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# The Proteome Profile of the Human Osteosarcoma U2OS Cell Line

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**Abstract.** *The human osteosarcoma U2OS cell line is one of the first generated cell lines and is used in various areas of biomedical research. Knowledge of its protein expression is limited and no comprehensive study on the proteome of this cell line has been reported to date. Proteomics technology was used in order to analyse the proteins of the U2OS cell line. Total protein extracts were separated by two-dimensional gel electrophoresis (2-DE) and analysed by matrix-assisted laser desorption ionisation-mass spectrometry (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 3,000 spots were excised from two 2-DE gels and were analysed, resulting in the identification of 237 different gene products. The majority of the identified proteins were enzymes, regulatory proteins and RNA-associated proteins, while leukocyte markers and oncogenes were also present. Our findings include 11 protooncogenes (FKBP4, SRC8, PSD10, FUBP1, PARK7, NPM, PDIA1, OXRP, SET, TCTP and GRP75) related to the cancerous state of the U2OS cell line. The U2OS 2-DE database provides the basis for future protein studies.*

Human osteosarcoma is a primary malignant tumor of the bone affecting children and young adults (age 15-29 years), as well as adults in later life (age >60 years). It is the 6th most common type of cancer in children and appears during the second decade of life in the adolescent growth period, with a high incidence in teenage boys who are experiencing growth spurts. The cause of osteosarcoma is unknown; however irradiation, genetic influences and rapid bone growth are considered to be related to its development.

In order to further understand the molecular basis of this disease, we applied proteomics technology to the human osteosarcoma U2OS cell line. Proteomics technologies are powerful analytical tools and, in combination with other high throughput screening techniques, are widely used for the analysis of biological specimens. Protein analysis by two-dimensional gel electrophoresis (2-DE) coupled to mass spectrometry nowadays comprises the standard approach for the construction of comprehensive protein databases (1). Protein identification is greatly enhanced and improved by robotic systems and automated mass spectra acquisition devices, which allow thorough analysis of biological specimens such as cell lines.

Proteome databases are primarily used to illuminate normal and disease states, and the information acquired can be used in diagnosis and treatment, and in biomarker discovery (2). Cancer cell lines are generally used for proteomic analysis since they exhibit equivalent changes to the tissue they derive from. Hence the screening of the proteome of some cancer cell lines has been performed, *e.g.* human immature T-cell line CCRF-CEM, HeLa cell line and osteosarcoma Saos2 cell line (3-5).

The human osteosarcoma U2OS cell line was derived in 1964 from a moderately differentiated sarcoma of the tibia of a 15-year-old girl. It is one of the first generated cell lines and is used very frequently. Spectral karyotyping analysis and cytogenetic analysis has revealed chromosomal instability, structural rearrangements and alterations and high incidence of aneuploidy (6). Spectral analysis indicated the near-triploidy state of U2OS cells which appeared to be a combination of tetraploidization and chromosomal losses. Other rearrangements mainly involved chromosomes 20 and 8, from simple translocations to highly complex rearrangements, but

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

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also to a less extent chromosome 17. Most of the centromeric breakpoints occurred in chromosomes 6, 13 and 17, but at least four in chromosomes 2, 4, 5, 8, 19 and 21.

Two tumor suppressive genes, *p53* and *pRb*, are functional in U2OS cells, whereas in other, more aggressive, osteosarcoma cell lines such as Saos2 these genes are mutated (7-9). Analysis of other oncogenes and tumor suppressor genes signalized 5 times amplification of *c-myc* with no apparent serum stimulations; additionally *c-fos* had no visible alterations. Compared with other osteosarcoma cell lines, U2OS cells have the lowest level of chromosomal numerical variations and only 2% of the cells have multipolar mitoses, similar to normal control fibroblasts, probably due to functional *p53* and *pRb* (9).

The U2OS cell line is widely used in biomedical research such as biochemistry, molecular biology, bone formation, arthritis (10-14). In this study, the proteome profile of U2OS cells was constructed, consisting of 237 single gene products.

## 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 supplemented with 5% $CO_2$.

*Two-dimensional gel electrophoresis.* U2OS 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, 2M thiourea, 4% CHAPS, 10 mM 1,4-dithioerythritol, 1 mM EDTA and a mixture of protease inhibitors [1 mM PhenylMethaneSulphonylFluoride (PMSF) and 1 tablet Complete™ (Roche Diagnostics, Basel, Swiss) per 50 ml of suspension buffer] and phosphate inhibitors (0.2 mM $Na_2VO_3$ and 1 mM NaF). The cells were lysed by sonication at 3x60 s in 35% amplification and the suspension was centrifuged at 13000xg for 30 min at 4˚C. The protein content of the supernatant was determined using the EXPERION Automated Electrophoresis Station (BioRad) in combination with Protein 260 Analysis Kit™ (Biorad) according to the manufacturer's instructions.

2D-Gel electrophoresis was performed as reported elsewhere (4). Total protein (1 mg) was applied to immobilized pH 3-10 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 8000 V at 3 V/min and remained constant for a further 22 h. The second-dimensional separation was performed in 12% SDS-polyacrylamide gels (180x200x1.5 mm), running at 40 mA per gel in a PROTEAN apparatus (Biorad). After fixation with 50% methanol, containing 10% acetic acid for at least 2 h, the gels were stained overnight with colloidal Coomassie blue (Novex, San Diego, CA, USA), washed twice with water 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 described elsewhere (15). Spots were automatically detected by Melanie 4.02 (GeneBio, Geneve Bioinformatics S.A., Geneva, Swiss) 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 resulting peptide mixture (1.5 μl) was simultaneously applied with 1 μl of matrix solution, consisting of 0.025% α-cyano-4-hydroxycinnamic acid (Sigma-Aldrich, USA), standard peptides *des*-Arg-bradykinin, (904.4681 Da; Sigma-Aldrich, USA) and adrenocorticotropic hormone fragment 18-39, (2465.1989 Da; Sigma-Aldrich, USA) in 65% ethanol, 35% acetonitrile and 0.03% trifluoroacetic acid. Samples were analyzed for PMF with matrix-assisted laser desorption-mass spectrometry (MALDI-MS) in a time-of-flight mass spectrometer (Ultraflex II, Bruker Daltonics, Bremen, Germany). Matching peptide and protein searches were performed automatically, as described by Berndt *et al.* (15). 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 (ftp://ftp.expasy.org/ databases/uniprot/knowledgebase), IPI (ftp://ftp.ebi.ac.uk/pub/databases/IPI/current/), and MSDB (ftp://ftp.ebi.ac.uk/pub/databases/MassSpecDB) databases which are updated bimonthly. 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 post-source decay (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 U2OS cell line was separated by 2-DE electrophoresis on IPG strips and the spots were visualized with colloidal Coomassie blue staining. In Figure 1, a representative example is shown of the proteins present in U2OS cells as separated on a pH 3-10 NL gel. Four pH 3-10 NL IPG gels were analyzed and

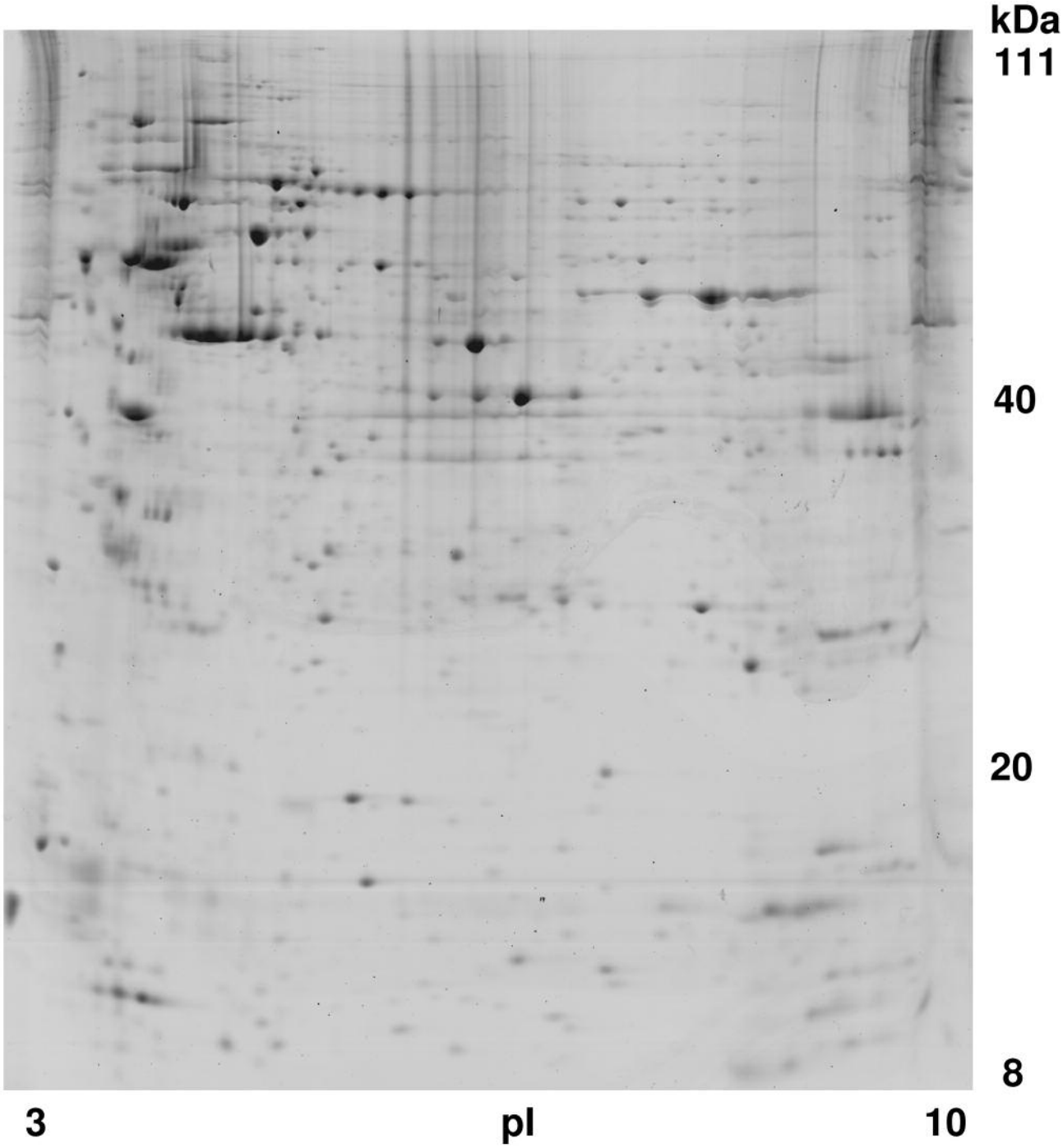


Figure 1. *Two-dimensional gel analysis of total protein extract from U2OS cells. Proteins were extracted and separated on IPG strip pH 3-10 non-linearly, followed by a 12% SDS-polyacrylamide gel. The gel was stained with Coomassie blue.*

approximately 2,500 spots were detected using the Melanie 4.02 software. Those spots were excised from the pH 3-10 gel and analyzed for protein identification following in-gel digestion with trypsin. Each spot was analyzed for PMF with MALDI-MS in a time-of-flight mass spectrometer and proteins were identified automatically by the peptide mass matching. Proteins not identified by PMF were subsequently selected for PSD-MS-MS and analyzed with MALDI-MS-MS. Using an internal peptide standard to correct the measured peptide masses, we were able to use very narrow windows of

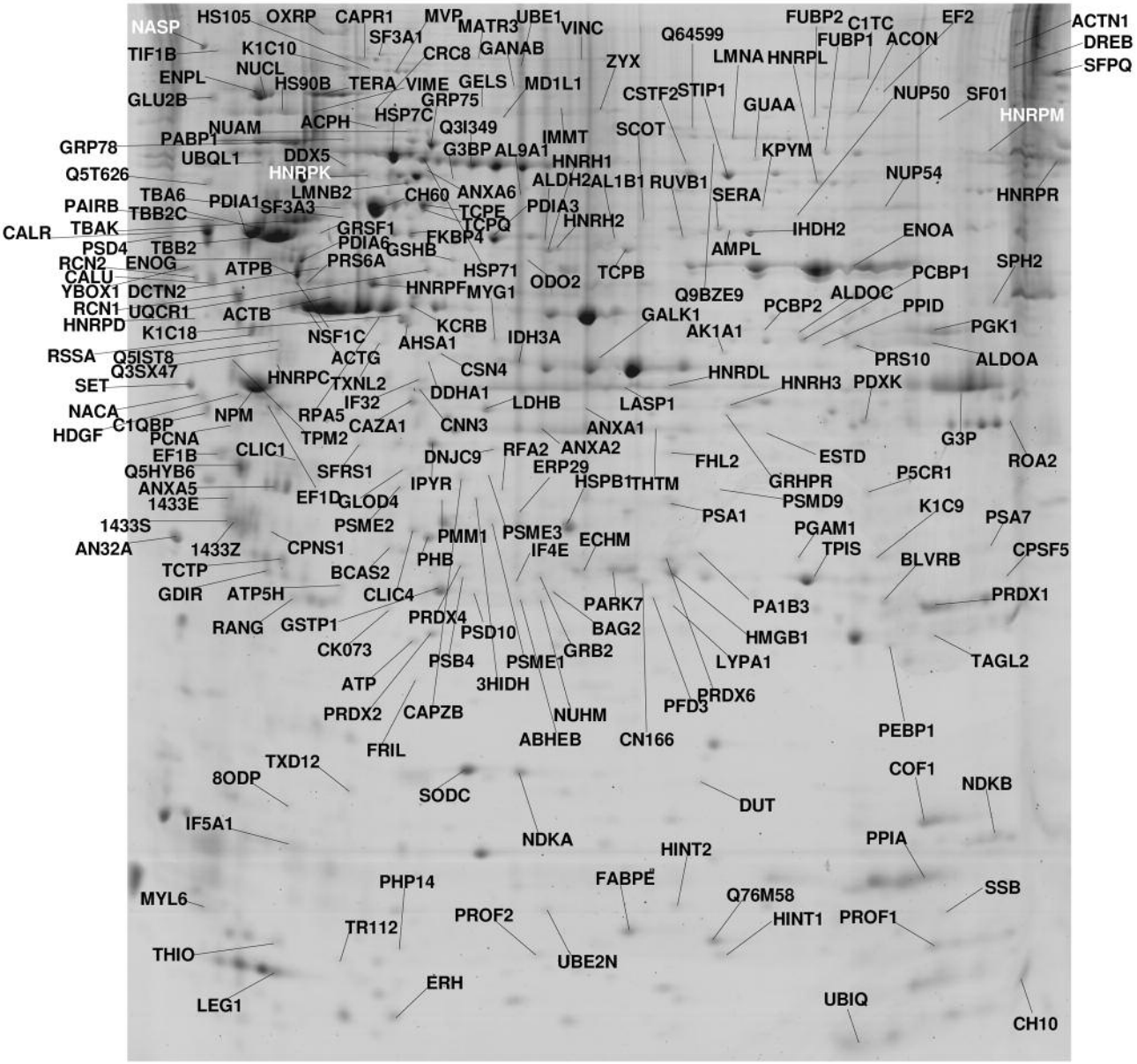


Figure 2. *Identification of protein spots present in the gel of Figure 1, analyzed by PMF and/or PSD-MS-MS. The identified proteins are annotated by their abbreviated names and are listed in Table I.*

mass tolerance (0.0025%) and hence, increase the confidence of identification, as well as the total identification rate up to 85%. This resulted in the identification of 237 different gene products which are annotated on the gel shown in Figure 2.

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. Furthermore, information on the subcellular localization and function of these proteins as given in publicly accessible databases are shown.

From the 2,500 spots analyzed 673 spots were identified resulting in 237 different gene products. Thus the most abundant proteins present in U2OS cells were 62 members of the heat shock protein family, 34 members of the actin family, 19 members of the lamin family, 18 members of the tubulin family and 16 members of the annexin family of proteins.

*Subcellular localization.* For 5% of the identified proteins, no data were found regarding their subcellular localization. Most of the proteins are cytoplasmic (37%), nuclear (27%), mitochondrial (12%) and endoplasmic reticular (8%).

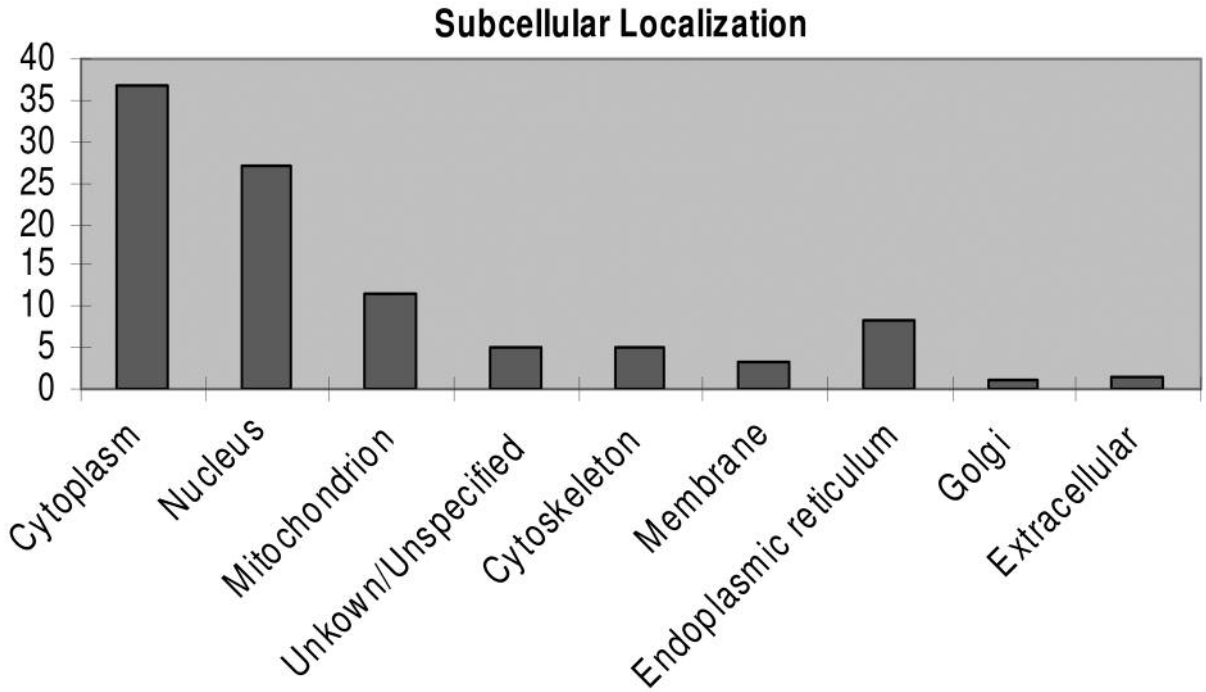


Figure 3. *Subcellular localization of the human osteosarcoma U2OS cell line proteins. The proteins in Table I were categorized according to their localization in the cells.*

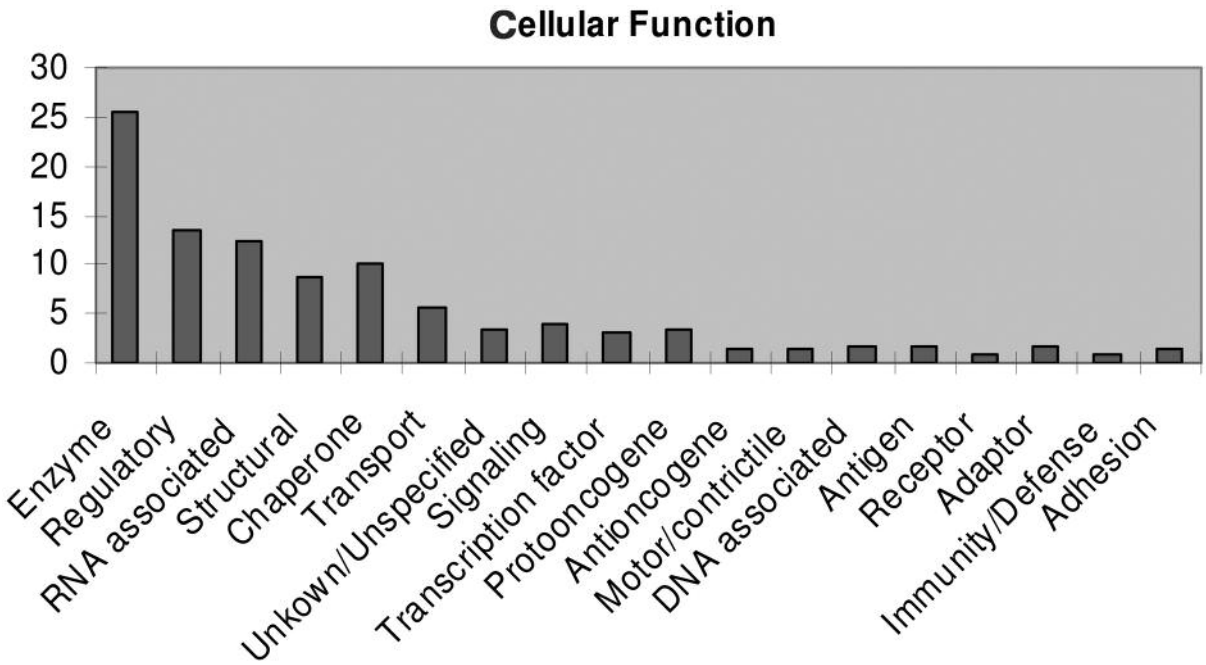


Figure 4. *Classification of the proteins present in U2OS cells into functional groups. Protein molecules and their function are given in Table I.*

Cytoskeletal proteins constitute 5% of the proteome and 3% are membranic proteins. The subcellular localization is also presented in Figure 3.

*Function.* As expected, most of the proteins are enzymes (25%), regulatory (13%) and RNA associated proteins (12%) as seen in Figure 4. There are chaperone/stress

Table I. *Proteins from the human osteosarcoma U2OS cell line 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, 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.*

| Acession no | Protein name | Protein symbol | Protein description | MW | Mascot score | Peptides matched | Cellular function | Subcellular localization |
|---|---|---|---|---|---|---|---|---|
| O00299 | CLIC1_HUMAN | CLIC1 | Chloride intracellular channel protein 1, NCC27 | 26792 | 141 | 11 | Transport Channel | Membranic, Nuclear membrane |
| P30086 | PEBP1_HUMAN | PEBP1 | Phosphatidylethanolamine-binding protein 1, Raf kinase inhibitor protein | 21057 | 94 | 9 | Antioncogene | Cytoplasmic |
| P08238 | HS90B_HUMAN | HS90B | Heat-shock protein HSP 90-beta | 83264 | 223 | 30 | Chaperone/Stress | Cytoplasmic |
| P48643 | TCPE_HUMAN | TCPE | T-complex protein 1 subunit epsilon | 59671 | 180 | 34 | Chaperone/Stress | Cytoplasmic |
| P50990 | TCPQ_HUMAN | TCPQ | T-complex protein 1 subunit theta, TCP-1-theta | 59621 | 163 | 27 | Chaperone/Stress | Cytoplasmic |
| P78371 | TCPB_HUMAN | TCPB | T-complex protein 1 subunit beta, CCT-beta | 57488 | 284 | 20 | Chaperone/Stress | Cytoplasmic |
| Q02790 | FKBP4_HUMAN | FKBP4 | FK506-binding protein 4 | 51805 | 282 | 33 | Chaperone/Stress, Enzyme, Protooncogene, Regulatory | |
| P61289 | PSME3_HUMAN | PSME3 | Proteasome activator complex subunit 3, Ki nuclear autoantigen, PA28g | 29506 | 81 | 10 | Chaperone/stress, | Cytoplasmic Regulatory |
| Q06323 | PSME1_HUMAN | PSME1 | Proteasome activator complex subunit 1, PA28alpha | 28723 | 86 | 9 | Chaperone/stress, Regulatory | Cytoplasmic |
| Q9UL46 | PSME2_HUMAN | PSME2 | Proteasome activator complex subunit 2, PA28beta | 27362 | 94 | 9 | Chaperone/stress, Regulatory | Cytoplasmic |
| O00764 | PDXK_HUMAN | PDXK | Pyridoxal kinase | 35102 | 60 | 5 | Enzyme | Cytoplasmic |
| O75608 | LYPA1_HUMAN | LYPA1 | Acyl-protein thioesterase 1 | 24670 | 58 | 4 | Enzyme | Cytoplasmic |
| P00558 | PGK1_HUMAN | PGK1 | Phosphoglycerate kinase 1 | 44615 | 160 | 18 | Enzyme | Cytoplasmic |
| P04075 | ALDOA_HUMAN | ALDOA | Fructose-bisphosphate aldolase A | 39420 | 107 | 14 | Enzyme | Cytoplasmic |
| P07195 | LDHB_HUMAN | LDHB | L-lactate dehydrogenase B chain | 36638 | 130 | 14 | Enzyme | Cytoplasmic |
| P07741 | APT_HUMAN | ATP | Adenine phosphoribosyltransferase | 19608 | 137 | 12 | Enzyme | Cytoplasmic |
| P09211 | GSTP1_HUMAN | GSTP1 | Glutathione S-transferase P | 23356 | 113 | 12 | Enzyme | Cytoplasmic |
| P09972 | ALDOC_HUMAN | ALDOC | Fructose-bisphosphate aldolase C | 39456 | 111 | 12 | Enzyme | Cytoplasmic |
| P10599 | THIO_HUMAN | THIO | Thioredoxin | 11737 | 70 | 6 | Enzyme | Cytoplasmic |
| P10768 | ESTD_HUMAN | ESTD | S-formylglutathione hydrolase, Esterase D | 31463 | 72 | 6 | Enzyme | Cytoplasmic |
| P11586 | C1TC_HUMAN | C1TC | C-1-tetrahydrofolate synthase, cytoplasmic | 101559 | 151 | 19 | Enzyme | Cytoplasmic |
| P12268 | IMDH2_HUMAN | IHDH2 | Inosine-5'-monophosphate dehydrogenase 2 | 55805 | 168 | 13 | Enzyme | Cytoplasmic |
| P12277 | KCRB_HUMAN | KCRB | Creatine kinase B-type | 42644 | 88 | 10 | Enzyme | Cytoplasmic |
| P13798 | ACPH_HUMAN | ACPH | Acylamino-acid-releasing enzyme, Acyl-peptide hydrolase | 81225 | 89 | 15 | Enzyme | Cytoplasmic |
| P14550 | AK1A1_HUMAN | AK1A1 | Alcohol dehydrogenase [NADP+] | 36573 | 92 | 10 | Enzyme | Cytoplasmic |
| P14618 | KPYM_HUMAN | KPYM | Pyruvate kinase isozymes M1/M2 | 57937 | 128 | 14 | Enzyme | Cytoplasmic |
| P18669 | PGAM1_HUMAN | PGAM1 | Phosphoglycerate mutase 1, Phosphoglycerate mutase isozyme B | 28804 | 103 | 10 | Enzyme | Cytoplasmic |
| P28838 | AMPL_HUMAN | AMPL | Cytosol aminopeptidase | 56166 | 132 | 13 | Enzyme | Cytoplasmic |
| P30043 | BLVRB_HUMAN | BLVRB | Flavin reductase, Biliverdin reductase B | 22119 | 88 | 9 | Enzyme | Cytoplasmic |
| P32119 | PRDX2_HUMAN | PRDX2 | Peroxiredoxin-2 | 21892 | 109 | 7 | Enzyme | Cytoplasmic |
| P49189 | AL9A1_HUMAN | AL9A1 | 4-trimethylaminobutyraldehyde dehydrogenase, Gamma-aminobutyraldehyde dehydrogenase | 53802 | 66 | 7 | Enzyme | Cytoplasmic |
| P49915 | GUAA_HUMAN | GUAA | GMP synthase [glutamine-hydrolyzing] | 76715 | 265 | 25 | Enzyme | Cytoplasmic |
| P51570 | GALK1_HUMAN | GALK1 | Galactokinase | 42272 | 74 | 6 | Enzyme | Cytoplasmic |

Table I. *continued*

Table I. *continued*

| Acession no | Protein name | Protein symbol | Protein description | MW | Mascot score | Peptides matched | Cellular function | Subcellular localization |
|---|---|---|---|---|---|---|---|---|
| P55036 | PSD4_HUMAN | PSD4 | 26S proteasome non-ATPase regulatory subunit 4 | 40737 | 87 | 10 | Enzyme | Cytoplasmic |
| Q06830 | PRDX1_HUMAN | PRDX1 | Peroxiredoxin-1 | 22110 | 201 | 20 | Enzyme | Cytoplasmic |
| Q13162 | PRDX4_HUMAN | PRDX4 | Peroxiredoxin-4 | 30540 | 105 | 9 | Enzyme | Cytoplasmic |
| Q15102 | PA1B3_HUMAN | PA1B3 | Platelet-activating factor acetylhydrolase IB subunit gamma | 25734 | 63 | 7 | Enzyme | Cytoplasmic |
| Q15181 | IPYR_HUMAN | IPYR | Inorganic pyrophosphatase | 32660 | 180 | 19 | Enzyme | Cytoplasmic |
| Q92871 | PMM1_HUMAN | PMM1 | Phosphomannomutase 1, PMM 1 | 29747 | 66 | 5 | Enzyme | Cytoplasmic |
| Q9BX68 | HINT2_HUMAN | HINT2 | Histidine triad nucleotide-binding protein 2, HINT-2 | 17162 | 69 | 4 | Enzyme | Cytoplasmic |
| P04632 | CPNS1_HUMAN | CPNS1 | Calpain small subunit 1, Calcium-dependent protease small subunit 1 | 28316 | 56 | 5 | Enzyme, Regulatory | Cytoplasmic |
| P30041 | PRDX6_HUMAN | PRDX6 | Peroxiredoxin-6, 1-Cys peroxiredoxin, Antioxidant protein 2 | 25035 | 191 | 17 | Enzyme, Regulatory | Cytoplasmic |
| P06733 | ENOA_HUMAN | ENOA | Alpha-enolase, C-myc promoter-binding protein, MBP-1 | 47169 | 211 | 20 | Enzyme, Structural, Antigen, Antioncogene | Cytoplasmic |
| Q14247 | SRC8_HUMAN | SRC8 | Src substrate cortactin, Oncogene EMS1 | 61636 | 75 | 11 | Protooncogene | Cytoplasmic |
| O76003 | TXNL2_HUMAN | TXNL2 | Thioredoxin-like protein 2, PKC-theta-interacting protein | 37432 | 90 | 11 | Regulatory | Cytoplasmic |
| P31947 | 1433S_HUMAN | 1433S | 14-3-3 protein sigma | 27774 | 137 | 19 | Regulatory | Cytoplasmic |
| P35080 | PROF2_HUMAN | PROF2 | Profilin-2 | 15046 | 56 | 4 | Regulatory | Cytoplasmic |
| P63104 | 1433Z_HUMAN | 1433Z | 14-3-3 protein zeta/delta | 27745 | 122 | 18 | Regulatory | Cytoplasmic |
| Q9BT78 | CSN4_HUMAN | CSN4 | COP9 signalosome complex subunit 4 | 46269 | 107 | 12 | Regulatory | Cytoplasmic |
| Q9UMX0 | UBQL1_HUMAN | UBQL1 | Ubiquilin-1, hPLIC-1 | 62519 | 77 | 14 | Regulatory | Cytoplasmic |
| P52565 | GDIR_HUMAN | GDIR | Rho GDP-dissociation inhibitor 1, Rho GDI 1 | 23207 | 74 | 9 | Regulatory, Signaling | Cytoplasmic |
| Q9Y696 | CLIC4_HUMAN | CLIC4 | Chloride intracellular channel protein 4, Intracellular chloride ion channel protein p64H1 | 28772 | 145 | 16 | Regulatory, Transport channel | Cytoplasmic |
| P13639 | EF2_HUMAN | EF2 | Elongation factor 2 | 95338 | 84 | 12 | RNA associated | Cytoplasmic |
| P62988 | UBIQ_HUMAN | UBIQ | Ubiquitin | 8565 | 75 | 5 | RNA associated | Cytoplasmic |
| Q12849 | GRSF1_HUMAN | GRSF1 | G-rich sequence factor 1, GRSF-1 | 50170 | 72 | 10 | RNA associated | Cytoplasmic |
| Q3SX47 | Q3SX47_BOVIN | Q3SX47 | Heterogeneous nuclear ribonucleoprotein C | 32428 | 66 | 9 | RNA associated | Cytoplasmic |
| Q76M58 | Q76M58_HUMAN | Q76M58 | 40S ribosomal protein S12 | 14515 | 102 | 10 | RNA associated | Cytoplasmic |
| Q13347 | IF32_HUMAN | IF32 | Eukaryotic translation initiation factor 3 subunit 2, eIF3i, TRIP-1 | 36502 | 133 | 12 | RNA associated, Antioncogene | Cytoplasmic |
| Q5IST8 | Q5IST8_MACFA | Q5IST8 | Heterogeneous nuclear ribonucleoprotein C [Fragment] | 29266 | 65 | 8 | RNA associated, DNA associated | Cytoplasmic |
| P02792 | FRIL_HUMAN | FRIL | Ferritin light chain | 20020 | 97 | 7 | Storage | Cytoplasmic |
| P07437 | TBB2_HUMAN | TBB2 | Tubulin beta chain | 49671 | 238 | 37 | Structural | Cytoplasmic |
| P12814 | ACTN1_HUMAN | ACTN1 | Alpha-actinin-1 | 103058 | 59 | 16 | Structural | Cytoplasmic |
| P63261 | ACTG_HUMAN | ACTG | Actin, cytoplasmic 2, Gamma-actin | 41793 | 172 | 21 | Structural | Cytoplasmic |
| Q16643 | DREB_HUMAN | DREB | Drebrin | 71425 | 92 | 15 | Structural | Cytoplasmic |
| Q01469 | FABPE_HUMAN | FABPE | Fatty acid-binding protein, epidermal, Psoriasis-associated fatty acid-binding protein homolog | 15164 | 154 | 18 | Transport Carrier | Cytoplasmic |
| Q53FT3 | CK073_HUMAN | CK073 | Uncharacterized protein C11orf73 | 21628 | 57 | 5 | Unknown/Unspecified | Cytoplasmic |
| Q9BZE9 | Q9BZE9_HUMAN | Q9BZE9 | Tether containing UBX domain for GLUT4, Alveolar soft part sarcoma locus | 60183 | 86 | 10 | Unknown/Unspecified | Cytoplasmic |

Table I. *continued*

Table I. *continued*

| Acession no | Protein name | Protein symbol | Protein description | MW | Mascot score | Peptides matched | Cellular function | Subcellular localization |
|---|---|---|---|---|---|---|---|---|
| P09104 | ENOG_HUMAN | ENOG | Gamma-enolase | 47269 | 106 | 11 | Enzyme | Cytoplasmic, Cell membrane |
| O94760 | DDAH1_HUMAN | DDAH1 | NG,NG-dimethylarginine dimethylaminohydrolase 1, DDAH-1 | 31122 | 72 | 8 | Enzyme | Cytoplasmic, Cytosolic |
| O95433 | AHSA1_HUMAN | AHSA1 | Activator of 90 kDa heat shock protein ATPase homolog 1, p38, AHA1 | 38274 | 59 | 7 | Chaperone/stress, Regulatory | Cytoplasmic, Endoplasmic reticulum |
| O75832 | PSD10_HUMAN | PSD10 | 26S proteasome non-ATPase regulatory subunit 10, Gankyrin | 24428 | 103 | 8 | Regulatory, Protooncogene | Cytoplasmic, Extracellular |
| P08758 | ANXA5_HUMAN | ANXA5 | Annexin A5 | 35937 | 221 | 18 | Regulatory, Transport channel | Cytoplasmic, Membranic |
| P23528 | COF1_HUMAN | COF1 | Cofilin-1, p18 | 18502 | 128 | 12 | Structural | Cytoplasmic, Mitochondrial, Nuclear |
| Q14847 | LASP1_HUMAN | LASP1 | LIM and SH3 domain protein 1, LASP-1 | 29717 | 82 | 13 | Adaptor | Cytoplasmic, Nuclear |
| P31948 | STIP1_HUMAN | STIP1 | Stress-induced-phosphoprotein 1, STI1, Hsc70/Hsp90-organizing protein | 62639 | 264 | 43 | Adaptor, Chaperone/Stress | Cytoplasmic, Nuclear |
| Q14192 | FHL2_HUMAN | FHL2 | Four and a half LIM domains protein 2, Skeletal muscle LIM-protein 3 | 32193 | 93 | 8 | Adaptor, Regulator, Signaling | Cytoplasmic, Nuclear |
| P04792 | HSPB1_HUMAN | HSPB1 | Heat-shock protein beta-1, Heat-shock 27 kDa protein | 22783 | 120 | 13 | Chaperone/stress | Cytoplasmic, Nuclear |
| P11142 | HSP7C_HUMAN | HSP7C | Heat-shock cognate 71 kDa protein | 70898 | 247 | 35 | Chaperone/Stress | Cytoplasmic, Nuclear |
| P49321 | NASP_HUMAN | NASP | Nuclear autoantigenic sperm protein, NASP | 85238 | 144 | 23 | Chaperone/Stress | Cytoplasmic, Nuclear |
| P61758 | PFD3_HUMAN | PFD3 | Prefoldin subunit 3, Von Hippel-Lindau-binding protein 1, VBP-1 | 22658 | 74 | 6 | Chaperone/stress | Cytoplasmic, Nuclear |
| P62333 | PRS10_HUMAN | PRS10 | 26S protease regulatory subunit S10B | 44173 | 94 | 12 | Chaperone/Stress, Enzyme | Cytoplasmic, Nuclear |
| P17980 | PRS6A_HUMAN | PRS6A | 26S protease regulatory subunit 6A, Tat-binding protein 1 | 49204 | 56 | 14 | Chaperone/Stress, Transcription factor | Cytoplasmic, Nuclear |
| O14818 | PSA7_HUMAN | PSA7 | Proteasome subunit alpha type 7 | 27887 | 73 | 7 | Enzyme | Cytoplasmic, Nuclear |
| P04406 | G3P_HUMAN | G3P | Glyceraldehyde-3-phosphate dehydrogenase, GAPDH | 36053 | 149 | 15 | Enzyme | Cytoplasmic, Nuclear |
| P22392 | NDKB_HUMAN | NDKB | Nucleoside diphosphate kinase B | 17298 | 97 | 12 | Enzyme | Cytoplasmic, Nuclear |
| P25786 | PSA1_HUMAN | PSA1 | Proteasome subunit alpha type 1 | 29556 | 75 | 10 | Enzyme | Cytoplasmic, Nuclear |
| P28070 | PSB4_HUMAN | PSB4 | Proteasome subunit beta type 4 [Precursor] | 29204 | 69 | 7 | Enzyme | Cytoplasmic, Nuclear |
| P48637 | GSHB_HUMAN | GSHB | Glutathione synthetase | 52385 | 75 | 9 | Enzyme | Cytoplasmic, Nuclear |
| Q9Y265 | RUVB1_HUMAN | RUVB1 | RuvB-like 1, TIP49a | 50228 | 104 | 15 | Enzyme, DNA associated, Antigen | Cytoplasmic, Nuclear |
| Q96AE4 | FUBP1_HUMAN | FUBP1 | Far upstream element-binding protein 1, FUSE-binding protein 1, FBP | 67560 | 210 | 26 | Protooncogene | Cytoplasmic, Nuclear |
| Q99497 | PARK7_HUMAN | PARK7 | Protein DJ-1, Oncogene DJ1 | 19891 | 134 | 19 | Protooncogene, Signaling neurotransmitter | Cytoplasmic, Nuclear |
| Q92945 | FUBP2_HUMAN | FUBP2 | Far upstream element-binding protein 2, KSRP, FUSE-binding protein 2 | 72709 | 135 | 15 | Regulatory, RNA associated | Cytoplasmic, Nuclear |
| Q15637 | SF01_HUMAN | SF01 | Splicing factor 1, Transcription factor ZFM1 | 68330 | 61 | 7 | Regulatory, Transcription factor | Cytoplasmic, Nuclear |
| O43390 | HNRPR_HUMAN | HNRPR | Heterogeneous nuclear ribonucleoprotein R | 70943 | 61 | 17 | RNA associated | Cytoplasmic, Nuclear |

Table I. *continued*

Table I. *continued*

| Acession no | Protein name | Protein symbol | Protein description | MW | Mascot score | Peptides matched | Cellular function | Subcellular localization |
|---|---|---|---|---|---|---|---|---|
| P07910 | HNRPC_HUMAN | HNRPC | Heterogeneous nuclear ribonucleoproteins C1/C2 | 33670 | 60 | 8 | RNA associated | Cytoplasmic, Nuclear |
| P14866 | HNRPL_HUMAN | HNRPL | Heterogeneous nuclear ribonucleoprotein L | 60187 | 174 | 20 | RNA associated | Cytoplasmic, Nuclear |
| P52272 | HNRPM_HUMAN | HNRPM | Heterogeneous nuclear ribonucleoprotein M, hnRNP M | 77516 | 108 | 30 | RNA associated | Cytoplasmic, Nuclear |
| P63241 | IF5A1_HUMAN | IF5A1 | Eukaryotic translation initiation factor 5A-1, eIF-5A-1 | 16832 | 77 | 7 | RNA associated | Cytoplasmic, Nuclear |
| Q14103 | HNRPD_HUMAN | HNRPD | Heterogeneous nuclear ribonucleoprotein D0, AUF1 | 38434 | 62 | 5 | RNA associated | Cytoplasmic, Nuclear |
| Q15365 | PCBP1_HUMAN | PCBP1 | Poly(rC)-binding protein 1, Alpha-CP1 | 37498 | 133 | 15 | RNA associated | Cytoplasmic, Nuclear |
| Q15366 | PCBP2_HUMAN | PCBP2 | Poly(rC)-binding protein 2, Alpha-CP2 | 38580 | 78 | 6 | RNA associated | Cytoplasmic, Nuclear |
| Q8NC51 | PAIRB_HUMAN | PAIRB | Plasminogen activator inhibitor 1 RNA-binding protein | 44965 | 99 | 16 | RNA associated | Cytoplasmic, Nuclear |
| P11940 | PABP1_HUMAN | PABP1 | Polyadenylate-binding protein 1 | 70671 | 108 | 15 | RNA associated, Receptor | Cytoplasmic, Nuclear |
| P19338 | NUCL_HUMAN | NUCL | Nucleolin | 76614 | 142 | 22 | RNA associated, Transcription factor | Cytoplasmic, Nuclear |
| P39687 | AN32A_HUMAN | AN32A | Acidic leucine-rich nuclear phosphoprotein 32 family member A, Mapmodulin, Acidic nuclear phosphoprotein pp32 | 28585 | 70 | 7 | Signaling | Cytoplasmic, Nuclear |
| P51858 | HDGF_HUMAN | HDGF | Hepatoma-derived growth factor | 26788 | 87 | 10 | Signaling growth factor | Cytoplasmic, Nuclear |
| Q14764 | MVP_HUMAN | MVP | Major vault protein | 99327 | 150 | 24 | Structural, RNA associated, Transport | Cytoplasmic, Nuclear |
| Q96IU4 | ABHEB_HUMAN | ABHEB | Abhydrolase domain-containing protein 14B | 22346 | 68 | 5 | Unknown/Unspecified | Cytoplasmic, Nuclear |
| O00233 | PSMD9_HUMAN | PSMD9 | 26S proteasome non-ATPase regulatory subunit 9 | 24654 | 81 | 10 | Enzyme | Cytoplasmic, Proteasome regulatory particle |
| P08865 | RSSA_HUMAN | RSSA | 40S ribosomal protein SA, 34/67 kDa laminin receptor | 32854 | 118 | 12 | Adhesion, Signaling, Receptor | Cytoplasmic, Ribosomal |
| P06396 | GELS_HUMAN | GELS | Gelsolin [Precursor], ADF | 85698 | 63 | 7 | Regulatory, Structural | Cytoplasmic, Secreted |
| P18206 | VINC_HUMAN | VINC | Vinculin | 123799 | 70 | 19 | Adhesion | Cytoskeletal |
| P07951 | TPM2_HUMAN | TPM2 | Tropomyosin beta chain | 32851 | 88 | 12 | Motor/Contractile | Cytoskeletal |
| P60709 | ACTB_HUMAN | ACTB | Actin, cytoplasmic 1 | 41737 | 133 | 22 | Motor/Contractile, Structural | Cytoskeletal |
| P05783 | K1C18_HUMAN | K1C18 | Keratin, type I cytoskeletal 18, Cytokeratin-18 | 48058 | 110 | 19 | Structural | Cytoskeletal |
| P08670 | VIME_HUMAN | VIME | Vimentin | 53652 | 334 | 39 | Structural | Cytoskeletal |
| P13645 | K1C10_HUMAN | K1C10 | Keratin, type I cytoskeletal 10 | 59519 | 67 | 12 | Structural | Cytoskeletal |
| P35527 | K1C9_HUMAN | K1C9 | Keratin, type I cytoskeletal 9, Keratin-9 | 62129 | 69 | 7 | Structural | Cytoskeletal |
| P47756 | CAPZB_HUMAN | CAPZB | F-actin capping protein subunit beta | 31350 | 103 | 10 | Structural | Cytoskeletal |
| P52907 | CAZA1_HUMAN | CAZA1 | F-actin capping protein subunit alpha-1 | 32923 | 147 | 11 | Structural | Cytoskeletal |
| Q15417 | CNN3_HUMAN | CNN3 | Calponin-3 | 36414 | 120 | 18 | Structural | Cytoskeletal |
| P06748 | NPM_HUMAN | NPM | Nucleophosmin | 32575 | 74 | 12 | Protooncogene | Cytoskeletal, Centrosome, Nuclear |
| Q13561 | DCTN2_HUMAN | DCTN2 | Dynactin subunit 2 | 44231 | 69 | 8 | Motor/Contractile, Transport | Cytoskeletal, Nuclear |
| P49773 | HINT1_HUMAN | HINT1 | Histidine triad nucleotide-binding protein 1, Protein kinase C inhibitor 1 | 13802 | 76 | 5 | Regulatory | Cytoskeletal, Nuclear |
| Q9Y6D9 | MD1L1_HUMAN | MD1L1 | Mitotic spindle assembly checkpoint protein MAD1 | 83067 | 72 | 15 | Regulatory, Antioncogene | Cytoskeletal, Nuclear |
| Q03252 | LMNB2_HUMAN | LMNB2 | Lamin-B2 | 67689 | 181 | 23 | Structural | Cytoskeletal, Nuclear |

Table I. *continued*

Table I. *continued*

| Acession no | Protein name | Protein symbol | Protein description | MW | Mascot score | Peptides matched | Cellular function | Subcellular localization |
|---|---|---|---|---|---|---|---|---|
| Q15084 | PDIA6_HUMAN | PDIA6 | Protein disulfide-isomerase A6 [Precursor] | 48121 | 136 | 17 | Chaperone/Stress | Endoplasmic reticulum |
| P14625 | ENPL_HUMAN | ENPL | Endoplasmin [Precursor], Heat-shock protein 90 kDa beta member 1, GRP94 | 92469 | 151 | 29 | Chaperone/Stress, Antigen | Endoplasmic reticulum |
| P27797 | CALR_HUMAN | CALR | Calreticulin [Precursor] | 48142 | 163 | 19 | Chaperone/Stress, Antigen | Endoplasmic reticulum |
| P00441 | SODC_HUMAN | SODC | Superoxide dismutase [Cu-Zn] | 15936 | 102 | 8 | Chaperone/stress, Enzyme | Endoplasmic reticulum |
| P30101 | PDIA3_HUMAN | PDIA3 | Protein disulfide-isomerase A3 [Precursor], ERp60 | 56782 | 204 | 24 | Chaperone/Stress, Enzyme | Endoplasmic reticulum |
| P30040 | ERP29_HUMAN | ERP29 | Endoplasmic reticulum protein ERp29 [Precursor], ERp28 | 28993 | 86 | 5 | Chaperone/Stress, Enzyme, Transport | Endoplasmic reticulum |
| P62937 | PPIA_HUMAN | PPIA | Peptidyl-prolyl cis-trans isomerase A, Cyclophilin A | 18012 | 161 | 13 | Enzyme, Immunity/defence | Endoplasmic reticulum |
| P07237 | PDIA1_HUMAN | PDIA1 | Protein disulfide-isomerase [Precursor] | 57116 | 214 | 26 | Enzyme, Protooncogene | Endoplasmic reticulum |
| P50454 | SPH2_HUMAN | SPH2 | Serpin H1 [Precursor], Collagen-binding protein, Colligin | 46441 | 68 | 10 | Enzyme, Regulatory | Endoplasmic reticulum |
| O95881 | TXD12_HUMAN | TXD12 | Thioredoxin domain-containing protein 12 [Precursor] | 19206 | 68 | 8 | Enzyme, Transport | Endoplasmic reticulum |
| Q15293 | RCN1_HUMAN | RCN1 | Reticulocalbin-1 [Precursor] | 38890 | 130 | 17 | Regulatory | Endoplasmic reticulum |
| P24534 | EF1B_HUMAN | EF1B | Elongation factor 1-beta, EF-1-beta | 24764 | 68 | 7 | RNA associated, Transcription factor | Endoplasmic reticulum |
| Q14257 | RCN2_HUMAN | RCN2 | Reticulocalbin-2 [Precursor], E6-binding protein | 36876 | 130 | 13 | Structural | Endoplasmic reticulum |
| P14314 | GLU2B_HUMAN | GLU2B | Glucosidase 2 subunit beta [Precursor] | 59425 | 95 | 16 | Unknown/Unspecified | Endoplasmic reticulum |
| Q9Y4L1 | OXRP_HUMAN | OXRP | 150 kDa oxygen-regulated protein [Precursor] | 111335 | 111 | 21 | Chaperone/Stress, Protooncogene | Endoplasmic reticulum, Cytoplasmic |
| Q14697 | GANAB_HUMAN | GANAB | Neutral alpha-glucosidase AB [Precursor] | 106874 | 150 | 15 | Enzyme | Endoplasmic reticulum, Golgi apparatus |
| O43852 | CALU_HUMAN | CALU | Calumenin [Precursor] | 37107 | 152 | 14 | Regulatory, Transport | Endoplasmic reticulum, Golgi apparatus |
| P55072 | TERA_HUMAN | TERA | Transitional endoplasmic reticulum ATPase, VCP, Valosin-containing protein | 89322 | 283 | 41 | Chaperone/Stress, Structural, Transport | Endoplasmic reticulum, Nuclear |
| Q01105 | SET_HUMAN | SET | Protein SET | 33489 | 56 | 7 | Regulatory, DNA associated, Transcription factor, Protooncogene | Endoplasmic reticulum, Nuclear |
| Q9UKX7 | NUP50_HUMAN | NUP50 | Nucleoporin 50 kDa | 50144 | 75 | 9 | Structural | Endoplasmic reticulum Nuclear |

Table I. *continued*

Table I. *continued*

| Acession no | Protein name | Protein symbol | Protein description | MW | Mascot score | Peptides matched | Cellular function | Subcellular localization |
|---|---|---|---|---|---|---|---|---|
| Q3I349 | Q3I349_BOSIN | Q3I349 | Serum albumin [Fragment] | 53925 | 237 | 25 | Transport | Extracellular |
| Q64599 | Q64599_RAT | Q64599 | Hemiferrin | 24091 | 74 | 7 | Transport | Extracellular |
| P13693 | TCTP_HUMAN | TCTP | Translationally-controlled tumor protein, Fortilin | 19595 | 70 | 12 | Enzyme, Protooncogene | Extracellular, Cytoplasmic |
| P11021 | GRP78_HUMAN | GRP78 | 78 kDa glucose-regulated protein [Precursor], GRP 78, BiP | 72333 | 312 | 35 | Chaperone/Stress, Signaling | Extracellular, Membranic, Endoplasmic reticulum |
| Q15942 | ZYX_HUMAN | ZYX | Zyxin | 61277 | 71 | 10 | Adhesion | Membranic |
| Q9NRX4 | PHP14_HUMAN | PHP14 | 14 kDa phosphohistidine phosphatase | 13833 | 73 | 6 | Regulatory | Membranic |
| P62993 | GRB2_HUMAN | GRB2 | Growth factor receptor-bound protein 2, Adapter protein GRB2 | 25206 | 113 | 10 | Regulatory, Signaling growth factor | Membranic |
| P07355 | ANXA2_HUMAN | ANXA2 | Annexin A2 | 38604 | 123 | 14 | Regulatory, Signaling, Transport channel | Membranic Membranic |
| Q14444 | CAPR1_HUMAN | CAPR1 | Caprin-1, p137GPI | 72752 | 60 | 10 | Transport | Membranic |
| P09382 | LEG1_HUMAN | LEG1 | Galectin-1 | 14716 | 154 | 13 | Adhesion, Regulatory | Membranic, Cytoplasmic, Nuclear |
| Q13283 | G3BP_HUMAN | G3BP | Ras GTPase-activating protein-binding protein 1, HDH-VIII, G3BP-1 | 52164 | 177 | 20 | Enzyme | Membranic, Nuclear |
| P10809 | CH60_HUMAN | CH60 | 60 kDa heat shock protein, mitochondrial [Precursor] | 61055 | 266 | 34 | Chaperone/Stress | Mitochondrial |
| P05091 | ALDH2_HUMAN | ALDH2 | Aldehyde dehydrogenase, mitochondrial | 56381 | 60 | 8 | Enzyme | Mitochondrial |
| P19404 | NUHM_HUMAN | NUHM | NADH dehydrogenase [ubiquinone] flavoprotein 2, mitochondrial [Precursor] | 27392 | 58 | 7 | Enzyme | Mitochondrial |
| P25325 | THTM_HUMAN | THTM | 3-mercaptopyruvate sulfurtransferase | 33178 | 79 | 6 | Enzyme | Mitochondrial |
| P28331 | NUAM_HUMAN | NUAM | NADH-ubiquinone oxidoreductase 75 kDa subunit, mitochondrial [Precursor] | 79468 | 159 | 17 | Enzyme | Mitochondrial |
| P30084 | ECHM_HUMAN | ECHM | Enoyl-CoA hydratase, mitochondrial [Precursor] | 31387 | 102 | 13 | Enzyme | Mitochondrial |
| P30837 | AL1B1_HUMAN | AL1B1 | Aldehyde dehydrogenase X, mitochondrial [Precursor] | 57238 | 76 | 7 | Enzyme | Mitochondrial |
| P31930 | UQCR1_HUMAN | UQCR1 | Ubiquinol-cytochrome-c reductase complex core protein 1, mitochondrial [Precursor], Core I protein | 52646 | 154 | 21 | Enzyme | Mitochondrial |
| P31937 | 3HIDH_HUMAN | 3HIDH | 3-hydroxyisobutyrate dehydrogenase, mitochondrial [Precursor] | 35329 | 69 | 9 | Enzyme | Mitochondrial |
| P32322 | P5CR1_HUMAN | P5CR1 | Pyrroline-5-carboxylate reductase 1 | 33361 | 101 | 8 | Enzyme | Mitochondrial |
| P36639 | 8ODP_HUMAN | 8ODP | 7,8-dihydro-8-oxoguanine triphosphatase, 8-oxo-dGTPase | 22552 | 60 | 5 | Enzyme | Mitochondrial |
| P36957 | ODO2_HUMAN | ODO2 | Dihydrolipoyllysine-residue succinyltransferase component of 2-oxoglutarate dehydrogenase complex, mitochondrial [Precursor], E2K | 48640 | 76 | 9 | Enzyme | Mitochondrial |
| P50213 | IDH3A_HUMAN | IDH3A | Isocitrate dehydrogenase [NAD] subunit alpha, mitochondrial [Precursor], NAD(+)-specific ICDH | 39592 | 56 | 7 | Enzyme | Mitochondrial |
| P55809 | SCOT_HUMAN | SCOT | Succinyl-CoA:3-ketoacid-coenzyme A transferase 1, mitochondrial [Precursor], Scot-S | 56158 | 59 | 8 | Enzyme | Mitochondrial |
| Q99798 | ACON_HUMAN | ACON | Aconitate hydratase, mitochondrial [Precursor] | 85425 | 98 | 11 | Enzyme | Mitochondrial |
| Q08752 | PPID_HUMAN | PPID | 40 kDa peptidyl-prolyl cis-trans isomerase, Cyclophilin-40, CYP-40 | 40764 | 65 | 5 | Enzyme, Immunity/defence, Receptor | Mitochondrial |

Table I. *continued*

Table I. *continued*

| Acession no | Protein name | Protein symbol | Protein description | MW | Mascot score | Peptides matched | Cellular function | Subcellular localization |
|---|---|---|---|---|---|---|---|---|
| P06576 | ATPB_HUMAN | ATPB | ATP synthase subunit beta, mitochondrial [Precursor] | 56560 | 184 | 26 | Enzyme, Transport | Mitochondrial |
| Q16891 | IMMT_HUMAN | IMMT | Mitochondrial inner membrane protein, Mitofilin | 83678 | 69 | 8 | Motor/Contractile, Structural | Mitochondrial |
| P07737 | PROF1_HUMAN | PROF1 | Profilin-1 | 15054 | 74 | 9 | Regulatory | Mitochondrial |
| P61088 | UBE2N_HUMAN | UBE2N | Ubiquitin-conjugating enzyme E2 N, Ubc13 | 17138 | 105 | 8 | Regulatory | Mitochondrial |
| Q04837 | SSB_HUMAN | SSB | Single-stranded DNA-binding protein, mitochondrial [Precursor] | 17260 | 100 | 9 | Regulatory | Mitochondrial |
| P62258 | 1433E_HUMAN | 1433E | 14-3-3 protein epsilon, 14-3-3E | 29174 | 111 | 14 | Adaptor, Regulator, Signaling neurotransmitter | Mitochondrial, Cytoskeletal |
| O43175 | SERA_HUMAN | SERA | D-3-phosphoglycerate dehydrogenase | 56519 | 135 | 16 | Enzyme | Mitochondrial, Endoplasmic reticulum |
| P38646 | GRP75_HUMAN | GRP75 | Stress-70 protein, mitochondrial [Precursor], Mortalin | 73680 | 286 | 40 | Chaperone/Stress, Transcription factor, Regulatory, Protooncogene | Mitochondrial, Endoplasmic reticulum, Cytoplasmic |
| P35232 | PHB_HUMAN | PHB | Prohibitin | 29804 | 132 | 12 | Antioncogene | Mitochondrial, Endoplasmic reticulum, Nuclear |
| P08107 | HSP71_HUMAN | HSP71 | Heat-shock 70 kDa protein 1 | 70052 | 161 | 18 | Chaperone/Stress | Mitochondrial, Endoplasmic reticulum, Nuclear |
| P61604 | CH10_HUMAN | CH10 | 10 kDa heat-shock protein, mitochondrial | 10932 | 137 | 11 | Chaperone/stress | Mitochondrial, Matrix |
| Q13765 | NACA_HUMAN | NACA | Nascent polypeptide-associated complex subunit alpha, NAC-alpha | 23384 | 58 | 5 | Chaperone/Stress | Mitochondrial, Nuclear |
| P15531 | NDKA_HUMAN | NDKA | Nucleoside diphosphate kinase A, nm23-H1 | 17149 | 152 | 15 | Enzyme | Mitochondrial, Nuclear |
| P33316 | DUT_HUMAN | DUT | Deoxyuridine 5'-triphosphate nucleotidohydrolase, mitochondrial [Precursor], dUTP pyrophosphatase | 26706 | 133 | 12 | Enzyme | Mitochondrial, Nuclear |
| Q07021 | C1QBP_HUMAN | C1QBP | Complement component 1 Q subcomponent-binding protein, mitochondrial [Precursor], Glycoprotein gC1qBP, p33 | 31362 | 57 | 7 | Immunity/Defence, RNA associated, Signaling | Mitochondrial, Nuclear |
| Q92598 | HS105_HUMAN | HS105 | Heat-shock protein 105 kDa | 96865 | 101 | 14 | Regulatory | Mitochondrial, Nuclear |
| Q9Y224 | CN166_HUMAN | CN166 | Protein C14orf166 | 28068 | 138 | 14 | Regulatory | Mitochondrial, Nuclear |
| P29692 | EF1D_HUMAN | EF1D | Elongation factor 1-delta | 31122 | 179 | 16 | RNA associated | Mitochondrial, Nuclear |
| Q8WXX5 | DNJC9_HUMAN | DNJC9 | DnaJ homolog subfamily C member 9 | 29910 | 65 | 6 | Chaperone/Stress | Nuclear |
| P15927 | RFA2_HUMAN | RFA2 | Replication protein A 32 kDa subunit, RP-A | 29247 | 68 | 4 | DNA associated | Nuclear |
| O15160 | RPA5_HUMAN | RPA5 | DNA-directed RNA polymerase I 40 kDa polypeptide | 39250 | 112 | 12 | Enzyme | Nuclear |
| P17844 | DDX5_HUMAN | DDX5 | Probable ATP-dependent RNA helicase DDX5, RNA helicase p68 | 69148 | 60 | 9 | Enzyme | Nuclear |
| P12004 | PCNA_HUMAN | PCNA | Proliferating cell nuclear antigen | 28769 | 89 | 10 | Regulatory, DNA associated, Antigen | Nuclear |
| P43487 | RANG_HUMAN | RANG | Ran-specific GTPase-activating protein, RanBP1 | 23310 | 60 | 9 | Regulatory, Signaling | Nuclear |

Table I. *continued*

Table I. *continued*

| Acession no | Protein name | Protein symbol | Protein description | MW | Mascot score | Peptides matched | Cellular function | Subcellular localization |
|---|---|---|---|---|---|---|---|---|
| Q13263 | TIF1B_HUMAN | TIF1B | Transcription intermediary factor 1-beta, KRAB-associated protein 1, KAP-1 | 88550 | 129 | 17 | Regulatory, Transcription factor | Nuclear |
| O43809 | CPSF5_HUMAN | CPSF5 | Cleavage and polyadenylation specificity factor 5 | 26227 | 56 | 5 | RNA associated | Nuclear |
| P06730 | IF4E_HUMAN | IF4E | Eukaryotic translation initiation factor 4E | 25097 | 63 | 6 | RNA associated | Nuclear |
| P22626 | ROA2_HUMAN | ROA2 | Heterogeneous nuclear ribonucleoproteins A2/B1 | 37430 | 148 | 11 | RNA associated | Nuclear |
| P31942 | HNRH3_HUMAN | HNRH3 | Heterogeneous nuclear ribonucleoprotein H3 | 36926 | 118 | 18 | RNA associated | Nuclear |
| P31943 | HNRH1_HUMAN | HNRH1 | Heterogeneous nuclear ribonucleoprotein H | 49229 | 124 | 17 | RNA associated | Nuclear |
| P33240 | CSTF2_HUMAN | CSTF2 | Cleavage stimulation factor 64 kDa subunit, CstF-64 | 60959 | 90 | 13 | RNA associated | Nuclear |
| P52597 | HNRPF_HUMAN | HNRPF | Heterogeneous nuclear ribonucleoprotein F, hnRNP F | 45672 | 68 | 11 | RNA associated | Nuclear |
| P55795 | HNRH2_HUMAN | HNRH2 | Heterogeneous nuclear ribonucleoprotein H' | 49264 | 129 | 13 | RNA associated | Nuclear |
| P61978 | HNRPK_HUMAN | HNRPK | Heterogeneous nuclear ribonucleoprotein K, hnRNP K | 50976 | 124 | 16 | RNA associated | Nuclear |
| Q07955 | SFRS1_HUMAN | SFRS1 | Splicing factor, arginine/serine-rich 1, ASF-1 | 27745 | 155 | 13 | RNA associated | Nuclear |
| Q12874 | SF3A3_HUMAN | SF3A3 | Splicing factor 3A subunit 3, SF3a60 | 58849 | 83 | 13 | RNA associated | Nuclear |
| Q15459 | SF3A1_HUMAN | SF3A1 | Splicing factor 3 subunit 1, SF3a120 | 88886 | 116 | 18 | RNA associated | Nuclear |
| Q96S43 | HNRDL_HUMAN | HNRDL | Heterogeneous nuclear ribonucleoprotein D-like, hnHNRP-DL, JKT41-binding protein | 46438 | 64 | 7 | RNA associated | Nuclear |
| P23246 | SFPQ_HUMAN | SFPQ | Splicing factor, proline- and glutamine-rich, PSF | 76149 | 85 | 20 | RNA associated, Antioncogene | Nuclear |
| P67809 | YBOX1_HUMAN | YBOX1 | Nuclease sensitive element-binding protein 1, Y-box-binding protein 1, YB-1 | 35924 | 123 | 13 | RNA associated, Transcription factor | Nuclear |
| O75934 | BCAS2_HUMAN | BCAS2 | Breast carcinoma amplified sequence 2 | 26131 | 81 | 8 | Structural | Nuclear |
| P43243 | MATR3_HUMAN | MATR3 | Matrin-3 | 94623 | 159 | 19 | Structural | Nuclear |
| P09429 | HMGB1_HUMAN | HMGB1 | High mobility group protein B1 | 24894 | 77 | 8 | Transcription factor | Nuclear |
| Q7Z3B4 | NUP54_HUMAN | NUP54 | Nucleoporin p54 | 55435 | 123 | 13 | Transport | Nuclear |
| Q9UNZ2 | NSF1C_HUMAN | NSF1C | NSFL1 cofactor p47 | 40573 | 90 | 10 | Adaptor | Nuclear, Golgi apparatus |
| P02545 | LMNA_HUMAN | LMNA | Lamin-A/C | 74139 | 295 | 36 | Structural | Nuclear, Nuclear matrix |
| P04083 | ANXA1_HUMAN | ANXA1 | Annexin A1 | 38714 | 68 | 4 | Regulatory, Transport | Membranic |
| O95816 | BAG2_HUMAN | BAG2 | BAG family molecular chaperone regulator 2, BAG-2 | 23772 | 77 | 8 | Chaperone/stress | |
| O75947 | ATP5H_HUMAN | ATP5H | ATP synthase D chain, mitochondrial | 18491 | 115 | 12 | Enzyme | Mitochondrial |
| P22314 | UBE1_HUMAN | UBE1 | Ubiquitin-activating enzyme E1 | 117849 | 102 | 13 | Enzyme | |
| P60174 | TPIS_HUMAN | TPIS | Triosephosphate isomerase | 26669 | 210 | 14 | Enzyme | |
| Q9UBQ7 | GRHPR_HUMAN | GRHPR | Glyoxylate reductase/hydroxypyruvate reductase | 35668 | 94 | 8 | Enzyme | |
| P84090 | ERH_HUMAN | ERH | Enhancer of rudimentary homolog | 12259 | 64 | 7 | Regulatory | |
| P08133 | ANXA6_HUMAN | ANXA6 | Annexin A6, P68 | 75873 | 184 | 30 | Regulatory, Transport | |
| P60660 | MYL6_HUMAN | MYL6 | Myosin light polypeptide 6, Smooth muscle and nonmuscle myosin light chain alkali 6 | 16930 | 94 | 8 | Structural | |
| P68363 | TBAK_HUMAN | TBAK | Tubulin alpha-1B chain | 50152 | 207 | 26 | Structural | |
| P68371 | TBB2C_HUMAN | TBB2C | Tubulin beta-2C chain | 49831 | 236 | 32 | Structural | |
| Q9HB07 | MYG1_HUMAN | MYG1 | UPF0160 protein MYG1 | 42445 | 82 | 9 | Unknown/Unspecified | |
| P37802 | TAGL2_HUMAN | TAGL2 | Transgelin-2 | 22391 | 121 | 14 | Unknown/Unspecified, Transcription factor | |
| Q5HYB6 | Q5HYB6_HUMAN | Q5HYB6 | Hypothetical protein DKFZp686J1372 | 27176 | 112 | 15 | | |
| Q5T626 | Q5T626_HUMAN | Q5T626 | Nuclear autoantigenic sperm protein | 48804 | 70 | 11 | | |
| Q96B89 | GLOD4_HUMAN | GLOD4 | Glyoxalase domain-containing protein 4 | 34793 | 123 | 13 | | |
| Q9BQE3 | TBA6_HUMAN | TBA6 | Tubulin alpha-1C chain | 49895 | 68 | 8 | | |
| Q9UI30 | TR112_HUMAN | TR112 | TRM112-like protein | 14199 | 64 | 4 | | |

proteins, structural proteins, such as tubulins and actins, and other major classes of identified proteins, such as transcription factors, transport/carrier and signal transduction. In addition, we found a number of protooncogenes and antioncogenes, representing 3% and 1%, respectively of the total proteome.

## Discussion

For the comprehensive analysis of the U2OS osteosarcoma cell line we decided to apply proteomics technology as it provides an insight into the relationships between genes, their products and cell function (16). Analytical methods used for proteomic research result most commonly in inclusive databases such as 2-DE maps. Genome sequence databases, complete catalogues of proteins expressed in organisms, mass spectrometry and software that match MS data with protein sequences databases helped us to successfully complete the construction of the protein database of the osteosarcoma U2OS cell line.

We identified 237 different gene products with several functions including regulatory, signal transduction, protooncogenes and antioncogenes, chaperone/stress and nucleic acid-binding proteins. The use of internal peptide standards allowed narrow windows of mass tolerance (0.0025%), increasing the confidence of identification by PMF and PSD mode of mass spectrometry. Thus the identification was based on three or more (up to 69) matching peptides. Most of them were localized in the cytoplasm, nucleus, mitochondria and some in the membrane.

Eleven protooncogenes were identified among them, TCTP, SRC8, FUBP1, NPM, PARK7, SET, FKBP4 and OXRP. The SRC8 (*EMS1*) protooncogene encodes a human homologue of cortactin, a c-Src substrate associated with 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 (17, 18). FUBP1, a far upstream element binding protein1 (Q96AE4), complexes with FUSE and inhibits c-myc expression which is also involved in cell growth, proliferation, differentiation and apoptosis (19). The location of the FUBP1 gene is in chromosome 1 p31.1, which is very often amplified in osteosarcomas (20). In addition, in this amplified chromosome area, (1p36.33-p36.12) is located protooncogene PARK7 (DJ-1). DJ-1 protein was shown (21) to be a potent inhibitor of the Daxx/ASK1 cell death signaling pathway, thus protecting cells from oxidative stress, and functions 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 (22). It is induced by genotoxic stress and stabilizes certain conformers of p53, binds pRb and synergistically stimulates DNA polymerase $\alpha$ (23). In addition, NPM protects cells from death and stress-induced apoptosis through inhibition of p53 (24, 25). TCTP is a ubiquitously expressed protein and is regulated both at the transcriptional and translational level. Thus TCTP protein cellular levels are highly regulated in response to numerous extracellular signals and cellular conditions. TCTP is believed to play an important role in cell growth and division as it is considerably up-regulated upon entry of cells into the cell cycle. Therefore, TCTP down-regulation was associated with reversion of transformed cells to a normal phenotype and suppression of malignant transformation (26). This strongly suggests an implication of TCTP in the malignant phenotype and tumor growth. This is strongly supported by the fact that TCTP is stabilized by the anti-apoptotic protein MCL1 and by a correlation of its level with drug resistance in melanoma cells (26).

A comparison of the U2OS proteome with that of Saos2 (5) revealed some differences despite the same origin of the two cancer cell lines. Concerning the whole proteome, 141 proteins were identical, while 304 proteins were different. Nevertheless both cancer cell lines have great similarities regarding the protooncogenes; they share 7 of the same protooncogenes; while 3 are exclusive to Saos2 and 4 to U2OS. Additionally, there were some differences in the presence of antioncogenes as 6 exist in U2OS and 9 in Saos2; five of these were identical. Consequently, in spite of similarities and the osteogenic origin of these two cell lines there were many differences concerning the proteome, which reflects the differences of the genome and character of the two types of cancer: Saos2 is more aggressive than U2OS.

Eleven proteins with unknown/unspecified subcellular function and/or localization were also identified. One was a hypothetical protein (Q5HYB6) with an interest for further investigation.

Summarizing, in the present study, we created the 2-DE database for the human osteosarcoma U2OS cell line. The 237 different gene products were identified using MALDI-MS and MALDI-MS-MS analysis of approximately 3,000 spots out of four 2-DE gels. This 2-DE database creates a useful tool in the study of molecular carcinogenesis.

## References


1 Görg A, Weiss W and Dunn MJ: Current two-dimensional electrophoresis technology for proteomics. Proteomics *4*: 3665-3685, 2004.

2 Friedman KM and Fox BA: The promising future of proteomics in cancer diagnosis and treatment. Eur J Gastroenterol Hepatol *17*: 701-703, 2005.

3 Anagnostopoulos AK, Vougas K, Kolialexi A, Mavrou A, Fountoulakis M and Tsangaris GT: The protein profile of the human immature T-cell line CCRF-CEM. Cancer Genom Proteom *2*: 1-29, 2005.

4 Fountoulakis M, Tsangaris G, Oh J, Maris A and Lubec G: Protein profile of the HeLa cell line. J Chrom A *1038*: 247-265, 2004.

5 Niforou KN, Anagnostopoulos AK, Vougas K, Kittas C, Gorgoulis VG and Tsangaris GT: The proteome profile of human osteosarcoma Saos2 cell line. Cancer Genom Proteom *3*: 325-346, 2006.

6 Bayani J, Zielenska M, Pandita A, Al-Romaih K, Karaskova J, Harrison K, Bridge JA, Sorensen P, Thorner P and Squire JA: Spectral karyotyping identifies recurrent complex rearrangement of chromosomes 8, 17, 20 in osteosarcomas. Genes Chromosomes Cancer *36*: 7-16, 2003.

7 Wesierska-Gadek J and Schmid G: The subcellular distribution of the p53 tumor suppressor, and organismal ageing. Cell Mol Biol Lett *10*: 439-453, 2005.

8 Zhu L: Tumour suppressor retinoblastoma protein Rb: a transcriptional regulator. Eur J Cancer *41*: 2415-2427, 2005.

9 Isfort RJ, Cody DB, Lovell G and Doersen CJ: Analysis of oncogenes, tumor suppressor genes, autocrine growth factor production and differentiation state of human osteosarcoma cell lines. Mol Carcinog *14*: 170-178, 1995.

10 Furuya K, Ozaki T, Hanamoto T, Hosoda M, Hayashi S, Barker PA, Takano K, Matsumoto M and Nakagawara A: Stabilization of p73 by nuclear I{kappa}B kinase-{alpha} mediates cisplatin-induced apoptosis. J Biol Chem *282(25)*: 18365-18378, 2007.

11 Kinsey CG, Bussolati G, Bosco M, Kimura T, Pizzorno MC, Chernin MI, Cassoni P and Novak JF: Constitutive and ligand-induced nuclear localization of oxytocin receptor. J Cell Mol Med *11(1)*: 96-110, 2007.

12 Mancini L, Paul-Clark MJ, Rosignoli G, Hannon R, Martin JE, Macintyre I and Perretti M: Calcitonin and prednisolone display antagonistic actions on bone and have synergistic effects in experimental arthritis. Am J Pathol *170(3)*: 1018-1027, 2007.

13 Gorgoulis VG, Vassiliou LV, Karakaidos P, Zacharatos P, Kotsinas A, Liloglou T, Venere M, Ditullio RA Jr, Kastrinakis NG, Levy B, Kletsas D, Yoneta A, Herlyn M, Kittas C and Halazonetis TD: Activation of DNA-damage checkpoint and genomic instability in human precancerous lesions. Nature *434*: 907-913, 2005.

14 Bartkova J, Rezaei N, Liontos M, Karakaidos P, Kletsas D, Issaeva N, Vassiliou LVF, Kolettas E, Niforou K, Zoumpourlis VC, Takaoka M, Nakagawa H, Tort F, Fugger K, Johansson F, Sehested M, Andersen CL, Dyrskjot L, Ørntoft T, Lukas J, Kittas C, Helleday T, Halazonetis TD, Bartek J and Gorgoulis VG: Oncogene-induced senescence is part of the tumorigenesis barrier imposed by DNA-damage checkpoints. Nature *444(7119)*: 633-637, 2006.

15 Berndt P, Hobohm U and Langen H: Reliable automatic protein identification from matrix-assisted laser desorption/ionization mass spectrometric peptide fingerprints. Electrophoresis *20*: 3521-3526, 1999.

16 Plebani M: Proteomics: The next revolution in laboratory medicine? Clin Chim Acta *357*: 113-122, 2005.

17 Ormandy CJ, Musgrove EA, Hui R, Daly RJ and Sutherland RL: Cyclin D1, EMS1 and 11q13 amplification in breast cancer. Breast Cancer Res Treat *78(3)*: 323-335, 2003.

18 Yuan BZ, Zhou X, Zimonjic DB, Durkin ME and Popescu NC: Amplification and overexpression of the EMS1 oncogene, a possible prognostic marker, in human hepatocellular carcinoma. J Mol Diagn *5(1)*: 48-53, 2003.

19 He L, Liu J, Collins I, Sanford S, O'Connell B, Benham CJ and Levens D: Loss of FBP function arrests cellular proliferation and extinguishes c-myc expression. EMBO J *19(5)*: 1034-1044, 2000.

20 Sandberg AA and Bridge J: Updates in the cytogenesis and molecular genetics of bone and soft tissue tumors: osteosarcoma and related tumors. Cancer Genet Cytogenet *145*: 1-30, 2003.

21 Junn E, Taniguchi H, Jeong BS, Zhao X, Ichijo H and Mouradian MM: Interaction of DJ-1 with Daxx inhibits apoptosis signal-regulating kinase 1 activity and cell death. Proc Natl Acad Sci USA *102*: 9691-9696, 2005.

22 Tarapore P, Shinmura K, Suzuki H, Tokuyama Y, Kim SH, Mayeda A and Fukasawa K: $Thr^{199}$ phosphorylation targets nucleophosmin to nuclear speckles and represses pre-mRNA processing. FEBS Lett *580*: 399-409, 2006.

23 Lambert B and Buckle M: Characterization of the interface between nucleophosmin (NPM) and p53: Potential role in p53 stabilization. FEBS Lett *580*: 345-350, 2006.

24 Li J, Zhang X, Sejas DP and Pang Q: Negative regulation of p53 by nucleophosmin antagonizes stress-induced apoptosis in human normal and malignant hematopoietic cells. Leuk Res *29*: 1415-1423, 2005.

25 Li J, Zhang X, Sejas DP, Bagby GC and Pang Q: Hypoxia-induced nucleophosmin protects cell death through inhibition of p53. J Biol Chem *279*: 41275-41279, 2004.

26 Bommer UA and Thiele BJ: The translationally controlled tumor protein (TCTP). Int J Biochem Cell Biol *36(3)*: 379-385 2004.


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