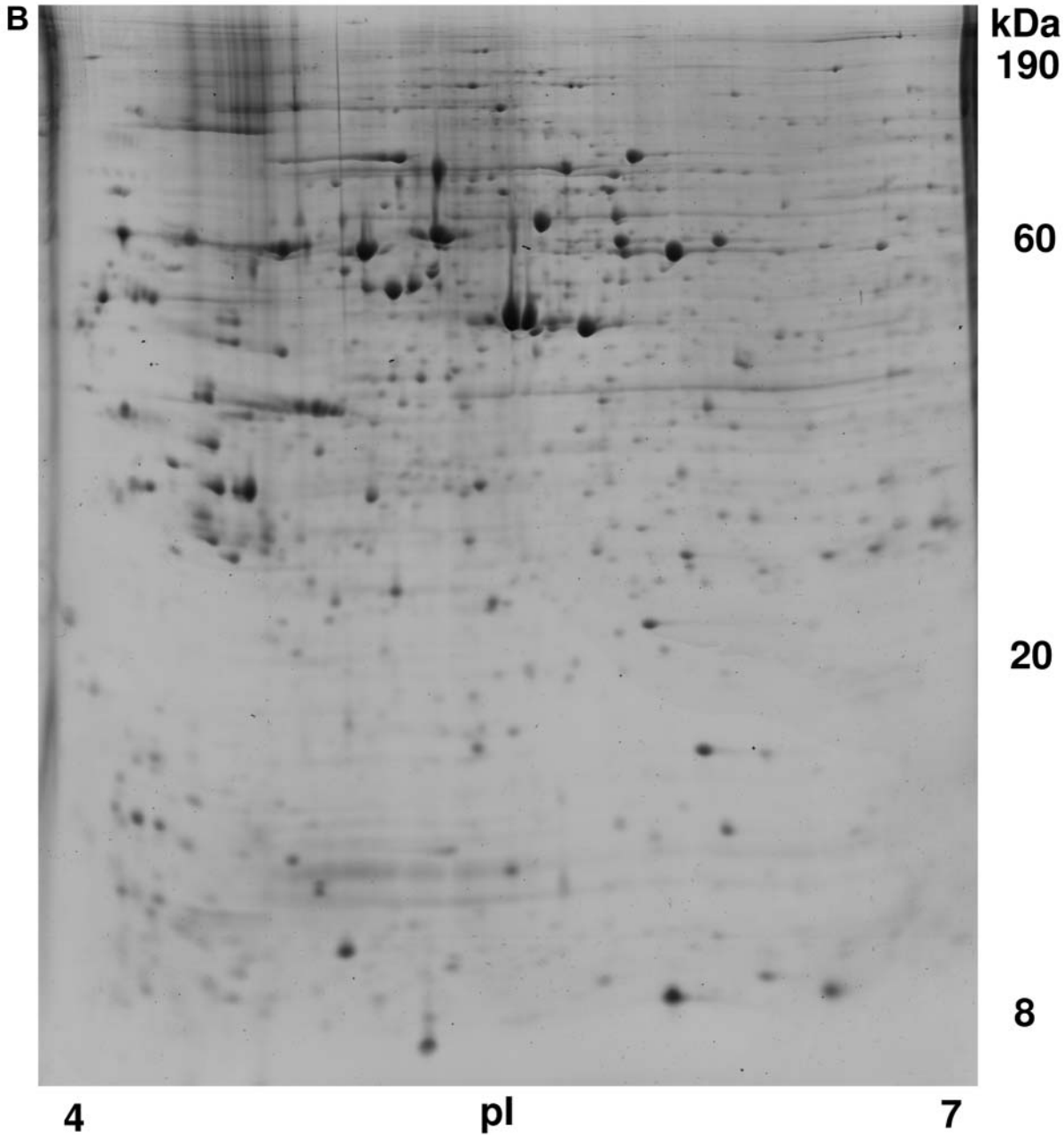

Figure 1. *continued*

349 single gene products and should provide a good reference point for further investigation and research in other biomedical fields.

Materials and Methods

Materials and reagents. Immobilized pH-gradient (IPG) strips and IPG buffers were purchased from Biorad Laboratories (Hercules, CA, USA). Acrylamide/piperazine-di-acrylamide (PDA) solution (37.5:1 w/v) was purchased from Biosolve Ltd. (Valkenswaard, The Netherlands) and the other reagents for the polyacrylamide gel preparation from BioRad CHAPS was obtained from Roche Diagnostics (Mannheim, Germany), urea from AppliChem (Darmstadt, Germany), thiourea from Fluka (Buchs, Switzerland), 1,4-dithioerythritol (DTE) and EDTA from Merck (Darmstadt, Germany). Except for CHAPS, which was kept at 23˚C, the other reagents were kept at 4˚C.

Cell cultures. Cells were grown in Dulbecco's modified Eagle's medium (DMEM) (Biochrome, Berlin, Germany) supplemented with 10% fetal calf serum (FCS) (Biochrome), at 37˚C in a humidified atmosphere with 5% CO_2.

Two-dimensional gel electrophoresis. Saos2 cells ($40x10^6$) were washed with normal saline and resuspended in 0.5 ml of urea buffer, consisting of 20 mM Tris, 7 M urea, 2 M thiourea, 4% CHAPS, 10 mM 1, 4-dithioerythritol (DTE), 1 mM EDTA and a mixture of protease inhibitors [1 mM PMSF and 1 tablet complete™ (Roche Diagnostics, Basel, Swiss) per 50 ml of suspension buffer] and phosphate inhibitors (0.2 mM Na2VO3 and 1 mM NaF). The cells were lysed by sonication at 3x60 sec at 35% amplification and the suspension was centrifuged at 14,000 xg for 30 min. The protein content of the supernatant was determined using the EXPERION Automated Electrophoresis Station in combination with Protein 260 Analysis Kit™ (Biorad) according to the manufacturer's instructions.

2D gel electrophoresis was performed as reported previously (5). One mg total protein was applied to immobilized pH 3-10 and pH 4-7 non-linear gradient strips in sample cups at their acidic and basic ends. Focusing started at 250 V for 30 min and the voltage was gradually increased to 6000 V at 3 V/min and remained constant for a further 18 h. The second-dimensional separation was performed in 12% SDS-polyacrylamide gels (180x200x1.5 mm), running at 50 mA per gel in a PROTEAN apparatus (Biorad). After fixation with 50% methanol, containing 10% acetic acid for 2 h, the gels were stained overnight with colloidal Coomassie blue (Novex, San Diego, CA, USA), washed twice with H_2O and scanned in a densitometer (GS-800 Calibrated Densitometer, Biorad).

Peptide mass fingerprint (PMF) and post source decay (PSD). Peptide analysis and protein identification were performed as previously described (20). Spots were automatically detected by Melanie 4.02 software on the Coomassie blue-stained gel, excised by the Proteiner SPII (Bruker Daltonics, Bremen, Germany), destained with 30% acetonitrile in 50 mM ammonium bicarbonate and dried in a speed vacuum concentrator (MaxiDry Plus, Heto, Allered, Denmark). Each dried gel piece was rehydrated with 5 μl of 1 mM ammonium bicarbonate containing 50 ng trypsin (Roche Diagnostics) and left in the dark overnight at room temperature. Twenty μl of 50% acetonitrile, containing 0.3% trifluoroacetic acid were added to each gel piece and incubated for 15 min with constant shaking. The peptide mixture (1.5 μl) was simultaneously applied with 1 μl of matrix solution, consisting of 0.025% α-cyano-4-hydroxycinnamic acid (Sigma), standard peptides des-Arg-bradykinin (Sigma, 904.4681 Da) and adrenocorticotropic hormone fragment 18-39 (Sigma, 2465.1989 Da) in 65% ethanol, 35% acetonitrile and 0.03% trifluoroacetic acid. Samples were analyzed for PMF with MALDI-MS in a time-of-flight mass spectrometer (Ultraflex II, Bruker Daltonics). Matching peptide and protein searches were performed automatically, as described by Berndt *et al.* (20). Each spectrum was interpreted by the Mascot Software (Matrix Sciences Ltd., London, UK). For peptide identification, the monoisotopic masses were used and a mass tolerance of 0.0025% (25 ppm) was allowed. Unmatched peptides or peptides with up to one miscleavage site were not considered. The peptide masses were compared with the theoretical peptide masses of all available proteins from all species using SWISS-PROT, IPI and MSDB databases. The probability score identified by the software was used as the criterion of the identification. Samples not identified by PMF (probability significance of $p<0.05$) were automatically selected for PSD MS-MS analysis or MALDI-MS-MS. The peptide masses chosen for PSD-MS-MS analysis had a signal intensity of >600 counts and were excluded from the trypsin autodigest, matrix and keratin peaks. The resulting PSD spectra were also interpreted by the Mascot Software and Mascot probability-based scores of $p<0.02$ were considered significant. The identified proteins were annotated on the gel image by hand.

Results

2-DE database. The protein extract from the osteosarcoma cell line Saos2 was separated by 2-DE electrophoresis on narrow and broad pH range IPG strips and the spots were visualized with colloidal Coomassie blue. In Figure 1A and 1B representative examples of the Saos2 cells proteins separated on a pH 3-10 NL and pH 4-7 NL gel respectively are shown. Four pH 3-10 NL and four pH 4-7 NL IPG gels were analyzed and approximately 4,000 spots were totally detected using 2D ImageMaker software. Two thousands and five hundred spots were excised from the pH 3-10 gels and analyzed for protein identification following in-gel digestion with trypsin. Each spot was analyzed for PMF with MALDI-MS and proteins were identified by the peptide mass matching automatically. Proteins not identified by PMF were subsequently selected for PSD-MS-MS. Using an internal peptide standard to correct the measured peptide masses very narrow windows of mass tolerance (0.0025%) could be used and hence, increase the confidence of identification, as well as the total identification rate up to 85%. The result of this was the identification of 298 different gene products (Figure 2). Using the same approach, from the four pH 4-7 gels 1,500 spots in total were excited and analyzed which resulted in the identification of 51 new gene products not previously detected in 3-10 NL gels (Figure 3). Therefore a total of 349 different gene products was found in all analyzed gels (Table I).

The SWISS-PROT accession numbers, the abbreviated and full names of the proteins, the theoretical MW as well as data from the mass spectrometry analysis, *i.e.,* the numbers of matching peptides and the probability that the identification is random are listed in Table I. The most abundant proteins in that cell line were heat shock proteins (39), actins (31) and lamins (23).

Subcellular localization. The subcellular localization of the identified proteins was obtained from public accessible data bases. For 9% of the identified proteins no data were found.The proteins are localized in the cytoplasm (61%), nucleus (26%), mitochondrion (10%) and cytoskeleton (7%) (Figure 4). Membranic proteins represent 6% of the total protein identified. The subcellular location of each protein is annotated in Table I.

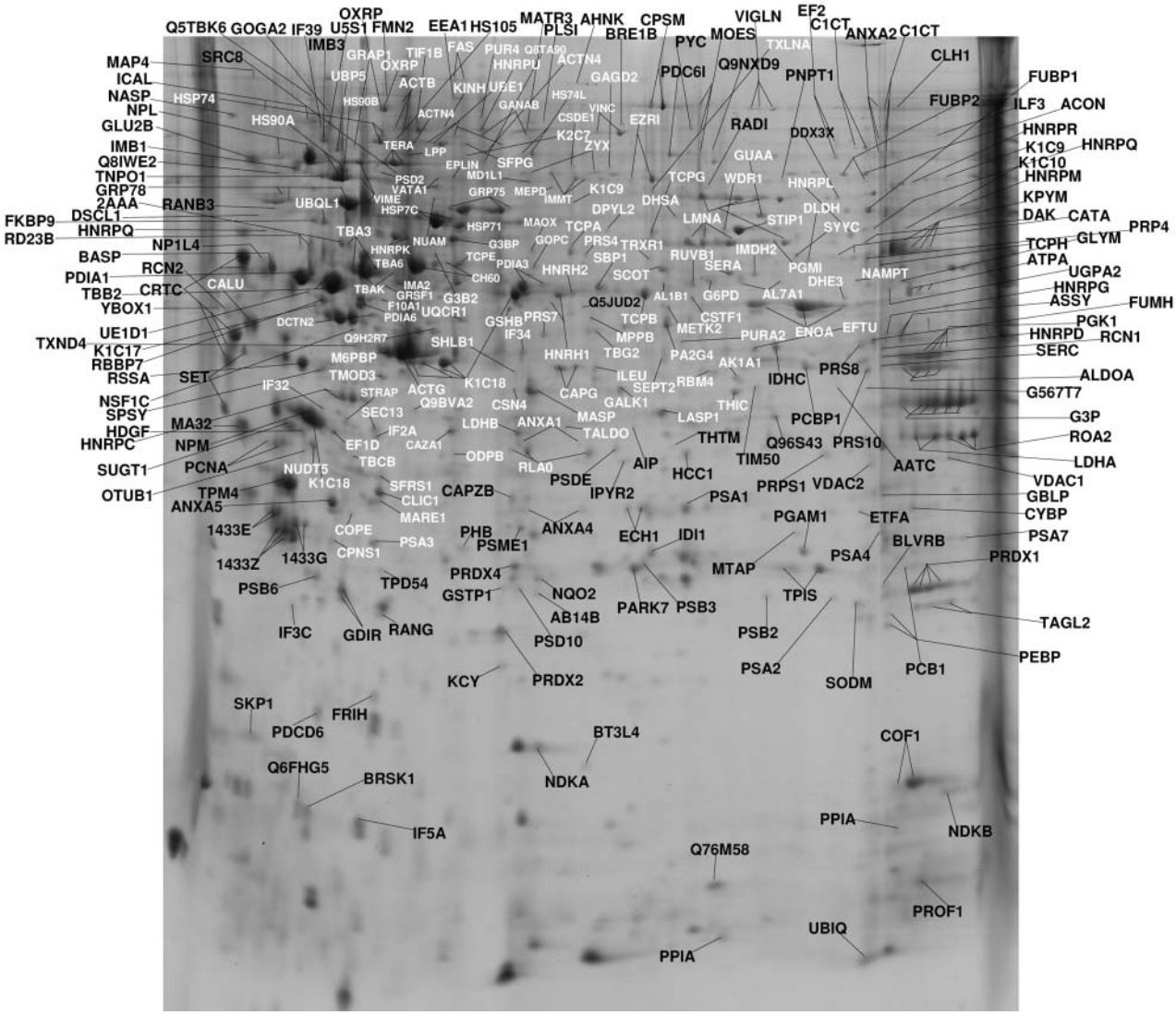

Figure 2. *Protein spot identification of the Figure 1A gel, analyzed by PMF and/or PSD-MS-MS. The identified proteins are annotated by their abbreviated names. Protein molecules are listed in Table I.*

Function. The identified proteins include enzymes (27%) and regulatory (14%) as seen in Table I. There are structural proteins like tubulins and actins and other major classes of identified proteins like DNA and RNA associated proteins, transcription factor, transport/carrier and signal transduction (Figure 5). In addition, as expected, some protooncogenes and antioncogenes representing 3% and 2% respectively of the total proteome, were found.

Discussion

Proteomics constitutes the linkage between genes and their products, providing evidence about gene role in cell function (21). Analytical methods used for proteomic research result most commonly in comprehensive databases such as 2-DE maps. Helpful tools for this effort include the usage of genome sequence databases, a complete catalogue of proteins expressed in organisms, mass spectrometry and software that match MS data with protein sequences in databases. Use of these resources allowed us to successfully complete the scope of this work, the construction of the protein database of the osteosarcoma cell line Saos2. Widespread use of Saos2 cells is known in all biomedical research fields and especially in drug development and discovery of novel diagnostic biomarkers (12-14).

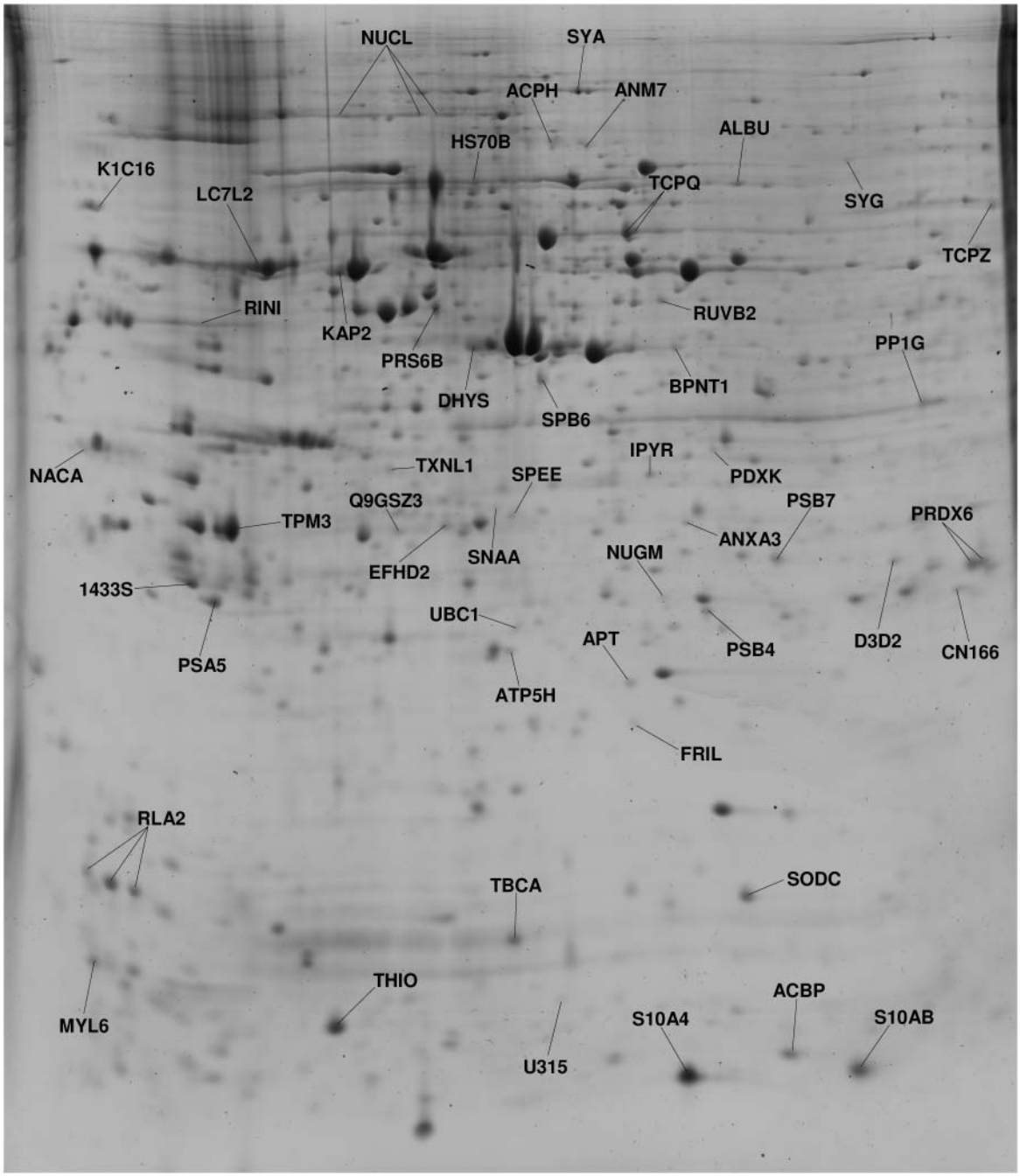

Figure 3. *Protein spot identification of the Figure 1B gel, analyzed by PMF and/or PSD-MS-MS. The proteins exclusively identified in 4-7 pH range strip are annotated in the gel image. Protein molecules are listed in Table I.*

Three hundred and forty-nine different gene products with several functions including regulatory, signal transduction, protooncogenes and antioncogenes, chaperone and nucleic acid binding proteins were identified. The use of an internal peptide standard allowed narrow windows of mass tolerance (0.0025%), increasing the confidence of identification by PMF and PSD mode of mass spectrometry. Identification was based on three or more (up to 69) matching peptides. These were localized in the cytoplasm, nucleus, mitochondrion and some in the membrane.

Our study resulted in the identification of 10 protooncogenes including STRAP, FUBP1, SRC8, NPM,

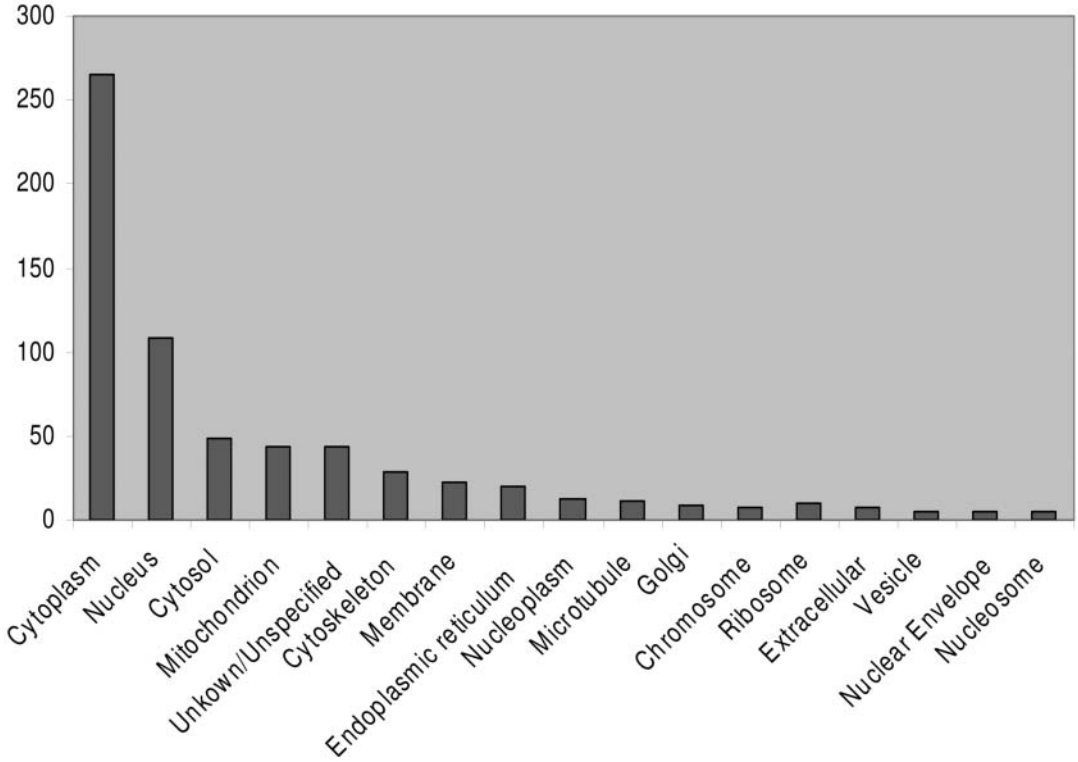

Figure 4. *Subcellular localization of the human osteosarcoma Saos2 cell line proteins. The proteins in Table I were categorized according to their location in the cells. Protein molecules and their localization are given in Table I.*

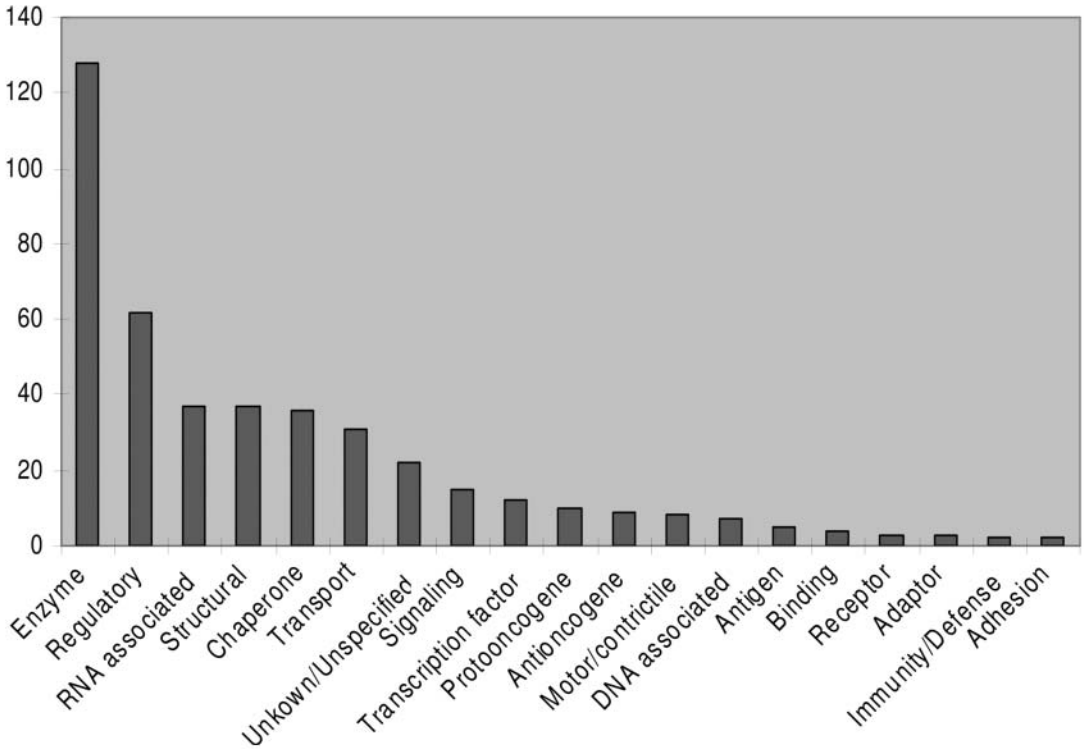

Figure 5. *Function of the human osteosarcoma Saos2 cell line proteins. The proteins identified in this study were classified into functional groups. The protein molecules and their functions are given in Table I.*

Table I. *Proteins from the human osteosarcoma cell line Saos2 were extracted and separated by 2-D gel electrophoresis, as described in Materials and Methods. The proteins were identified by PMF and/or PSD, following in-gel digestion with trypsin. The spots representing the identified proteins are indicated in Figures 2 and 3 and are designated with their abbreviated names or the SWISS-PROT accession numbers or the accession numbers of the other databases. The theoretical Mr and pI values, the matching peptides and the probability of a random identification (Score), as well the annotated subcellular location and function are listed. Score is -10*log(P), where P is the probability that the observed match is a random event (MASCOT, http:/www.matrixscience.com). Score >53 indicate p<0.05.*

Accession No	Protein name	Protein symbol	Protein description	MW	Mascot score	Peptide matched	Function	Subcellular localisation
Q15293	RCN1_HUMAN	RCN1	Reticulocalbin-1 [Precursor]	38890	134	10 of 15	Regulatory	Cytoplasm, Endoplasmic reticulum
P55809	SCOT_HUMAN	SCOT	Succinyl-CoA:3-ketoacid-coenzyme A transferase 1, mitochondrial [Precursor]	56158	99	10 of 18	Enzyme	Cytoplasm, Mitochondrion
P31930	UQCR1_HUMAN	UQCR1	Ubiquinol-cytochrome-c reductase complex core protein I, mitochondrial [Precursor]	52619	112	10 of 20	Enzyme	Cytoplasm, Mitochondrion
P11413	G6PD_HUMAN	G6PD	Glucose-6-phosphate 1-dehydrogenase	59135	88	10 of 22	Enzyme	Cytoplasm, Cytosol
P40121	CAPG_HUMAN	CAPG	Macrophage capping protein	38518	86	10 of 24	Structural	Cytoplasm, Cytoskeleton, Nucleus
P00966	ASSY_HUMAN	ASSY	Argininosuccinate synthase, Citrulline --aspartate ligase	46530	103	10 of 26	Enzyme	Cytoplasm, Cytosol
O00487	PSDE_HUMAN	PSDE	26S proteasome non-ATPase regulatory subunit 14	34577	72	10 of 27	Enzyme,	Unkown/Unspecified Regulatory
Q01105	SET_HUMAN	SET	SET protein, Phosphatase 2A inhibitor I2PP2A	33489	123	10 of 29	Regulatory, DNA associated, Transcription factor	Cytoplasm, Endoplasmic reticulum, Nucleus
Q16881	TRXR1_HUMAN	TRXR1	Thioredoxin reductase 1, cytoplasmic [Precursor]	54707	85	10 of 31	Enzyme, Signaling	Cytoplasm
P67809	YBOX1_HUMAN	YBOX1	Nuclease sensitive element-binding protein 1	35924	112	10 of 31	Regulatory, Transcription factor	Cytoplasm, Nucleus
P51858	HDGF_HUMAN	HDGF	Hepatoma-derived growth factor	26788	118	10 of 31	Signaling	Cytoplasm, Nucleus
Q00839	HNRPU_HUMAN	HNRPU	Heterogenous nuclear ribonucleoprotein U, Scaffold attachment factor A	90479	66	10 of 34	DNA associated, RNA associated	Nucleus, Nucleoplasm, Chromosome, Nucleosome
P05198	IF2A_HUMAN	IF2A	Eukaryotic translation initiation factor 2 subunit 1	35981	88	10 of 35	RNA associated	Unkown/Unspecified
Q99497	PARK7_HUMAN	PARK7	Protein DJ-1, Oncogene DJ1	19891	113	10 of 39	Protooncogene, Signaling	Cytoplasm, Nucleus
Q13162	PRDX4_HUMAN	PRDX4	Peroxiredoxin-4, Thioredoxin peroxidase AO372	30540	105	10 of 47	Enzyme	Cytoplasm
Q14103	HNRPD_HUMAN	HNRPD	Heterogeneous nuclear ribonucleoprotein D0	38434	76	10 of 50	RNA associated	Cytoplasm, Nucleus, Chromosome, Nucleosome
P08779	K1C16_HUMAN	K1C16	Keratin, type I cytoskeletal 16, CK-16	51137	68	10 of 52	Structural	Cytoplasm, Cytoskeleton, Intermed filament
P12004	PCNA_HUMAN	PCNA	Proliferating cell nuclear antigen	28769	163	11 of 16	Regulatory, DNA associated, Antigen	Nucleus, Nucleoplasm, Chromosome, Nucleus bodies
Q96C19	EFHD2_HUMAN	EFHD2	EF-hand domain-containing protein 2, Swiprosin-1	26697	120	11 of 24	Regulatory	Unkown/Unspecified
Q13228	SBP1_HUMAN	SBP1	Selenium-binding protein 1	52313	100	11 of 25	Transport carrier	Cytoplasm, Cytosol, Golgi
P15531	NDKA_HUMAN	NDKA	Nucleoside diphosphate kinase A, EC 2.7.4.6	17149	130	11 of 25	Enzyme	Cytoplasm, Mitochondrion, Nucleus
Q12906	ILF3_HUMAN	ILF3	Interleukin enhancer-binding factor 3, NF-AT-90	95338	89	11 of 26	Transcription factor	Cytoplasm, Cytosol, Ribosome, Nucleus
O43390	HNRPR_HUMAN	HNRPR	Heterogeneous nuclear ribonucleoprotein R	70943	100	11 of 26	RNA associated	Cytoplasm, Nucleus, Nucleoplasm
P17174	AATC_HUMAN	AATC	Aspartate aminotransferase, cytoplasmic, Transaminase A	46116	129	11 of 26	Enzyme	Cytoplasm, Cytosol

continued

Table I. *continued*

Accession No	Protein name	Protein symbol	Protein description	MW	Mascot score	Peptide matched	Function	Subcellular localisation
Q13011	ECH1_HUMAN	ECH1	Delta3,5-delta2,4-dienoyl-CoA isomerase, mitochondrial [Precursor]	35816	128	11 of 26	Enzyme, Regulatory	Cytoplasm, Peroxisome, Mitochondrion
P00367	DHE3_HUMAN	DHE3	Glutamate dehydrogenase 1, mitochondrial [Precursor]	61398	100	11 of 27	Enzyme, Signaling	Cytoplasm, Mitochondrion
P49419	AL7A1_HUMAN	AL7A1	Aldehyde dehydrogenase family 7 member A1	55235	138	11 of 33	Enzyme, Regulatory	Unkown/Unspecified
P09211	GSTP1_HUMAN	GSTP1	Glutathione S-transferase P	23225	110	11 of 37	Enzyme	Cytoplasm, Cytosol
Q99832	TCPH_HUMAN	TCPH	T-complex protein 1 subunit eta	59367	121	11 of 38	Chaperone	Cytoplasm
P48163	MAOX_HUMAN	MAOX	NADP-dependent malic enzyme, Malic enzyme 1	64150	81	11 of 40	Enzyme	Cytoplasm, Cytosol
Q9UHB6	EPLIN_HUMAN	EPLIN	Epithelial protein lost in neoplasm	85226	76	11 of 42	Unknown/ Unspecified	Cytoplasm
P06748	NPM_HUMAN	NPM	Nucleophosmin, Nucleolar phosphoprotein B23	32575	75	11 of 52	Protooncogene	Cytoplasm, Cytoskeleton, Microtubule, Centrosome, Nucleus, Nucleoplasm, Nucleolus
Q9Y617	SERC_HUMAN	SERC	Phosphoserine aminotransferase, PSAT	40423	90	11 of 54	Enzyme	Unkown/Unspecified
P37802	TAGL2_HUMAN	TAGL2	Transgelin-2, SM22-alpha homolog	22260	145	12 of 17	Unknown/ unspecified, Transcription factor	Unkown/Unspecified
Q06830	PRDX1_HUMAN	PRDX1	Peroxiredoxin-1, Natural killer cell-enhancing factor A	22110	164	12 of 20	Enzyme	Cytoplasm
P43686	PRS6B_HUMAN	PRS6B	26S protease regulatory subunit 6B, TAT-binding protein 7	47366	116	12 of 21	Chaperone, Enzyme	Cytoplasm, Nucleus
P63104	1433Z_HUMAN	1433Z	14-3-3 protein zeta/delta, Protein kinase C inhibitor protein 1	27745	134	12 of 21	Regulatory	Cytoplasm
P48643	TCPE_HUMAN	TCPE	T-complex protein 1 subunit epsilon	59671	79	12 of 22	Chaperone	Cytoplasm, Cytosol
P43490	NAMPT_HUMAN	NAMPT	Nicotinamide phosphoribosyltransferase	55521	147	12 of 22	Enzyme	Cytoplasm
P33176	KINH_HUMAN	KINH	Kinesin heavy chain, UKHC	109685	101	12 of 24	Transport carrier	Cytoplasm, Cytoskeleton, Microtubule
P14314	GLU2B_HUMAN	GLU2B	Glucosidase II beta subunit [Precursor]	59296	111	12 of 24	Unknown/ Unspecified	Cytoplasm, Endoplasmic reticulum
P19338	NUCL_HUMAN	NUCL	Nucleolin, Protein C23	76483	124	12 of 25	RNA associated, Transcription factor	Cytoplasm, Cytosol, Ribosome, Nucleus, Nucleolus
Q09666	AHNK_HUMAN	AHNK	Neuroblast differentiation-associated protein AHNAK [Fragments], Desmoyokin	312493	56	12 of 25	Chaperone, Regulatory, Structural	Cytoplasm, Cytosol, Vesicle, Membrane, Nucleus
Q13126	MTAP_HUMAN	MTAP	S-methyl-5-thioadenosine phosphorylase	31250	134	12 of 33	Enzyme	Cytoplasm
P25788	PSA3_HUMAN	PSA3	Proteasome subunit alpha type 3, Proteasome component C8	28302	106	12 of 37	Enzyme	Extracellular, Cytoplasm, Nucleus
Q14847	LASP1_HUMAN	LASP1	LIM and SH3 domain protein 1	29717	94	12 of 41	Adaptor	Cytoplasm, Nucleus
P62191	PRS4_HUMAN	PRS4	26S protease regulatory subunit 4	49185	96	12 of 41	Chaperone, Enzyme	Cytoplasm, Nucleus
P18669	PGAM1_HUMAN	PGAM1	Phosphoglycerate mutase 1, EC 5.4.2.1	28673	128	12 of 41	Enzyme	Cytoplasm, Cytosol

continued

Table I. *continued*

Accession No	Protein name	Protein symbol	Protein description	MW	Mascot score	Peptide matched	Function	Subcellular localisation
P47756	CAPZB_HUMAN	CAPZB	F-actin capping protein beta subunit	31219	105	12 of 47	Structural	Cytoplasm, Cytoskeleton
O15067	PUR4_HUMAN	PUR4	Phosphoribosylformylglycinamidine synthase, EC 6.3.5.3	144664	100	12 of 48	Enzyme	Cytoplasm
P61981	1433G_HUMAN	1433G	14-3-3 protein gamma	28171	79	12 of 53	Regulatory	Cytoplasm
Q9H6Z4	RANB3_HUMAN	RANB3	Ran-binding protein 3	60210	71	12 of 55	Chaperone, Structural	Cytoplasm, Nucleus
Q15942	ZYX_HUMAN	ZYX	Zyxin	61277	86	12 of 58	Adhesion	Membrane, Cytoplasm
P78371	TCPB_HUMAN	TCPB	T-complex protein 1 subunit beta	57357	133	13 of 20	Chaperone	Cytoplasm, Cytosol
P08865	RSSA_HUMAN	RSSA	40S ribosomal protein SA, p40	32723	163	13 of 22	Adhesion, Signaling, Receptor	Cytoplasm, Cytosol, Ribosome
P31947	1433S_HUMAN	1433S	14-3-3 protein sigma, Stratifin	27774	116	13 of 28	Regulatory	Extracellular, Cytoplasm
Q9Y6D9	MD1L1_HUMAN	MD1L1	Mitotic spindle assembly checkpoint protein MAD1, MAD1-like 1	83067	90	13 of 29	Regulatory, Antioncogene	Cytoplasm, Cytoskeleton, Microtubule, Centrosome, Nucleus
Q06323	PSME1_HUMAN	PSME1	Proteasome activator complex subunit 1, PA28alpha	28723	137	13 of 36	Chaperone, Regulatory	Unkown/Unspecified
Q15075	EEA1_HUMAN	EEA1	Early endosome antigen 1, Endosome-associated protein p162	162466	78	13 of 37	Binding	Endosome, Cytoplasm, Membrane
P09622	DLDH_HUMAN	DLDH	Dihydrolipoyl dehydrogenase, mitochondrial [Precursor]	54150	106	13 of 37	Enzyme	Cytoplasm, Mitochondrion
Q16576	RBBP7_HUMAN	RBBP7	Histone-binding protein RBBP7, Retinoblastoma-binding protein p46	47820	108	13 of 40	Regulatory	Nucleus, Chromosome
Q14247	SRC8_HUMAN	SRC8	Src substrate cortactin, Amplaxin	61636	114	13 of 42	Protooncogene	Cytoplasm, Cytosol
Q9NXD9	Q9NXD9_HUMAN	Q9NXD9	Hypothetical protein FLJ20303	59352	108	13 of 42	Unknown/ Unspecified	Unkown/Unspecified
Q05048	CSTF1_HUMAN	CSTF1	Cleavage stimulation factor, 50 kDa subunit	48358	112	13 of 43	RNA associated	Nucleus
Q5TBK6	Q5TBK6_HUMAN	Q5TBK6	LIM domain only 7	153670	60	13 of 45	Unkown/ Unspecified	Unkown/Unspecified
Q13200	PSD2_HUMAN	PSD2	26S proteasome non-ATPase regulatory subunit 2	100200	62	13 of 53	Regulatory	Unkown/Unspecified
P08729	K2C7_HUMAN	K2C7	Keratin, type II cytoskeletal 7, CK-7	51287	90	13 of 53	Structural	Cytoplasm, Cytoskeleton, Intermed filament
P35527	K1C9_HUMAN	K1C9	Keratin, type I cytoskeletal 9	62129	87	13 of 63	Structural	Cytoplasm, Cytoskeleton, Intermed filament
P25786	PSA1_HUMAN	PSA1	Proteasome subunit alpha type 1, EC 3.4.25.1	29556	69	13 of 71	Enzyme	Cytoplasm, Nucleus
O43172	PRP4_HUMAN	PRP4	U4/U6 small nuclear ribonucleoprotein Prp4, WD splicing factor Prp4	58449	71	13 of 72	RNA associated	Nucleus
P52292	IMA2_HUMAN	IMA2	Importin alpha-2 subunit, SRP1-alpha	57862	81	13 of 80	Transport carrier	Cytoplasm, Cytosol, Nucleus
P35237	SPB6_HUMAN	SPB6	Serpin B6, Protease inhibitor 6	42590	124	14 of 21	Enzyme, Regulatory	Cytoplasm, Cytosol
P13489	RINI_HUMAN	RINI	Ribonuclease inhibitor, RAI	49842	183	14 of 21	RNA associated	Cytoplasm
P54920	SNAA_HUMAN	SNAA	Alpha-soluble NSF attachment protein, SNAP-alpha	33247	201	14 of 23	Transport carrier	Cytoplasm, Golgi
O00410	IMB3_HUMAN	IMB3	Importin beta-3, RanBP5	123630	100	14 of 28	Transport, Signal transduction	Cytoplasm, Cytosol, Nucleus, Nucleus Envelope

continued

Table I. *continued*

Accession No	Protein name	Protein symbol	Protein description	MW	Mascot score	Peptide matched	Function	Subcellular localisation
P00338	LDHA_HUMAN	LDHA	L-lactate dehydrogenase A chain	36558	118	14 of 30	Enzyme	Cytoplasm
P52888	MEPD_HUMAN	MEPD	Thimet oligopeptidase	78709	118	14 of 32	Enzyme	Cytoplasm
Q9Y3F4	STRAP_HUMAN	STRAP	Serine-threonine kinase receptor-associated protein, MAP activator with WD repeats	38438	144	14 of 32	Protooncogene	Cytoplasm, Nucleus
P12268	IMDH2_HUMAN	IMDH2	Inosine-5'-monophosphate dehydrogenase 2	55805	136	14 of 38	Enzyme	Cytoplasm
P28066	PSA5_HUMAN	PSA5	Proteasome subunit alpha type 5, Macropain zeta chain	26411	109	14 of 39	Enzyme	Cytoplasm, Nucleus
P51570	GALK1_HUMAN	GALK1	Galactokinase	42272	139	14 of 40	Enzyme	Cytoplasm
Q8IWE2	Q8IWE2_HUMAN	Q8IWE2	Hypothetical protein LOC92689	60825	109	14 of 41	Unknown/ Unspecified	Unkown/Unspecified
P48637	GSHB_HUMAN	GSHB	Glutathione synthetase	52385	114	14 of 44	Enzyme	Cytoplasm, Nucleus
O60664	M6PBP_HUMAN	M6PBP	Mannose-6-phosphate receptor-binding protein 1, PP17	47047	119	14 of 46	Transport	Cytoplasm
Q12849	GRSF1_HUMAN	GRSF1	G-rich sequence factor 1	50170	77	14 of 59	RNA associated	Cytoplasm
Q8TA90	Q8TA90_HUMAN	Q8TA90	Similar to Elongation factor 2b [Fragment]	57500	67	14 of 81	Unknown/ Unspecified	Mitochondrion
Q92945	FUBP2_HUMAN	FUBP2	Far upstream element-binding protein 2, FUSE-binding protein 2	72709	181	15 of 23	Regulatory, RNA associated	Cytoplasm, Nucleus
Q9UMX0	UBQL1_HUMAN	UBQL1	Ubiquilin-1, PLIC-1	62519	119	15 of 24	Regulatory	Cytoplasm, Membrane, Nucleus
P04406	G3P_HUMAN	G3P	Glyceraldehyde-3-phosphate dehydrogenase, GAPDH	35922	158	15 of 25	Enzyme	Cytoplasm, Nucleus
O43175	SERA_HUMAN	SERA	D-3-phosphoglycerate dehydrogenase	56519	139	15 of 33	Enzyme	Cytoplasm, Mitochondrion, Endoplasmic reticulum
P45974	UBP5_HUMAN	UBP5	Ubiquitin carboxyl-terminal hydrolase 5, EC 3.1.2.15	95786	122	15 of 34	Enzyme	Cytoplasm
Q71U36	TBA3_HUMAN	TBA3	Tubulin alpha-3 chain	50136	131	15 of 35	Structural	Cytoplasm, Microtubule
O75534	CSDE1_HUMAN	CSDE1	Cold shock domain-containing protein E1, UNR protein	88885	123	15 of 36	RNA associated	Cytoplasm
P29692	EF1D_HUMAN	EF1D	Elongation factor 1-delta	30991	132	15 of 38	RNA associated	Cytoplasm, Mitochondrion, Nucleus
Q9BS26	TXND4_HUMAN	TXND4	Thioredoxin domain-containing protein 4 [Precursor], Endoplasmic reticulum resident protein ERp44	46971	154	15 of 41	Regulatory	Cytoplasm, Endoplasmic reticulum
P55795	HNRH2_HUMAN	HNRH2	Heterogeneous nuclear ribonucleoprotein H', FTP-3	49264	90	15 of 45	RNA associated	Nucleus, Nucleoplasm
O00299	CLIC1_HUMAN	CLIC1	Chloride intracellular channel protein 1, Chloride channel ABP	26792	188	15 of 45	Transport channel	Nucleus
O43852	CALU_HUMAN	CALU	Calumenin [Precursor], Crocalbin	37107	138	15 of 47	Regulatory, Transport	Cytoplasm, Endoplasmic reticulum
P06576	ATPB_HUMAN	ATPB	ATP synthase beta chain, mitochondrial [Precursor]	56560	67	15 of 56	Enzyme, Transport	Cytoplasm, Mitochondrion
P13645	K1C10_HUMAN	K1C10	Keratin, type I cytoskeletal 10, CK-10	59519	89	15 of 63	Structural	Cytoplasm, Cytoskeleton
P54727	RD23B_HUMAN	RD23B	UV excision repair protein RAD23 homolog B, p58	43171	104	15 of 65	DNA associated	Nucleus
P00558	PGK1_HUMAN	PGK1	Phosphoglycerate kinase 1, PRP 2	44483	157	16 of 30	Enzyme	Cytoplasm
P19623	SPEE_HUMAN	SPEE	Spermidine synthase, Putrescine aminopropyltransferase	33825	188	16 of 36	Enzyme	Unkown/Unspecified
P62258	1433E_HUMAN	1433E	14-3-3 protein epsilon	29174	138	16 of 37	Regulatory, Signaling neurotransmitter	Cytoplasm

continued

Table I. *continued*

Accession No	Protein name	Protein symbol	Protein description	MW	Mascot score	Peptide matched	Function	Subcellular localisation
P02769	ALBU_BOVIN	ALBU	Serum albumin [Precursor], BSA	69293	178	16 of 39	Regulatory	Secreted
Q9UQ80	PA2G4_HUMAN	PA2G4	Proliferation-associated protein 2G4, Cell cycle protein p38-2G4 homolog	43787	97	16 of 51	Regulatory	Cytoplasm, Nucleus, Nucleolus
P60174	TPIS_HUMAN	TPIS	Triosephosphate isomerase, EC 5.3.1.1	26538	177	16 of 51	Enzyme	Unkown/Unspecified
P16083	NQO2_HUMAN	NQO2	Ribosyldihydronicotinamide dehydrogenase [quinone], EC 1.10.99.2	25821	141	16 of 51	Enzyme	Cytoplasm
P27797	CRTC_HUMAN	CRTC	Calreticulin [Precursor], HACBP	48142	159	16 of 55	Chaperone, Antigen	Cytoplasm, Endoplasmic reticulum
P60709	ACTB_HUMAN	ACTB	Actin, cytoplasmic 1, Beta-actin	41737	98	16 of 63	Structural	Cytoplasm
O60506	HNRPQ_HUMAN	HNRPQ	Heterogeneous nuclear ribonucleoprotein Q	69633	146	16 of 66	RNA associated	Cytoplasm, Nucleus
P36952	MASP_HUMAN	MASP	Maspin [Precursor], Protease inhibitor 5	42138	102	16 of 89	Enzyme, Regulatory, Antioncogene	Extracellular
P35998	PRS7_HUMAN	PRS7	26S protease regulatory subunit 7, MSS1 protein	48503	190	17 of 25	Chaperone, Enzyme	Cytoplasm, Cytosol, Nucleus
P14550	AK1A1_HUMAN	AK1A1	Alcohol dehydrogenase [NADP+], Aldehyde reductase	36442	216	17 of 28	Enzyme	Cytoplasm, Cytosol
Q9Y4L1	OXRP_HUMAN	OXRP	150 kDa oxygen-regulated protein [Precursor], Orp150	111335	65	17 of 31	Chaperone, Protooncogene	Cytoplasm, Cytosol, Endoplasmic reticulum
P54577	SYYC_HUMAN	SYYC	Tyrosyl-tRNA synthetase, cytoplasmic	59012	156	17 of 35	Enzyme, Signaling	Extracellular, Cytoplasm
Q13347	IF32_HUMAN	IF32	Eukaryotic translation initiation factor 3 subunit 2, TGF-beta receptor-interacting protein 1	36502	175	17 of 36	RNA associated, Antioncogene	Cytoplasm, Cytosol, Ribosome
Q4V328	GRAP1_HUMAN	GRAP1	GRIP1-associated protein 1	95990	180	17 of 43	Regulatory	Unkown/Unspecified
O75150	BRE1B_HUMAN	BRE1B	Ubiquitin ligase protein BRE1B, RING finger protein 40	113678	91	17 of 50	Regulatory, Transcription factor	Nucleus, Nucleoplasm, Nucleus bodies
P04040	CATA_HUMAN	CATA	Catalase	59625	142	17 of 53	Enzyme	Cytoplasm, Peroxisome
P30101	PDIA3_HUMAN	PDIA3	Protein disulfide-isomerase A3 [Precursor], ERp60	56782	134	17 of 56	Chaperone, Enzyme	Membrane, Cytoplasm, Endoplasmic reticulum
P55884	IF39_HUMAN	IF39	Eukaryotic translation initiation factor 3 subunit 9, eIF3 p116	92492	80	17 of 60	RNA associated	Cytoplasm, Cytosol, Ribosome
P20810	ICAL_HUMAN	ICAL	Calpastatin, Calpain inhibitor	76501	82	17 of 84	Regylatory	Unkown/Unspecified
Q92598	HS105_HUMAN	HS105	Heat-shock protein 105 kDa	96865	71	17 of 87	Regulatory	Cytoplasm, Mitochondrion, Nucleus
P22626	ROA2_HUMAN	ROA2	Heterogeneous nuclear ribonucleoproteins A2/B1	37430	190	18 of 38	RNA associated	Nucleus, Ribonucleosomes
P50990	TCPQ_HUMAN	TCPQ	T-complex protein 1 subunit theta	59489	145	18 of 40	Chaperone	Cytoplasm
Q15365	PCBP1_HUMAN	PCBP1	Poly(rC)-binding protein 1	37498	137	18 of 41	RNA associated	Cytoplasm, Nucleus
P52272	HNRPM_HUMAN	HNRPM	Heterogeneous nuclear ribonucleoprotein M	77384	92	18 of 47	RNA associated	Nucleus, Nucleolus
P40222	TXLNA_HUMAN	TXLNA	Alpha-taxilin	61891	100	18 of 74	Unknown/ Unspecified	Unkown/Unspecified
P38606	VATA1_HUMAN	VATA1	Vacuolar ATP synthase catalytic subunit A, ubiquitous isoform	68304	86	18 of 84	Transport carrier, Enzyme	Membrane, Cytoplasm, Lysosome

continued

Table I. *continued*

Accession No	Protein name	Protein symbol	Protein description	MW	Mascot score	Peptide matched	Function	Subcellular localisation
Q9BQE3	TBA6_HUMAN	TBA6	Tubulin alpha-6 chain	49895	216	19 of 30	Structural	Cytoplasm, Mitochondrion
P49321	NASP_HUMAN	NASP	Nuclear autoantigenic sperm protein	85238	162	19 of 36	Chaperone	Cytoplasm, Golgi, Nucleus, Chromosome, Nucleosome
Q15084	PDIA6_HUMAN	PDIA6	Protein disulfide-isomerase A6 [Precursor]	48121	196	19 of 58	Chaperone	Cytoplasm, Endoplasmic reticulum
P07237	PDIA1_HUMAN	PDIA1	Protein disulfide-isomerase [Precursor], p55	57116	140	19 of 81	Enzyme, Protooncogene	Cytoplasm, Endoplasmic reticulum
Q13263	TIF1B_HUMAN	TIF1B	Transcription intermediary factor 1-beta. KAP-1	88550	81	19 of 87	Regulatory, Transcription factor	Nucleus, Chromosome, Heterochromosome
Q7KZF4	SND1_HUMAN	SND1	Staphylococcal nuclease domain-containing protein 1, p100 co-activator	101997	172	20 of 37	Transcription cofactor	Cytoplasm, Nucleus
P04075	ALDOA_HUMAN	ALDOA	Fructose-bisphosphate aldolase A, Muscle-type aldolase	39289	178	20 of 58	Enzyme	Cytoplasm, Cytosol
O95302	FKBP9_HUMAN	FKBP9	FK506-binding protein 9 [Precursor], EC 5.2.1.8	63084	134	20 of 65	Unknown/ Unspecified	Cytoplasm, Endoplasmic reticulum
Q13283	G3BP_HUMAN	G3BP	Ras-GTPase-activating protein binding protein 1, ATP-dependent DNA helicase VIII	52164	130	20 of 88	Enzyme	Membrane, Cytoplasm, Cytosol, Nucleus
P14866	HNRPL_HUMAN	HNRPL	Heterogeneous nuclear ribonucleoprotein L	60187	223	21 of 32	RNA associated	Cytoplasm, Nucleus, Nucleoplasm
P35241	RADI_HUMAN	RADI	Radixin	68564	114	21 of 80	Motor/Contractile	Membrane
P29401	TKT_HUMAN	TKT	Transketolase, (EC 2.2.1.1), TK	67878	75	21 of 82	Enzyme	Unkown/Unspecified
Q99798	ACON_HUMAN	ACON	Aconitate hydratase, mitochondrial [Precursor], Citrate hydro-lyase	85425	234	22 of 33	Enzyme	Cytoplasm, Mitochondrion
P10809	CH60_HUMAN	CH60	60 kDa heat shock protein, mitochondrial [Precursor], Hsp60	61055	180	22 of 47	Chaperone	Cytoplasm, Mitochondrion
Q92973	TNPO1_HUMAN	TNPO1	Transportin-1, Importin beta-2	101310	107	22 of 82	Transport carrier	Cytoplasm, Cytosol, Nucleus, Nucleolus
O00571	DDX3X_HUMAN	DDX3X	ATP-dependent RNA helicase DDX3X, DEAD box protein 3, X-chromosomal	73112	226	23 of 38	Enzyme, RNA associated	Cytoplasm, Nucleus
P62333	PRS10_HUMAN	PRS10	26S protease regulatory subunit S10B	44173	148	23 of 51	Enzyme, Chaperone	Cytoplasm, Nucleus
P08758	ANXA5_HUMAN	ANXA5	Annexin A5, Thromboplastin inhibitor	35806	182	23 of 79	Regulatory, Transport channel	Membrane
P61978	HNRPK_HUMAN	HNRPK	Heterogeneous nuclear ribonucleoprotein K	50976	124	23 of 90	RNA associated	Cytoplasm, Nucleus, Nucleoplasm
P30041	PRDX6_HUMAN	PRDX6	Peroxiredoxin-6, Antioxidant protein 2	24904	271	24 of 49	Enzyme, Regulatory	Cytoplasm, Cytosol, Lysosome
Q93052	LPP_HUMAN	LPP	Lipoma-preferred partner	65746	180	24 of 60	Unknown/ Unspecified	Cytoplasm, Nucleus
P07355	ANXA2_HUMAN	ANXA2	Annexin A2, Lipocortin II, Calpactin I heavy chain, p36	38473	98	24 of 63	Regulatory, Signaling, Transport channel	Membrane
P34897	GLYM_HUMAN	GLYM	Serine hydroxymethyltransferase, mitochondrial [Precursor]	55993	175	24 of 69	Enzyme	Cytoplasm, Mitochondrion
P31943	HNRH1_HUMAN	HNRH1	Heterogeneous nuclear ribonucleoprotein H	49098	140	24 of 86	RNA associated	Nucleus, Nucleoplasm

continued

Table I. *continued*

Accession No	Protein name	Protein symbol	Protein description	MW	Mascot score	Peptide matched	Function	Subcellular localisation
P63261	ACTG_HUMAN	ACTG	Actin, cytoplasmic 2, Gamma-actin	41793	135	24 of 94	Structural	Cytoplasm
Q16555	DPYL2_HUMAN	DPYL2	Dihydropyrimidinase-related protein 2, DRP-2	62294	208	25 of 52	Enzyme, Signaling	Cytoplasm
P13639	EF2_HUMAN	EF2	Elongation factor 2	95207	184	25 of 56	RNA associated	Cytoplasm, Cytosol, Ribosome
P68363	TBAK_HUMAN	TBAK	Tubulin alpha-ubiquitous chain	50152	174	25 of 75	Structural	Microtubule
P31040	DHSA_HUMAN	DHSA	Succinate dehydrogenase [ubiquinone] flavoprotein subunit, mitochondrial [Precursor]	72692	200	26 of 68	Enzyme	Cytoplasm, Mitochondrion, Nucleus
P26038	MOES_HUMAN	MOES	Moesin, Membrane-organizing extension spike protein	67689	151	26 of 71	Structural	Cytoplasm, Cytoskeleton
P07437	TBB2_HUMAN	TBB2	Tubulin beta-2 chain	49671	186	26 of 71	Structural	Microtubule
P06733	ENOA_HUMAN	ENOA	Alpha-enolase, 2-phospho-D-glycerate hydro-lyase	47038	213	26 of 78	Enzyme, Structural protein, Antigen, Antioncogene	Cytoplasm
P31150	GDIA_HUMAN	GDIA	Rab GDP dissociation inhibitor alpha	50583	243	27 of 52	Regulatory, Signaling, Transport	Cytoplasm
P07900	HS90A_HUMAN	HS90A	Heat shock protein HSP 90-alpha	84543	213	27 of 58	Chaperone	Cytoplasm
P22314	UBE1_HUMAN	UBE1	Ubiquitin-activating enzyme E1, A1S9 protein	117849	132	27 of 65	Enzyme	Unkown/Unspecified
P43243	MATR3_HUMAN	MATR3	Matrin-3	94623	178	28 of 74	Structural	Nucleus, Nucleoplasm
O75083	WDR1_HUMAN	WDR1	WD-repeat protein 1, Actin-interacting protein 1	66062	192	28 of 76	Structural	Cytoplasm, Cytoskeleton
O43707	ACTN4_HUMAN	ACTN4	Alpha-actinin-4	104854	183	28 of 78	Structural, Motor/ Contractile	Cytoplasm, Membrane, Cytoskeleton, Microfilament, Nucleus
P28331	NUAM_HUMAN	NUAM	NADH-ubiquinone oxidoreductase 75 kDa subunit, mitochondrial [Precursor]	79516	180	28 of 78	Enzyme	Cytoplasm, Mitochondrion
P36871	PGM1_HUMAN	PGM1	Phosphoglucomutase-1	61318	252	29 of 62	Enzyme	Cytoplasm
Q14697	GANAB_HUMAN	GANAB	Neutral alpha-glucosidase AB [Precursor	106874	166	29 of 86	Enzyme	Cytoplasm, Endoplasmic reticulum, Golgi
Q96K17	BT3L4_HUMAN	BT3L4	Transcription factor BTF3 homolog 4	17271	56	3 of 15	Unknown/ Unspecified	Nucleus
Q8TDC3	BRSK1_HUMAN	BRSK1	BR serine/threonine-protein kinase 1, SAD1 kinase	86753	61	3 of 3	Enzyme	Cytoplasm, Nucleus
P40227	TCPZ_HUMAN	TCPZ	T-complex protein 1 subunit zeta, T-complex protein 1 subunit zeta	57893	95	3 of 4	Chaperone	Cytoplasm
Q9HB71	CYBP_HUMAN	CYBP	Calcyclin-binding protein, Siah-interacting protein	26210	61	3 of 4	Binding	Cytoplasm, Nucleus
P49411	EFTU_HUMAN	EFTU	Elongation factor Tu, mitochondrial [Precursor], P43	49542	58	3 of 5	Regulatory, Transcription factor	Cytoplasm, Mitochondrion
P02792	FRIL_HUMAN	FRIL	Ferritin light chain	19888	53	3 of 5	Storage	Cytoplasm, Cytosol
Q9UI30	U315_HUMAN	U315	UPF0315 protein AD-001	14199	63	3 of 7	Unkown/ Unspecified	Unkown/Unspecified
P49588	SYA_HUMAN	SYA	Alanyl-tRNA synthetase	106801	177	30 of 75	Enzyme	Cytoplasm
Q15029	U5S1_HUMAN	U5S1	116 kDa U5 small nuclear ribonucleoprotein component, U5-116 kDa	109436	183	30 of 87	RNA associated	Nucleus

continued

Table I. *continued*

Accession No	Protein name	Protein symbol	Protein description	MW	Mascot score	Peptide matched	Function	Subcellular localisation
P49327	FAS_HUMAN	FAS	Fatty acid synthase EC 2.3.1.85	273400	215	31 of 42	Enzyme	Cytoplasm, Cytosol
Q8WUM4	PDC6I_HUMAN	PDC6I	Programmed cell death 6-interacting protein	96023	287	31 of 50	Regulatory	Cytoplasm, Cytosol
P17987	TCPA_HUMAN	TCPA	T-complex protein 1 subunit alpha	60344	282	31 of 52	Chaperone	Cytoplasm
P49368	TCPG_HUMAN	TCPG	T-complex protein 1 subunit gamma	60534	236	31 of 65	Chaperone	Cytoplasm, Cytosol
Q16891	IMMT_HUMAN	IMMT	Mitochondrial inner membrane protein, Mitofilin	83678	276	31 of 67	Structural	Cytoplasm, Mitochondrion
P08107	HSP71_HUMAN	HSP71	Heat shock 70 kDa protein 1	70052	227	31 of 83	Chaperone	Cytoplasm, Nucleus
P05783	K1C18_HUMAN	K1C18	Keratin, type I cytoskeletal 18, CK-18	47927	270	32 of 67	Structural	Cytoplasm, Cytoskeleton, Intermed filament
P30153	2AAA_HUMAN	2AAA	Serine/threonine-protein phosphatase 2A 65 kDa regulatory subunit A alpha isoform	65092	211	32 of 77	Regulatory, Antioncogene	Unkown/Unspecified
Q96AE4	FUBP1_HUMAN	FUBP1	Far upstream element-binding protein 1	67473	199	33 of 83	Protooncogene	Cytoplasm, Nucleus
P08238	HS90B_HUMAN	HS90B	Heat shock protein HSP 90-beta, HSP 90	83133	153	34 of 77	Chaperone	Cytoplasm
P11586	C1TC_HUMAN	C1TC	C-1-tetrahydrofolate synthase, cytoplasmic, C1-THF synthase	101428	326	35 of 50	Enzyme	Cytoplasm
P18206	VINC_HUMAN	VINC	Vinculin, Metavinculin	123668	214	35 of 56	Adhesion	Cytoplasm, Cytoskeleton, Membrane
P34932	HSP74_HUMAN	HSP74	Heat shock 70 kDa protein 4	94300	266	35 of 59	Chaperone	Cytoplasm, Cytosol, Nucleus
P11021	GRP78_HUMAN	GRP78	78 kDa glucose-regulated protein [Precursor], Immunoglobulin heavy chain binding protein	72333	363	35 of 60	Chaperone, Signaling	Extracellular, Membrane, Cytoplasm, Endoplasmic reticulum
Q14974	IMB1_HUMAN	IMB1	Importin beta-1 subunit, Nuclear factor P97	97170	178	35 of 84	Nuclear receptor, Transport carrier	Cytoplasm, Nucleus Envelope
P11498	PYC_HUMAN	PYC	Pyruvate carboxylase, mitochondrial [Precursor], EC 6.4.1.1	129634	310	36 of 51	Enzyme	Cytoplasm, Mitochondrion
P38646	GRP75_HUMAN	GRP75	Stress-70 protein, mitochondrial [Precursor], Mortalin	73680	261	36 of 77	Chaperone, Regulatory, Transcription factor	Cytoplasm, Mitochondrion, Endoplasmic reticulum, Cytosol, Vesicle
Q00341	VIGLN_HUMAN	VIGLN	Vigilin, HDL-binding protein	141440	105	38 of 69	Transport	Cytoplasm, Nuclear Envelope
P55072	TERA_HUMAN	TERA	Transitional endoplasmic reticulum ATPase, VCP	89191	362	38 of 81	Chaperone, Structural, Transport	Cytoplasm, Endoplasmic reticulum, Golgi, Cytosol, Vesicle, Nucleus
P49915	GUAA_HUMAN	GUAA	GMP synthase [glutamine-hydrolyzing], EC 6.3.5.2	76715	329	39 of 82	Enzyme	Cytoplasm
Q07955	SFRS1_HUMAN	SFRS1	Splicing factor, arginine/serine-rich 1	27613	72	4 of 10	RNA associated	Cytoplasm, Nucleus, Nucleolar
P49721	PSB2_HUMAN	PSB2	Proteasome subunit beta type 2, EC 3.4.25.1	22836	62	4 of 10	Enzyme	Cytoplasm, Nucleus
Q6S4N2	HS70B_PIG	HS70B	Heat shock 70 kDa protein 1B	70098	58	4 of 11	Unkown/ Unspecified	Unkown/Unspecified
O75832	PSD10_HUMAN	PSD10	26S proteasome non-ATPase regulatory subunit 10, Gankyrin	24428	61	4 of 11	Regulatory, Protooncogene	Extracellular, Cytoplasm
P04083	ANXA1_HUMAN	ANXA1	Annexin A1, Chromobindin-9	38583	68	4 of 12	Regulatory, Transport	Membrane
Q9HD26	GOPC_HUMAN	GOPC	Golgi-associated PDZ and coiled-coil motif-containing protein, PIST	50520	58	4 of 14	Signaling, Transport	Cytoplasm, Golgi
O95861	BPNT1_HUMAN	BPNT1	3'(2'),5'-bisphosphate nucleotidase 1, PIP	33392	63	4 of 14	Enzyme	Unkown/Unspecified
Q14651	PLSI_HUMAN	PLSI	Plastin-1, Intestine-specific plastin	70353	53	4 of 15	Structural	Membrane, Cytoplasm, Cytoskeleton

continued

Table I. *continued*

Accession No	Protein name	Protein symbol	Protein description	MW	Mascot score	Peptide matched	Function	Subcellular localisation
Q9Y371	SHLB1_HUMAN	SHLB1	SH3 domain GRB2-like protein B1, Bax-interacting factor 1	40796	54	4 of 15	Regulatory	Cytoplasm
Q6FHG5	Q6FHG5_HUMAN	Q6FHG5	SNCG protein	13331	64	4 of 15	Enzyme	Unkown/Unspecified
O75874	IDHC_HUMAN	IDHC	Isocitrate dehydrogenase [NADP] cytoplasmic, Oxalosuccinate decarboxylase	46659	213	4 of 17	Enzyme	Cytoplasm, Cytosol, Peroxisome
P07954	FUMH_HUMAN	FUMH	Fumarate hydratase, mitochondrial [Precursor]	54637	56	4 of 17	Enzyme, Antioncogene	Cytoplasm, Mitochondrion
Q99733	NP1L4_HUMAN	NP1L4	Nucleosome assembly protein 1-like 4, NAP2	42823	60	4 of 20	Chaperone	Nucleus
P80723	BASP_HUMAN	BASP	Brain acid soluble protein 1	22562	57	4 of 20	Signaling	Membrane, Cytoplasm, Cytoskeleton
Q9H2U2	IPYR2_HUMAN	IPYR2	Inorganic pyrophosphatase 2, mitochondrial [Precursor], EC 3.6.1.1	37962	53	4 of 21	Enzyme	Cytoplasm, Mitochondrion
P30085	KCY_HUMAN	KCY	UMP-CMP kinase	22222	60	4 of 24	Enzyme	Cytoplasm, Nucleus
Q96IU4	AB14B_HUMAN	AB14B	Abhydrolase domain-containing protein 14B	22346	59	4 of 26	Unknown/ Unspecified	Cytoplasm, Nucleus
P13804	ETFA_HUMAN	ETFA	Electron transfer flavoprotein alpha-subunit, mitochondrial [Precursor]	35080	53	4 of 29	Transport carrier	Cytoplasm, Mitochondrion
Q9UNZ2	NSF1C_HUMAN	NSF1C	NSFL1 cofactor p47	40573	68	4 of 5	Adaptor	Nucleus
P35232	PHB_HUMAN	PHB	Prohibitin	29804	71	4 of 5	Antioncogene	Cytoplasm, Mitochondrion, Endoplasmic reticulum, Nucleus, Nucleoplasm
Q9GZS3	Q9GZS3_HUMAN	Q9GZS3	Hypothetical protein FLJ21101, Meiotic recombination protein REC14	33581	64	4 of 7	Unkown/ Unspecified	Unkown/Unspecified
P61086	UBC1_HUMAN	UBC1	Ubiquitin-conjugating enzyme E2-25 kDa, Ubiquitin carrier protein	22275	69	4 of 8	Enzyme	Cytoplasm
P63244	GBLP_HUMAN	GBLP	Guanine nucleotide-binding protein beta subunit 2-like 1, Receptor of activated protein kinase C 1	35077	65	4 of 8	Signaling, Receptor membrane G	Membrane
Q9H2R7	Q9H2R7_HUMAN	Q9H2R7	NPD011	24353	65	4 of 8	Regulatory	Unkown/Unspecified
P04179	SODM_HUMAN	SODM	Superoxide dismutase [Mn], mitochondrial [Precursor]	24722	67	4 of 8	Enzyme	Cytoplasm, Mitochondrion
Q9Y383	LC7L2_HUMAN	LC7L2	Putative RNA-binding protein Luc7-like 2	46514	54	4 of 9	Unkown/ Unspecified	Unkown/Unspecified
P02545	LMNA_HUMAN	LMNA	Lamin-A/C	74139	309	41 of 78	Structural	Cytoplasm, Cytoskeleton, Intermed filament, Nucleus Envelope
P14618	KPYM_HUMAN	KPYM	Pyruvate kinase isozymes M1/M2, Cytosolic thyroid hormone-binding protein	57806	315	41 of 90	Enzyme	Cytoplasm, Cytosol
P11142	HSP7C_HUMAN	HSP7C	Heat shock cognate 71 kDa protein	70898	310	41 of 93	Chaperone	Cytoplasm, Cytosol, Nucleus
P15311	EZRI_HUMAN	EZRI	Ezrin, p81, Cytovillin	69268	281	42 of 86,	Structural	Cytoplasm
P31948	STIP1_HUMAN	STIP1	Stress-induced-phosphoprotein 1, Hsc70/Hsp90-organizing protein	62639	276	43 of 72	Adaptor, Chaperone	Cytoplasm, Golgi, Cytosol, Vesicle, Nucleus
P08670	VIME_HUMAN	VIME	Vimentin	53520	142	46 of 88	Structural	Cytoplasm, Cytoskeleton, Intermed filament

continued

Table I. *continued*

Accession No	Protein name	Protein symbol	Protein description	MW	Mascot score	Peptide matched	Function	Subcellular localisation
P30086	PEBP_HUMAN	PEBP	Phosphatidylethanolamine-binding protein, Prostatic binding protein	20926	84	5 of 10	Binding	Cytoplasm
O00170	AIP_HUMAN	AIP	AH receptor-interacting protein, HBV-X-associated protein 2	37664	63	5 of 11	Receptor	Cytoplasm
Q13765	NACA_HUMAN	NACA	Nascent polypeptide-associated complex alpha subunit	23384	58	5 of 11	Chaperone	Cytoplasm, Nucleus
P13798	ACPH_HUMAN	ACPH	Acylamino-acid-releasing enzyme, APH	81225	72	5 of 12	Enzyme	Cytoplasm
P60660	MYL6_HUMAN	MYL6	Myosin light polypeptide 6	16799	78	5 of 12	Structural	Unkown/Unspecified
Q9Y265	RUVB1_HUMAN	RUVB1	RuvB-like 1, 49-kDa TATA box-binding protein-interacting protein	50228	59	5 of 13	Enzyme, DNA associated, Antigen	Cytoplasm, Nucleus, Nucleoplasm
P00441	SODC_HUMAN	SODC	Superoxide dismutase [Cu-Zn]	15805	81	5 of 13	Chaperone, Enzyme	Cytoplasm, Cytosol
Q9BVA2	Q9BVA2_HUMAN	Q9BVA2	Four and a half LIM domains 3	31192	63	5 of 14	Unknown/ Unspecified	Unkown/Unspecified
P45880	VDAC2_HUMAN	VDAC2	Voltage-dependent anion-selective channel protein 2	38093	63	5 of 14	Transport channel	Cytoplasm, Mitochondrion
P21796	VDAC1_HUMAN	VDAC1	Voltage-dependent anion-selective channel protein 1, Plasmalemmal porin	30641	70	5 of 14	Transport channel	Cytoplasm, Mitochondrion
P62988	UBIQ_HUMAN	UBIQ	Ubiquitin	8565	87	5 of 14	RNA associated	Cytoplasm, Cytosol, Ribosome, Nucleus
Q9Y224	CN166_HUMAN	CN166	Protein C14orf166	28068	62	5 of 15	Regulatory	Cytoplasm, Mitochodrion, Nucleus
O75821	IF34_HUMAN	IF34	Eukaryotic translation initiation factor 3 subunit 4, eIF3 p44	35611	57	5 of 17	RNA associated	Cytoplasm, Cytosol, Ribosome
P49366	DHYS_HUMAN	DHYS	Deoxyhypusine synthase	40971	56	5 of 17	Enzyme	Unkown/Unspecified
P27816	MAP4_HUMAN	MAP4	Microtubule-associated protein 4	121019	55	5 of 17	Structural	Cytoplasm, Cytoskeleton, Microtubule
O14818	PSA7_HUMAN	PSA7	Proteasome subunit alpha type 7	27887	63	5 of 18	Enzyme	Cytoplasm, Nucleus
P50502	F10A1_HUMAN	F10A1	Hsc70-interacting protein, Hip	41332	62	5 of 23	Antioncogene	Cytoplasm
P63208	SKP1_HUMAN	SKP1	S-phase kinase-associated protein 1A, Cyclin A/CDK2-associated protein p19	18527	65	5 of 24	Regulatory	Cytoplasm
P04632	CPNS1_HUMAN	CPNS1	Calpain small subunit 1, Calcium-dependent protease small subunit 1	28316	73	5 of 25	Enzyme, Regulatory	Cytoplasm, Membrane
P43487	RANG_HUMAN	RANG	Ran-specific GTPase-activating protein	23310	54	5 of 28	Regulatory, Enzyme	Nucleus
P42126	D3D2_HUMAN	D3D2	3,2-trans-enoyl-CoA isomerase, mitochondrial [Precursor], Dodecenoyl-CoA isomerase	32816	58	5 of 7	Enzyme	Cytoplasm, Mitochodrion, Matrix
Q3LXA3	DAK_HUMAN	DAK	Dihydroxyacetone kinase	58977	70	5 of 8	Enzyme	Unkown/Unspecified
P05388	RLA0_HUMAN	RLA0	60S acidic ribosomal protein P0, L10E	34274	85	5 of 8	RNA associated	Cytoplasm, Cytosol, Ribosome
P14625	ENPL_HUMAN	ENPL	Endoplasmin [Precursor], GRP94	92469	333	53 of 94	Chaperone	Extracellular, Endoplasmic reticulum
Q14257	RCN2_HUMAN	RCN2	Reticulocalbin-2 [Precursor], E6-binding protein	36876	91	6 of 11	Structural	Cytoplasm, Endoplasmic reticulum
O00764	PDXK_HUMAN	PDXK	Pyridoxal kinase	35102	82	6 of 12	Enzyme	Cytoplasm
P10599	THIO_HUMAN	THIO	Thioredoxin, Surface-associated sulphydryl protein	11606	66	6 of 13	Enzyme	Cytoplasm
Q9NZ56	FMN2_HUMAN	FMN2	Formin-2, Fragments	70340	64	6 of 15	Regulatory	Cytoplasm, Nucleus
P38159	HNRPG_HUMAN	HNRPG	Heterogeneous nuclear ribonucleoprotein G, Glycoprotein p43	42332	62	6 of 15	RNA associated	Nucleus

continued

Table I. *continued*

Accession No	Protein name	Protein symbol	Protein description	MW	Mascot score	Peptide matched	Function	Subcellular localisation
P62195	PRS8_HUMAN	PRS8	26S protease regulatory subunit 8, p45/SUG	45626	53	6 of 15	Chaperone, Enzyme, Regulatory	Cytoplasm, Nucleus
P62937	PPIA_HUMAN	PPIA	Peptidyl-prolyl cis-trans isomerase A, Cyclophilin A	17881	77	6 of 16	Enzyme, Immunity/ defense	Cytoplasm, Cytosol, Microsome, Endoplasmic reticulum
P07108	ACBP_HUMAN	ACBP	Acyl-CoA-binding protein, Endozepine, DBI	9913	61	6 of 16	Regulatory, Transport carrier	Unkown/Unspecified
O75439	MPPB_HUMAN	MPPB	Mitochondrial-processing peptidase beta subunit, mitochondrial [Precursor]	54366	63	6 of 17	Enzyme	Cytoplasm, Mitochondrion
Q16851	UGPA2_HUMAN	UGPA2	UTP--glucose-1-phosphate uridylyltransferase 2	56809	68	6 of 17	Enzyme	Cytoplasm
P23528	COF1_HUMAN	COF1	Cofilin-1, p18	18371	89	6 of 18	Structural, Signal transduction	Cytoplasm, Nucleus
Q99426	TBCB_HUMAN	TBCB	Tubulin-specific chaperone B, Cytoskeleton associated protein 1	27326	73	6 of 20	Structural	Cytoplasm, Cytoskeleton
Q76M58	Q76M58_HUMAN	Q76M58	40S ribosomal protein S12	14515	64	6 of 21	Unknown/ Unspecified	Unkown/Unspecified
Q9BWF3	RBM4_HUMAN	RBM4	RNA-binding protein 4, Lark homolog	40314	56	6 of 24	Transcription factor	Nucleus, Nucleolar
Q3ZCQ8	TIM50_HUMAN	TIM50	Import inner membrane translocase subunit TIM50, mitochondrial [Precursor]	39646	60	6 of 24	Transport channel	Cytoplasm, Mitochondrion
P31949	S10AB_HUMAN	S10AB	Calgizzarin, S100 calcium-binding protein A11	11740	62	6 of 24	Motor/Contractile	Cytoplasm, Nucleus
P36873	PP1G_HUMAN	PP1G	Serine/threonine-protein phosphatase PP1-gamma catalytic subunit	36984	59	6 of 26	Enzyme	Cytoplasm
Q9HD64	GAGD2_HUMAN	GAGD2	G antigen family D 2 protein	17751	57	6 of 51	Antigen, Protooncogenex	Cytoplasm, Nucleus
P28070	PSB4_HUMAN	PSB4	Proteasome subunit beta type 4 [Precursor], Macropain beta chain	29192	97	6 of 6	Enzyme	Cytoplasm, Nucleus
Q9NVM4	ANM7_HUMAN	ANM7	Protein arginine N-methyltransferase 7, EC 2.1.1.-	78459	83	6 of 7	Enzyme, Transcription factor	Cytoplasm, Mitochodrion
P26447	S10A4_HUMAN	S10A4	S100 calcium-binding protein A4, Metastasin, Calvasculin	11729	84	6 of 9	Regulatory	Cytoplasm, Nucleus
P31327	CPSM_HUMAN	CPSM	Carbamoyl-phosphate synthase [ammonia], mitochondrial Precursor (EC 6.3.4.16)	164939	70	69 of 92	Enzyme	Cytoplasm, Mitochondrion
Q15181	IPYR_HUMAN	IPYR	Inorganic pyrophosphatase, EC 3.6.1.1	32660	116	7 of 10	Enzyme	Cytoplasm
O43396	TXNL1_HUMAN	TXNL1	Thioredoxin-like protein 1	32120	121	7 of 10	Enzyme, Transport	Cytoplasm
P07741	APT_HUMAN	APT	Adenine phosphoribosyltransferase, APRT	19477	106	7 of 11	Enzyme	Cytoplasm
Q9NRH3	TBG2_HUMAN	TBG2	Tubulin gamma-2 chain	51092	85	7 of 12	Regylatory, Structural	Microtubule
Q9BT78	CSN4_HUMAN	CSN4	COP9 signalosome complex subunit 4, JAB1-containing signalosome subunit 4	46269	78	7 of 12	Regulatory	Cytoplasm, Cytosol, Nucleus
Q9GZZ9	UE1D1_HUMAN	UE1D1	Ubiquitin-activating enzyme E1 domain-containing protein 1	44863	68	7 of 12	Enzyme	Unkown/Unspecified
Q15019	SEPT2_HUMAN	SEPT2	Septin-2, Protein NEDD5	41487	91	7 of 12	Motor/Contractile	Unkown/Unspecified

continued

Table I. *continued*

Accession No	Protein name	Protein symbol	Protein description	MW	Mascot score	Peptide matched	Function	Subcellular localisation
P12429	ANXA3_HUMAN	ANXA3	Annexin A3, Lipocortin III, PAP-III	36244	90	7 of 13	Enzyme	Cytoplasm
Q9Y230	RUVB2_HUMAN	RUVB2	RuvB-like 2, 48-kDa TATA box-binding protein-interacting protein	51025	80	7 of 14	DNA associated	Nucleus, Nucleoplasm, Nuclear matrix
Q15691	MARE1_HUMAN	MARE1	Microtubule-associated protein RP/EB family member 1, End-binding protein 1	29868	108	7 of 14	Structural	Cytoplasm, Cytoskeleton, Microtubule
Q8TCS8	PNPT1_HUMAN	PNPT1	Polyribonucleotide nucleotidyltransferase 1, mitochondrial [Precursor], PNPase 1	85937	68	7 of 15	Enzyme, RNA associated	Cytoplasm, Mitochondial
P41250	SYG_HUMAN	SYG	Glycyl-tRNA synthetase	83140	68	7 of 15	Enzyme	Cytoplasm, Mitochodrion
O75947	ATP5H_HUMAN	ATP5H	ATP synthase D chain, mitochondrial	18360	106	7 of 16	Enzyme	Unkown/Unspecified
P05387	RLA2_HUMAN	RLA2	60S acidic ribosomal protein P2	11665	127	7 of 17	RNA associated	Cytoplasm, Cytosol, Ribosome
Q13561	DCTN2_HUMAN	DCTN2	Dynactin subunit 2, p50 dynamitin	44100	84	7 of 19	Motor/Contractile, Transport	Cytoplasm, Cytoskeleton, Nucleus, Chromosome, Kinetochore
O95757	HS74L_HUMAN	HS74L	Heat shock 70 kDa protein 4L, Osmotic stress protein 94	94486	203	7 of 20	Chaperone	Cytoplasm, Nucleus
O43399	TPD54_HUMAN	TPD54	Tumor protein D54	22238	105	7 of 20	Protooncogene	Unkown/Unspecified
Q99436	PSB7_HUMAN	PSB7	Proteasome subunit beta type 7 [Precursor], Multicatalytic endopeptidase complex chain Z	29965	63	7 of 21	Enzyme	Cytoplasm, Nucleus
P30837	AL1B1_HUMAN	AL1B1	Aldehyde dehydrogenase X, mitochondrial [Precursor]	57217	58	7 of 22	Enzyme	Cytoplasm, Mitochondrion
P63241	IF5A_HUMAN	IF5A	Eukaryotic translation initiation factor 5A	16701	104	7 of 22	RNA associated	Cytoplasm, Nucleus, Nucleus Envelope
O75347	TBCA_HUMAN	TBCA	Tubulin-specific chaperone A, CFA	12724	53	7 of 23	Chaperone	Cytoplasm, Cytoskeleton, Microtubule
Q567T7	Q567T7_HUMAN	Q567T7	Methionine adenosyltransferase II, beta, isoform 1	37480	75	7 of 25	Regulatory	Unkown/Unspecified
P25787	PSA2_HUMAN	PSA2	Proteasome subunit alpha type 2, EC 3.4.25.1	25767	72	7 of 29	Enzyme	Cytoplasm, Nucleus
P02794	FRIH_HUMAN	FRIH	Ferritin heavy chain	21094	87	7 of 30	Regulatory, Storage	Cytoplasm
Q9UBQ5	IF3C_HUMAN	IF3C	Eukaryotic translation initiation factor 3 subunit 12, Muscle-specific gene M9 protein	25060	78	7 of 34	RNA associated	Cytoplasm, Membrane
Q07021	MA32_HUMAN	MA32	Complement component 1, Q subcomponent-binding protein, mitochondrial [Precursor], p32	31362	56	7 of 40	Immunity/defense RNA associated, Signaling	Membrane, Cytoplasm, Cytosol, Mitochondrion, Nucleus
P07737	PROF1_HUMAN	PROF1	Profilin-1	14923	68	7 of 49	Regulatory	Cytoplasm, Mitochondrion
P09525	ANXA4_HUMAN	ANXA4	Annexin A4, Chromobindin-4	35752	102	8 of 14	Regulatory, Transport	Unkown/Unspecified
Q9NYL9	TMOD3_HUMAN	TMOD3	Tropomodulin-3	39595	109	8 of 16	Motor/ Contractile	Cytoplasm, Cytoskeleton
Q9Y2Z0	SUGT1_HUMAN	SUGT1	Suppressor of G2 allele of SKP1 homolog, Sgt1	40893	101	8 of 19	Regulatory, DNA associated	Nucleus, Chromosome, Kinetochore
P11177	ODPB_HUMAN	ODPB	Pyruvate dehydrogenase E1 component beta subunit, mitochondrial [Precursor], EC 1.2.4.1	39219	75	8 of 19	Enzyme	Cytoplasm, Mitochondrion
P13861	KAP2_HUMAN	KAP2	cAMP-dependent protein kinase type II-alpha regulatory subunit	45387	95	8 of 19	Regulatory	Cytoplasm
O14579	COPE_HUMAN	COPE	Coatomer subunit epsilon, Epsilon-coat protein	34351	74	8 of 20	Transport carrier	Cytoplasm, Golgi
Q5JUD2	Q5JUD2_HUMAN	Q5JUD2	OTTHUMP00000018608 [Fragment]	77059	60	8 of 21	Unknown/ Unspecified	Unkown/Unspecified
P67936	TPM4_HUMAN	TPM4	Tropomyosin alpha-4 chain, TM30p1	28391	94	8 of 22	Motor/contractile, Structural protein	Cytoplasm, Cytoskeleton, Microfilament
P37837	TALDO_HUMAN	TALDO	Transaldolase, EC 2.2.1.2	37540	68	8 of 23	Enzyme	Cytoplasm
P30740	ILEU_HUMAN	ILEU	Leukocyte elastase inhibitor, Serpin B1	42742	84	8 of 23	Enzyme,Regulatory	Cytoplasm

continued

Table I. *continued*

Accession No	Protein name	Protein symbol	Protein description	MW	Mascot score	Peptide matched	Function	Subcellular localisation
P60891	PRPS1_HUMAN	PRPS1	Ribose-phosphate pyrophosphokinase I, EC 2.7.6.1	34703	96	8 of 23	Enzyme	Unkown/Unspecified
P25789	PSA4_HUMAN	PSA4	Proteasome subunit alpha type 4, EC 3.4.25.1	29484	79	8 of 25	Enzyme	Cytoplasm, Nucleus
P82979	HCC1_HUMAN	HCC1	Nuclear protein Hcc-1, Proliferation-associated cytokine-inducible protein CIP29	23540	99	8 of 25	Unknown/ Unspecified	Nucleus
P55735	SEC13_HUMAN	SEC13	SEC13-related protein	35409	89	8 of 26	Transport carrier	Unkown/Unspecified
Q13907	IDI1_HUMAN	IDI1	Isopentenyl-diphosphate delta-isomerase 1, EC 5.3.3.2	26319	103	8 of 26	Enzyme	Cytoplasm, Peroxisome
Q96S43	Q96S43_HUMAN	Q96S43	JKTBP1delta6	27191	82	8 of 29	Unknown/ Unspecified	Unkown/Unspecified
P49720	PSB3_HUMAN	PSB3	Proteasome subunit beta type 3, EC 3.4.25.1	22949	58	8 of 31	Enzyme	Cytoplasm, Nucleus
P23246	SFPQ_HUMAN	SFPQ	Splicing factor, proline- and glutamine-rich, PSF	76149	84	8 of 32	RNA associated, Antioncogene	Nucleus, Nucleus Matrix
P06753	TPM3_HUMAN	TPM3	Tropomyosin alpha-3 chain, hTM5	32819	64	8 of 33	Motor/ Contractile	Cytoplasm, Cytoskeleton, Microfilament
P31153	METK2_HUMAN	METK2	S-adenosylmethionine synthetase isoform type-2, MAT-II	43661	76	8 of 33	Enzyme	Unkown/Unspecified
P52788	SPSY_HUMAN	SPSY	Spermine synthase, EC 2.5.1.22	41268	80	8 of 34	Enzyme	Unkown/Unspecified
Q9UKK9	NUDT5_HUMAN	NUDT5	ADP-sugar pyrophosphatase, Nudix motif 5	24328	89	8 of 35	Enzyme	Unkown/Unspecified
P20618	PSB1_HUMAN	PSB1	Proteasome subunit beta type 1, EC 3.4.25.1	26489	57	8 of 36	Enzyme	Cytoplasm, Nucleus
P22392	NDKB_HUMAN	NDKB	Nucleoside diphosphate kinase B, EC 2.7.4.6	17298	84	8 of 44	Enzyme	Cytoplasm, Nucleus
Q08379	GOGA2_HUMAN	GOGA2	Golgin subfamily A member 2, Gm130 autoantigen	111658	57	8 of 46	Transport	Cytoplasm, Golgi, Membrane
P28072	PSB6_HUMAN	PSB6	Proteasome subunit beta type 6 [Precursor], EC 3.4.25.	125358	57	8 of 62	Enzyme	Cytoplasm, Nucleus
P25705	ATPA_HUMAN	ATPA	ATP synthase alpha chain, mitochondrial [Precursor]	59751	106	9 of 11	Enzyme, Transport	Cytoplasm, Mitochondrion
O75489	NUGM_HUMAN	NUGM	NADH-ubiquinone oxidoreductase 30 kDa subunit, mitochondrial [Precursor], Complex I-30KD	30242	158	9 of 11	Enzyme	Cytoplasm, Mitochondrion
Q9BWD1	THIC_HUMAN	THIC	Acetyl-CoA acetyltransferase, cytosolic	41351	120	9 of 14	Enzyme, Regulatory	Cytoplasm, Cytosol
P52907	CAZA1_HUMAN	CAZA1	F-actin capping protein alpha-1 subunit	32923	124	9 of 15	Structural	Cytoplasm, Cytoskeleton
P07195	LDHB_HUMAN	LDHB	L-lactate dehydrogenase B chain	36507	78	9 of 18	Enzyme	Cytoplasm
Q96FW1	OTUB1_HUMAN	OTUB1	Ubiquitin thiolesterase protein OTUB1, Otubain 1	31284	127	9 of 18	Regulatory	Cytoplasm, Mitochondrion
P25325	THTM_HUMAN	THTM	3-mercaptopyruvate sulfurtransferase, MST	33047	115	9 of 23	Enzyme	Cytoplasm, Mitochondrion
P07910	HNRPC_HUMAN	HNRPC	Heterogeneous nuclear ribonucleoproteins C1/C2	33688	73	9 of 26	RNA associated	Cytoplasm, Nucleus, Ribonucleosomes
P30043	BLVRB_HUMAN	BLVRB	Flavin reductase, NADPH-dependent diaphorase	21988	94	9 of 26	Enzyme	Cytoplasm
P30520	PURA2_HUMAN	PURA2	Adenylosuccinate synthetase 2, IMP--aspartate ligase 2	50097	85	9 of 32	Enzyme	Cytoplasm
O75340	PDCD6_HUMAN	PDCD6	Programmed cell death protein 6	21868	100	9 of 32	Regulatory	Cytoplasm, Nucleus
Q00610	CLH1_HUMAN	CLH1	Clathrin heavy chain 1, CLH-17	191483	65	9 of 37	Binding	Membrane, Cytosol, Vesicle, Cytoplasm
Q8TD84	DSCL1_HUMAN	DSCL1	Down syndrome cell adhesion molecule-like protein 1 [Precursor]	224463	62	9 of 38	Regulatory	Membrane
P32119	PRDX2_HUMAN	PRDX2	Peroxiredoxin-2, TSA	21761	104	9 of 42	Enzyme	Cytoplasm
P52565	GDIR_HUMAN	GDIR	Rho GDP-dissociation inhibitor 1	23207	66	9 of 45	Regulatory, Signaling	Cytoplasm
Q04695	K1C17_HUMAN	K1C17	Keratin, type I cytoskeletal 17	47974	56	9 of 52	Structural	Cytoplasm, Cytoskeleton, Intermed Filament
Q9UN86	G3B2_HUMAN	G3B2	Ras-GTPase-activating protein binding protein 2	54111	77	9 of 64	Unknown/ Unspecified	Cytoplasm
P05787	K2C8_HUMAN	K2C8	Keratin, type II cytoskeletal 8	53543	143		Structural	Cytoplasm, Cytoskeleton, Intermed filament

PARK7, DJ-1, PSD10, OXRP, GAGD2 and TPD54. The SRC8 (EMS1) protooncogene encodes a human homologue of cortactin, a c-Src substrate associated with the cortical cytoskeleton (Q14247). This protein binds components of the actin-related protein (Arp) 2/3 complex which regulates the assembly and structure of actin networks. Cortactin also interacts with a variety of proteins depending on the cell type. The gene encoding SRC8, named as EMS1, is very often overexpressed and amplified in many tumors. Dysregulation may lead to increased tumor cell motility and invasiveness (22, 23). Protooncogene STRAP, a WD40 domain-containing protein (Q9Y3F4), synergizes with Smad7 and inhibits TGF-β which controls proliferation, differentiation, apoptosis, extracellular matrix production and other biological processes (24). FUBP1, a far upstream element binding protein1 (Q96AE), complexes with FUSE and inhibits c-myc expression which is also involved in cell growth, proliferation, differentiation and apoptosis (25). The location of the FUBP1 gene is in chromosome 1 p31.1, which is very often amplified in osteosarcomas (26). In addition, in this amplified chromosome area is located protooncogene PARK7 (DJ-1) (1p36.33-p36.12). DJ-1 protein has been shown (27) to be a potent inhibitor of the Daxx/ASK1 cell death signaling pathway, thus protecting cells from oxidative stress and functioning as a survival factor, hence promoting tumor growth.

Another protooncogene identified is nucleophosmin (NPM, P06748) which is present in actively proliferating cells including tumor cells. NPM is a multifunctional protein involved in ribosome assembly, pre-ribosomal RNA processing, DNA duplication, nucleocytoplasmic protein trafficking and centrosome duplication (28). It is induced by genotoxic stress, stabilizes certain conformers of p53, binds pRb and synergistically stimulates DNA polymerase α (29). In addition NPM protects cells from death and stress-induced apoptosis through inhibition of p53 (30, 31). Although Saos2 cells are p53 and pRb null, the existence of NPM gives evidence for another possible pathway by which cell survival may be maintained.

17 proteins with unknown/unspecified function and/or subcellular location were also identified. Two of them were hypothetical proteins (Q8IWE2, Q9NXD9) which merit further investigation.

Summarizing, in the present study the 2-DE database for the human osteosarcoma cell line Saos2 was created. Three hundred and forty-nine different gene products were identified using MALDI-MS and MALDI-MS-MS analysis of approximately 4,000 spots out of eight 2-DE gels. In that datadase ten oncogenes were included, while the function of 16 and the subcellular location of 43 of the identified proteins was unknown. Hopefully, this 2-DE database will form a useful tool in the study of molecular carcinogenesis and anticancer research.

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Received July 28, 2006
Accepted August 23, 2006