

Protein Profiling of the Supratentorial Primitive Neuroectodermal Tumor (PNET) Cell Line PFSK-1

ANDREAS PEYRL^{1*}, KURT KRAPPENBAUER^{2*}, LEILA AFJEHI-SADAT¹,
THOMAS STROBEL³, IRENE SLAVC¹ and GERT LUBEC¹

¹Department of Pediatrics and ³Institute of Neurology, Medical University of Vienna, Vienna, Austria;

²Roche Center for Medical Genomics Ltd, Basel, Switzerland

Abstract. *Background:* Supratentorial primitive neuroectodermal tumors (PNETs) are rare embryonal cerebral hemispheric tumors proposed to arise from primitive neuroepithelial cells. The permanent cell line PFSK-1 is widely used in studies of this tumor entity and it was the aim of this study to generate a proteome map to serve as a basis for further studies to search for tumor-related proteins. *Materials and Methods:* The PNET-related cell line PFSK-1 was cultivated and proteins from cell lysates were subject to two-dimensional gel electrophoresis with in-gel-digestion of protein spots and subsequent MALDI-MS identification. *Results:* Among the 157 proteins identified by this method we observed structural, metabolic, chaperone, antioxidant, transcriptional / translational proteases as well as miscellaneous proteins. Hypothetical proteins similar to pyrroline-5-carboxylate reductase isoform, similar to 3-hydroxyisobutyryl-Coenzyme A hydrolase, thioredoxin domain containing protein 5 precursor, potential helicase with zinc-finger domain, an unnamed protein product and proteins P1.11659_4 and Pro1512 were detected. *Conclusion:* No neuronal, glial or other specific markers were found; the presence of vimentin may point to a mesenchymal rather than an epithelial origin; expression of developmentally expressed potential helicase P42694 indicates immaturity and FUSE binding protein 1 provides a link to myc, a major protooncogene, and to differentiation per se. We provide an analytical tool unambiguously identifying structures of several protein classes and show the existence of several hypothetical proteins, that had so far been predicted from nucleic acid sequences only and never detected in mammalian cell lines or tissues at the protein level.

*A.P. and K.K. contributed equally to the study

Correspondence to: Prof. Dr. Irene Slavic, Medical University of Vienna, Department of Pediatrics, Waehringer Guertel 18, A-1090 Vienna, Austria. Fax: +43 1 40400 3093, e-mail: irene.slavic@meduniwien.ac.at

Key Words: stPNET, brain tumor, PFSK-1, proteomics.

Supratentorial cerebral primitive neuroectodermal tumors (PNETs) are rare embryonal tumors which most commonly occur in early childhood, accounting for approximately 5 % of cerebral hemispheric tumors and 3 % of all brain tumors in children (1).

The term PNET was coined by Hart and Earl to describe a cerebral hemispheric tumor composed of primitive neuroepithelial cells with the capacity for differentiation along neuronal, astrocytic, ependymal, or mesenchymal lines (2). Based on their presumed common origin from pluripotential neuroepithelial cells and similar light microscopic features, Rorke later proposed that cerebral PNETs were the supratentorial counterparts of medulloblastoma and should therefore be grouped along with similar tumors in other locations, such as pinealoblastoma and retinoblastoma (3). An opposing opinion, proposed by Rubinstein, is that these tumors are distinct entities arising from progenitor cells (4). Despite their obvious morphological similarities and their common propensity to spread within the central nervous system through subarachnoid pathways, these tumors differ significantly in terms of their prognosis, with an approximately 30% five-year progression-free survival of supratentorial PNET versus 60-80% for medulloblastoma (5).

Recently, Pomeroy and co-workers provided additional evidence that cerebral PNET is a tumor entity distinct from medulloblastoma by using DNA microarray gene expression profiling (6).

So far, however, very little is known about the protein profiles of these tumors and only few cell lines exist. The well-studied permanent cell line PFSK-1 is derived from a primitive neuroectodermal tumor of the right frontal lobe of a 22-month-old boy and was established in 1992 by Fults and co-workers (7). PFSK-1 shows abundant nestin immunoreactivity, an intermediate filament protein also expressed by neuroectodermal stem cells. No immunoreactivity was detected with antibodies against neurofilaments, galactocerebroside or glial fibrillary acidic protein, indicating that none of the major cell types of the mature nervous system, neurons and astrocytes was present (7).

We applied a protein-chemical method, two-dimensional gel electrophoresis with subsequent mass spectrometrical analysis of spots, unequivocally identifying proteins in tissues or cells. This method enables the concomitant identification of more than a hundred different proteins, *i.e.* generation of expressional patterns, and is a valuable tool for identifying so far unknown, hypothetical proteins (8-13).

It was the goal of this study to provide a comprehensive proteome map of a supratentorial PNET cell line to serve as a reference database for further studies on primary tumor tissue as well as forming the basis for studies to search for marker candidates, putative pharmaceutical targets and clues for potential pathomechanisms of specific tumor biology.

Materials and Methods

Cell lines. PFSK-1 was purchased from the American Type Culture Collection (ATCC 2060, Manassas, VA, USA). PFSK-1 was cultured in Minimum Essential Medium (Eagle) with 2 mM L-glutamine and Earle's BSS adjusted to contain 1.5 g/L sodium bicarbonate, 0.1 mM non-essential amino acids and 1.0 mM sodium pyruvate, with 10% fetal bovine serum. The cell culture was maintained in a humidified atmosphere of 5% v/v CO₂ in air at 37°C.

Two-dimensional electrophoresis (2-DE). PFSK-1 cells were washed three times with 10 mL PBS (Gibco BRL, Gaithersburg, MD, USA) and centrifuged for 10 min at 300 x g at room temperature. The supernatant was discarded and the pellet was suspended in 0.5 ml of sample buffer consisting of 40 mM Tris-HCl, 5 M urea (Merck, Darmstadt, Germany), 2 M thiourea (Sigma, St. Louis, MO, USA), 4% CHAPS (3-[(3-cholamidopropyl)dimethylammonio]-1-propane-sulfonate) (Sigma), 10 mM 1,4-dithioerythritol (Merck), 1 mM EDTA (ethylenediaminetetraacetic acid) (Merck) and protease inhibitor complete (Roche, Basel, Switzerland). The suspension was left at room temperature for 1 h and centrifuged at 14,000xg for 60 min. Desalting was done with Ultrafree-4 centrifugal filter unit (Millipore). The protein content in the supernatant was determined by the Coomassie blue method (14).

2-DE was performed as reported previously (15). Samples of 1 mg protein were applied on immobilized pH 3-10 nonlinear gradient strips in sample cups at their basic and acidic ends. Focusing started at 200 V and the voltage was gradually increased to 8000 V at 4 V/min and kept constant for a further 3 h (approximately 150000 Vh totally). The second-dimensional separation was performed on 9-16% gradient sodium dodecyl sulfate polyacrylamide gels. The gels (180x200x1.5 mm) were run at 40 mA per gel. After protein fixation for 12 h in 50% methanol, containing 10% phosphoric acid, the gels were stained with colloidal Coomassie blue (Novex, San Diego, CA, USA) for 12 h. Molecular masses were determined by running standard protein markers (Biorad, Hercules, CA, USA), covering the range 10-250 kDa. pI values were used as given by the supplier of the immobilized pH gradient strips. Excess of dye was washed out from the gels with distilled water and the gels were scanned with an ImageScanner (Amersham Pharmacia Biosciences, Uppsala, Sweden). Electronic images of the gels were recorded using Photoshop (Adobe) and PowerPoint (Microsoft) software.

MALDI-MS. MALDI-MS analysis was performed as described elsewhere (9) with minor modifications. Spots were excised with a spot picker and placed into 96-well microtiter plates. Each spot was destained with 100 µl of 30% acetonitrile in 50 mM ammonium bicarbonate and dried in a speedvac evaporator. Each dried gel piece was rehydrated with 4 µl of 3 mM Tris-HCl, pH 9.0, containing 50 ng trypsin (Promega, Madison, WI, USA). After 16 h at room temperature, 7 µl of distilled water were added to each gel piece and the samples were shaken for 10 min. Four ml of 50% acetonitrile, containing 0.3% trifluoroacetic acid and the standard peptides, des-Arg-bradykinin (Sigma, 904.4681 Da) and adrenocorticotrophic hormone fragment 18-39 (Sigma, 2465.1989 Da), were added to each gel piece and shaken for 10 min. Sample application was performed using SymBiot I sample processor (PE Biosystems, Framingham, MA, USA). 1.5 µl of the peptide mixture were simultaneously applied on 1 µl of matrix, consisting of a saturated solution of α -cyano-4-hydroxycinnamic acid (Sigma) in 50% acetonitrile, containing 0.1% trifluoroacetic acid. Samples were analyzed in a time-of-flight mass spectrometer (Reflex 3, Bruker Analytics, Bremen, Germany). An accelerating voltage of 20 kV was used. Peptide matching and protein searches were performed automatically. The peptide masses were compared with the theoretical peptide masses of all available proteins from all species. Monoisotopic masses were used and a mass tolerance of 0.0025% was allowed. Spectra were analyzed and protein sequence databases were searched using the programs Fragment 21 and MSROFIT, respectively, developed in-house. Databases queried were SWISS-PROT (<http://www.expasy.ch>) and PIR (<http://www.nbrf.georgetown.edu/pir>). The algorithm used for determining the probability of a false-positive match with a given MS-spectrum was published (16) and can be described as follows: Baseline correction: The baseline of the MALDI-MS spectrum was found by splitting the spectrum into sequential mass segments of a size 0.05 mass range. For each of these segments we calculated a robust linear fit (17) and derived the slope and offset and their respective errors. The baseline was then approximated by cubic spline interpolation in-between the midpoints of the segments, and the baseline was subtracted from the spectrum. Peak detection and isotope distribution fit: After baseline correction, the maximum y-coordinate was taken as the starting point for peak fitting. A standard implementation of the Levenberg-Marquardt algorithm (17) was used to fit the isotope distribution of an average peptide at a given mass (calculated using the algorithm of Rockwood *et al.* (18)) which is parametrized by the monoisotopic mass position, the instrument resolution and the peak height. The fit was characterized by the usual fit quality estimates (Chi-square statistics). Subtraction of fit: From the fitted isotope distribution parameters we calculated the fit isotope distribution multiplied by a safety margin of 1.2 and subtracted the fit from the spectrum. The procedure was restarted and looped until the desired number of monoisotopic masses had been found. The algorithm was implemented on a standard personal computer.

Results

Proteins from the supratentorial PNET cell line PFSK-1 were separated by 2-DE and protein spots were visualized following staining with Coomassie blue. Figure 1 shows a representative gel of PFSK-1 proteins, where 1 mg of total protein was applied.

Table I. Proteins from Supratentorial PNET cell line PFSK-1 were extracted and separated by 2-DE. The protein spots were excised from the gels, digested with trypsin and the peptides generated were analyzed by MALDI-MS as stated in Materials and Methods. Search in the SWISS-PROT and ^aNCBI database resulted in the identification of the proteins listed in the table. The probability of assignment of a wrong identity was calculated to be 10⁻⁹. For protein search, corrected peptide masses and a window of mass tolerance of 0.0025% were used. The proteins have been grouped with regard to their functional classification. List of identified proteins in the supratentorial PNET cell line PFSK-1.

SW.-Nr	Access. Nr	Protein Name	Matches	Probability	Observed pI	Theoretical MW	Theoretical pI
Structural proteins							
P02570	SW:ACTB_HUMAN	Actin, cytoplasmic 1 (beta-actin).	7	pMism:10.67	4.6/5.0/5.2/ 5.3(2)/5.4	41736.73	5.29
P23528	SW:COF1_HUMAN	Cofilin, non-muscle isoform (18 kDa phosphoprotein) (p18).	5	pMism:10.60	7.5/8.0	18502.49	8.22
P50454	SW:CBP2_HUMAN	Collagen-binding protein 2 precursor (colligin 2) (rheumatoid arthritis related antigen ra-a47)	6	pMism:12.43	7.8	46440.54	8.75
Q16658	SW:FSC1_HUMAN	Fascin (singled-like protein) (55 kDa actin bundling protein) (p55).	7	pMism:10.60	6.8./6.9	54398.81	6.81
O14926	CHR7-FSC2	Fascin 2 (Retinal fascin).	4	6,69E-05	6.9	55057.08	7.95
P02545	SW:LAMA_HUMAN	Lamin a (70 kD lamin).	6	1,18E-07	6.9	74139	6.57
P07737	SW:PRO1_HUMAN	Profilin I.	4	pMism:8.62	8.1	14923.04	8.47
P05209	SW:TBA1_CRIGR	Tubulin alpha-1 chain.	9	4,35E-15	5.3(2)	50151.63	4.94
Q13748	SW:TBA2_HUMAN	Tubulin alpha-2 chain (alpha-tubulin 2).	6	7,92E-08	5.6	49959.55	4.98
P05215	SW:TBA4_HUMAN	Tubulin alpha-4 chain.	7	5,44E-10	5.3	49924.40	4.95
Q9bqe3	SW:TBA6_HUMAN	Tubulin alpha-6 chain (alpha-tubulin 6).	9	4,35E-15	5.3	49895.33	4.96
Q9ny65	SW:TBA8_HUMAN	Tubulin alpha-8 chain (alpha-tubulin 8).	6	8,21E-08	5.6	50093.55	4.94
P07437	SW:TBB1_HUMAN	Tubulin beta-1 chain.	6	1,08E-07	5.1(2)	49758.90	4.75
P05217	SW:TBB2_HUMAN	Tubulin beta-2 chain.	6	4,10E-06	5.1	49831.01	4.79
Q13509	SW:TBB3_HUMAN	Tubulin beta-3 chain.	6	3,13E-05	5.1	50432.68	4.83
P05218	SW:TBB5_MOUSE	Tubulin beta-5 chain.	6	3,98E-06	5.1(2)	49670.82	4.78
P08670	SW:VIME_HUMAN	Vimentin.	6	1,77E-08	5.2	53554.51	5.06
Transcription and translation							
P08865	SW:RSP4_HUMAN	40s ribosomal protein sa (p40) (34/67 kD laminin receptor)	5	4,59E-05	4.8	32854.08	4.79
Q15233	SW:NR54_HUMAN	54 kD nuclear rna-binding protein (p54(nrb)).	5	8,59E-05	8.4/8.6/8.8	54100.35	9.01
P49406	SW:RM19_HUMAN	60s ribosomal protein l19, mitochondrial precursor (l19mt) (mrp-l15).	4	pMism:8.79	5.3	32380.59	9.60
Q92841	SW:DD17_HUMAN	Dead-box protein 17 (dead-box protein p72) (probable rna-dependent helicase p72).	6	pMism:9.26	8.3	72371.44	8.82
P17844	SW:DDX5_HUMAN	Dead-box protein 5 (dead-box protein p68) (probable rna-dependent helicase p68).	6	pMism:8.39	8.5	69148.08	9.06
P13639	SW:EF2_HUMAN	Elongation factor 2 (ef-2).	4	pMism:7.30	7.0	95206.95	6.42
P49411	SW:EFTU_HUMAN	Elongation factor tu, mitochondrial precursor (p43).	9	2,71E-11	6.7	49541.54	7.26
P04765	SW:IF41_HUMAN	Eukaryotic initiation factor 4a-i (eif-4a-i).	6	1,59E-05	5.8	46153.93	5.32
O60812	CHR14-O60812	DJ845O24.4 (Heterogenous Nuclear Ribonucleoprotein HNRNP C1 LIKE protein).	4	6,30E-05	5.1	32142.36	4.93
Q99729	HUMANGP: CHR5-Q8N7U3	Heterogeneous nuclear ribonucleoprotein A/B hnRNP A/B APOBEC-1 binding protein 1 ABBP-1.	5	pMism:8.72	6.7	36612.57	9.04
P09651	SW:ROA1_HUMAN	Heterogeneous nuclear ribonucleoprotein a1 (helix-destabilizing protein).	7	4,88E-08	8.3	38714.59	9.26
P55795	SW:ROH2_HUMAN	Heterogeneous nuclear ribonucleoprotein h' (hnRNP h').	5	5,70E-06	6.1	49263.57	5.89
P31943	SW:ROH1_HUMAN	Heterogeneous nuclear ribonucleoprotein h (hnRNP h).	7	1,77E-05	5.9/6.2	49229.47	5.89
P31942	SW:ROH3_HUMAN	Heterogeneous nuclear ribonucleoprotein h3 (hnRNP h3) (hnRNP 2h9).	4	pMism:8.50	6.6	36926.49	6.37
Q07244	SW:ROK_HUMAN	Heterogeneous nuclear ribonucleoprotein k (hnRNP k)	7	3,24E-08	5.6	50976.25	5.39
P14866	SW:ROL_HUMAN	Heterogeneous nuclear	10	2,61E-11	7.0	60187.23	6.65

Table I. *continued*

SW.-Nr	Access. Nr	Protein Name	Matches	Probability	Observed pI	Theoretical MW	Theoretical pI
P22626	SW:ROA2_HUMAN	ribonucleoprotein l (hnrrnp l). Heterogeneous nuclear ribonucleoproteins	7	2,64E-10	8.1/8.2/ 8.3/8.4	37429.70	8.97
P07910	SW:ROC_HUMAN	a2/b1 (hnrrnp a2 and hnrrnp b1). Heterogeneous nuclear ribonucleoproteins	5	1,33E-06	5.2	33688.04	4.95
P82650	SW:RT22_HUMAN	c1/c2 (hnrrnp c1 and hnrrnp c2). Mitochondrial 28s ribosomal	7	5,21E-09	6.5	41280.38	7.70
Q15365	SW:PCB1_HUMAN	protein s22 (mrp-s22). Poly(rc)-binding protein 1 (hnrrnp-e1)	8	3,53E-11	6.8	37525.86	6.66
Q9uq80	SW:P2G4_HUMAN	(nucleic acid binding protein sub2.3). Proliferation-associated protein 2g4	9	2,11E-12	6.5	43786.86	6.13
P23246	SW:PSF_HUMAN	(cell cycle protein p38-2g4 homolog). Ptb-associated splicing factor (psf).	8	6,70E-05	6.5	76149.35	9.45
Q07955	SW:SFR1_HUMAN	Splicing factor, arginine/serine-rich 1.	7	5,82E-10	5.4	27613.39	10.37
P09661	SW:RU2A_HUMAN	U2 small nuclear ribonucleoprotein a' (u2 snrrnp-a').	6	pMism:11.52	8.5	28415.57	8.71
O28029	SW:SYA_ARCFU	Alanyl-tRNA synthetase (ec 6.1.1.7) (alanine--tRNA ligase) (alars).	6	pMism:7.70	5.8	102536.36	5.28
Q12828	HUMANGP: CHR1-Q12828	Fuse binding protein.	8	pMism:11.72	7.0	67473.31	7.18
Q92945	HSUGP: 091142-17-0	KH-type splicing regulatory protein (FUSE binding protein 2).	6	6,14E-07	7.5	72709.02	8.02
Signaling and apoptosis							
O00299	SW:CL1I_HUMAN	Chloride intracellular channel protein 1 (nuclear chloride ion channel 27) (ncc27) (p64 clcp) (chloride channel abp).	4	pMism:13.11	5.1	26922.73	5.09
P25388	SW:GBLP_HUMAN	Guanine nucleotide-binding protein beta subunit-like protein 12.3 (p205) (receptor of activated protein kinase c 1) (rack1).	10	pMism:16.17	6.9/7.0	35076.73	7.60
P21796	SW:POR1_HUMAN	Voltage-dependent anion-selective channel protein 1 (vdac1).	6	1,44E-09	8.0	30641.40	8.63
P45880	SW:POR2_HUMAN	Voltage-dependent anion-selective channel protein 2 (vdac2).	4	8,19E-06	7.5	38092.73	6.32
P12268	SW:IMD2_HUMAN	Inosine-5'-monophosphate dehydrogenase 2 (ec 1.1.1.205).	7	1,04E-08	6.7	55804.98	6.44
Metabolism							
Q99714	SW:HCD2_HUMAN	3-hydroxyacyl-CoA dehydrogenase type II (ec 1.1.1.35) (type II hadh) (endoplasmic reticulum-associated amyloid beta-peptide binding protein) (short-chain type dehydrogenase/reductase xh98g2).	6	pMism:12.24	6.7	26923.08	7.65
Q92931	TR_HUM:Q92931	3-hydroxyisobutyryl-Coenzyme A hydrolase.	6	pMism:8.75	6.4	42907.60	8.34
P42765	SW:THIM_HUMAN	3-ketoacyl-coa thiolase mitochondrial (ec 2.3.1.16)	7	3,35E-09	7.6	42039.29	8.51
Q13126	SW:MTAP_HUMAN	5'-methylthioadenosine phosphorylase (ec 2.4.2.28)	6	1,60E-05	6.9	31250.05	6.75
Q8tdm4	HUMANGP: CHR6-Q8TDM4	Acetyl coa transferase-like protein.	6	pMism:11.91	6.6	41251.69	6.27
P24752	SW:THIL_HUMAN	Acetyl-CoA acetyltransferase, mitochondrial precursor (ec 2.3.1.9) (acetoacetyl-CoA thiolase) (t2).	6	pMism:8.38	7.6/8.0	45199.55	8.98
Q99798	SW:ACON_HUMAN	Aconitate hydratase, mitochondrial precursor (ec 4.2.1.3).	9	2,08E-13	7.0/7.1	85425.41	7.36
P25705	SW:ATPA_HUMAN	ATP synthase alpha chain, mitochondrial precursor (ec 3.6.1.34).	10	2,63E-16	5.1	59750.63	9.16
P06576	SW:ATPB_HUMAN	ATP synthase beta chain,	10	7,99E-15	5.6	56559.90	5.26

Table I. *continued*

SW.-Nr	Access. Nr	Protein Name	Matches	Probability	Observed pI	Theoretical MW	Theoretical pI
O43175	SW:SERA_HUMAN	mitochondrial precursor (ec 3.6.1.34). D-3-phosphoglycerate dehydrogenase (ec 1.1.1.95).	11	2,38E-18	6.6	56650.50	6.29
P30038	SW:PUT2_HUMAN	Delta-1-pyrroline-5-carboxylate dehydrogenase precursor (ec 1.5.1.12).	5	1,38E-05	7.0	61751.53	8.25
Q13011	SW:ECH1_HUMAN	Delta 3,5-delta 2,4-dienoyl-coa isomerase, mitochondrial precursor (ec 5.3.3.-).	4	pMism:9.06	6.4	35994.34	6.61
P09622	SW:DLDH_HUMAN	Dihydrolipoamide dehydrogenase precursor (ec 1.8.1.4).	5	1,77E-05	6.8	54150.18	7.59
P04075	SW:ALFA_HUMAN	Fructose-bisphosphate aldolase (ec 4.1.2.13).	6	1,58E-08	6.9	39288.83	8.39
P07954	SW:FUMH_HUMAN	Fumarate hydratase, mitochondrial precursor (ec 4.2.1.2).	8	2,75E-13	7.8	54636.99	8.85
P51570	SW:GAL1_HUMAN	Galactokinase (ec 2.7.1.6) (galactose kinase).	10	pMism:19.42	6.1	42272.23	6.04
P11413	SW:G6PD_HUMAN	Glucose-6-phosphate 1-dehydrogenase (ec 1.1.1.49) (g6pd).	5	pMism:10.13	6.7	59134.57	6.44
P04406	SW:G3P2_HUMAN	Glyceraldehyde 3-phosphate dehydrogenase, liver (ec 1.2.1.12).	6	6,89E-07	8.2	35922.02	8.58
O75874	SW:IDHC_HUMAN	Isocitrate dehydrogenase [NADP] cytoplasmic (ec 1.1.1.42) (oxalosuccinate decarboxylase) (idh) (NADP+-specific icdh) (idp).	5	pMism:6.95	6.8	46659.30	6.53
P40926	SW:MDHM_HUMAN	Malate dehydrogenase, mitochondrial precursor (ec 1.1.1.37).	8	1,61E-12	8.1/8.2/8.3	35531.34	8.92
Q9hcc0	SW:MCCB_HUMAN	Methylcrotonyl-coa carboxylase beta chain, mitochondrial precursor (ec 6.4.1.4).	7	8,33E-08	6.8	61333.20	7.58
P00558	SW:PGK1_HUMAN	Phosphoglycerate kinase 1 (ec 2.7.2.3) (primer recognition protein 2) (prp 2).	5	pMism:8.69	7.8	44596.65	8.30
P18669	SW:PMGB_HUMAN	Phosphoglycerate mutase, brain form (ec 5.4.2.1).	6	1,80E-08	6.8	28672.74	6.75
P08559	SW:ODPA_HUMAN	Pyruvate dehydrogenase e1 component alpha subunit, somatic form, mitochondrial precursor (ec 1.2.4.1) (pdhe1-a type i).	6	pMism:10.10	6.8	43295.63	8.35
P11177	SW:ODPB_HUMAN	Pyruvate dehydrogenase e1 component, beta subunit precursor (ec 1.2.4.1).	5	0,00013819	5.5	39219.37	6.20
P14618	SW:KPY1_HUMAN	Pyruvate kinase, m1 (muscle) isozyme (ec 2.7.1.40).	6	2,46E-07	7.5/7.6	57805.70	7.95
Q16836	SW:HCDH_HUMAN	Short chain 3-hydroxyacyl-CoA dehydrogenase precursor (ec 1.1.1.35).	6	8,45E-08	8.2	34277.50	8.88
P31040	SW:DHSA_HUMAN	Succinate dehydrogenase [ubiquinone] flavoprotein subunit, mitochondrial precursor (ec 1.3.5.1) (fp) (flavoprotein subunit of complex II).	10	pMism:12.57	6.5	72691.51	7.06
P55809	SW:SCOT_HUMAN	Succinyl-CoA:3-ketoacid-Coenzyme A transferase precursor.	7	8,02E-09	6.4	56157.62	7.13
P40939	SW:ECHA_HUMAN	Trifunctional enzyme alpha subunit, mitochondrial precursor (tp-alpha) (78 kda gastrin-binding protein).	10	2,30E-08	8.0/8.3	82999.65	9.16
P00938	SW:TPIS_HUMAN	Triosephosphate isomerase (ec 5.3.1.1) (tim).	6	6,03E-06	6.6	26538.30	6.51
P13995	SW:MTDC_HUMAN	Bifunctional methylenetetrahydrofolate dehydrogenase/cyclohydrolase, mitochondrial precursor [includes: nad-dependent methylenetetrahydrofolate dehydrogenase (ec 1.5.1.15); methenyltetrahydrofolate cyclohydrolase (ec 3.5.4.9)].	4	pMism:7.27	7.5	37320.38	8.86
P00568	SW:KAD1_HUMAN	Adenylate kinase isoenzyme 1 (ec 2.7.4.3) (atp-amp transphosphorylase) (ak1) (myokinase).	4	pMism:7.51	8.7	21634.84	8.73
P30837	SW:DHA5_HUMAN	Aldehyde dehydrogenase, mitochondrial precursor (ec 1.2.1.3) (aldh class 2).	7	pMism:11.69	6.4	57217.40	6.41
P00505	SW:AATM_HUMAN	Aspartate aminotransferase, mitochondrial precursor (ec 2.6.1.1) (transaminase a) (glutamate oxaloacetate transaminase-2).	4	pMism:8.71	8.7/8.5	47475.57	9.14

Table I. *continued*

SW.-Nr	Access. Nr	Protein Name	Matches	Probability	Observed pI	Theoretical MW	Theoretical pI
P09382	SW:LEG1_HUMAN	Galectin-1 (beta-galactoside-binding lectin I-14-i) 6 (lactose-binding lectin 1) (s-lac lectin 1) (galaptin) (14 kDa lectin) (hpl) (hbl).		pMism:14.35	5.1	14584.51	5.34
P00367	SW:DHE3_HUMAN	Glutamate dehydrogenase 1 precursor (ec 1.4.1.3).	8	4,49E-09	6.1	61397.87	7.66
P49915	SW:GUAA_HUMAN	GMP synthase (glutamine-hydrolysing) (ec 6.3.5.2).	7	0,00019179	6.9	76715.41	6.42
P04181	SW:OAT_HUMAN	Ornithine aminotransferase, mitochondrial precursor (ec 2.6.1.13) (ornithine-oxo-acid aminotransferase).	5	pMism:9.85	6.2	48534.84	6.57
Q9y617	SW:SERC_HUMAN	Phosphoserine aminotransferase (ec 2.6.1.52) (psat).	9	pMism:14.20	6.8	40422.68	7.56
P32322	SW:PROC_HUMAN	Pyrroline-5-carboxylate reductase (ec 1.5.1.2) (p5cr).	5	1,23E-05	7.3	33374.65	7.18
P34897	SW:GLYM_HUMAN	Serine hydroxymethyltransferase, mitochondrial precursor (ec 2.1.2.1).	8	2,60E-09	7.5/7.6	55992.98	8.76
Q15102	SW:PA1G_HUMAN	Platelet-activating factor acetylhydrolase Ib gamma subunit (ec 3.1.1.47).	5	0,00019054	6.6	25734.24	6.33
P06733	SW:ENOA_HUMAN	Alpha enolase (ec 4.2.1.11) (2-phospho-d-glycerate hydro-lyase).	10	1,64E-12	6.8(2)/ 6.9(2)	47037.77	6.99
Q05524	SW:ENOL_HUMAN	Alpha enolase, lung specific (ec 4.2.1.11) (2-phospho-d-glycerate hydro-lyase) (non-neural enolase) (nne) (phosphopyruvate hydratase).	5	pMism:7.59	6.8/6.9(2)	49477.34	5.78
P13929	SW:ENOB_HUMAN	Beta enolase (ec 4.2.1.11) (2-phospho-d-glycerate hydro-lyase) (skeletal muscle enolase) (mse) (enolase 3).	6	pMism:8.32	6.7	46855.69	7.73
P09104	SW:ENOG_HUMAN	Gamma enolase (ec 4.2.1.11) (2-phospho-d-glycerate hydro-lyase)	6	6,77E-10	5.0	47137.39	4.91
Chaperones							
P10809	SW:P60_HUMAN	60 kDa heat shock protein, mitochondrial precursor (hsp60) (60 kDa chaperonin) (cpn60) (heat shock protein 60) (hsp-60) (mitochondrial matrix protein p1) (p60 lymphocyte protein) (hucha60). (p60 lymphocyte protein)	7	2,75E-07	5.4/5.5	61054.64	5.70
P11021	SW:GR78_HUMAN	78 kD glucose regulated protein precursor (grp 78)	9	7,27E-13	5.2	72332.96	5.07
P25685	SW:DJB1_HUMAN	DnaJ homolog subfamily b member 1 (heat shock 40 kDa protein 1) (heat shock protein 40) (hsp40) (DNAJ protein homolog 1) (hdj-1).	7	pMism:11.60	8.6	38044.11	8.74
P14625	SW:ENPL_HUMAN	Endoplasmic precursor (94 kD glucose-regulated protein)	8	1,62E-06	5.0/5.1	92468.87	4.76
P04792	SW:HS27_HUMAN	Heat shock 27 kD protein (hsp 27) (stress-responsive protein 27).	4	4,32E-06	6.1	22782.52	5.98
P11142	SW:HS7C_HUMAN	Heat shock cognate 71 kD protein.	5	7,80E-05	5.2	70898.09	5.37
P08238	SW:HS9B_HUMAN	Heat shock protein hsp 90-beta (hsp 84)	6	1,32E-07	5.5/5.6	83133.01	4.97
P30101	SW:ER60_HUMAN	Protein disulfide isomerase a3 precursor (ec 5.3.4.1) (disulfide isomerase er-60) (erp60) (58 kDa microsomal protein) (p58) (erp57) (58 kDa glucose regulated protein).	11	4,10E-18	5.7/5.8(2)	56782.39	5.98
Q15084	SW:ERP5_HUMAN	Protein disulfide isomerase a6 precursor (ec 5.3.4.1) (protein disulfide isomerase p5).	5	1,44E-05	5.5	48121.32	4.95
P07237	SW:PDI_HUMAN	Protein disulfide isomerase precursor (pdi).	8	1,71E-09	4.8	57116.37	4.76
P38646	SW:GR75_HUMAN	Stress-70 protein, mitochondrial precursor (75 kDa glucose regulated protein) (grp 75) (peptide-binding protein 74) (pbp74) (mortalin) (mot).	11	7,46E-18	5.6/5.7	73680.50	5.87

Table I. *continued*

SW.-Nr	Access. Nr	Protein Name	Matches	Probability	Observed pI	Theoretical MW	Theoretical pI
P31948	SW:IEFS_HUMAN - Matches ()	Stress-induced-phosphoprotein 1 (stil) (hsp70/hsp90-organizing protein) (transformation-sensitive protein ief ssp 3521).	8	pMism:12.32	6.7	62639.26	6.40
P78371	SW:TCPB_HUMAN	T-complex protein 1, beta subunit (tcp-1-beta).	6	7,79E-09	6.2	57488.21	6.01
P48643	SW:TCPE_HUMAN	T-complex protein 1, epsilon subunit (tcp-1-epsilon).	5	0,00015145	5.5	59671.02	5.45
Q99832	SW:TCPH_HUMAN	T-complex protein 1, eta subunit (tcp-1-eta) (cct-eta) (hiv-1 nef interacting protein).	7	pMism:12.42	6.9	59366.62	7.55
P40227	SW:TCPZ_HUMAN	T-complex protein 1, zeta subunit (tcp-1-zeta)	8	1,17E-09	6.3	58024.17	6.24
P55072	SW:TERA_HUMAN	Transitional endoplasmic reticulum atpase (ter atpase) (15s mg(2+)- atpase p97 subunit) (valosin containing protein) (vcp) [contains: valosin].	9	pMism:10.58	5.3	89321.80	5.14
P05092	SW:CYPH_HUMAN	Peptidyl-prolyl cis-trans isomerase a (ec 5.2.1.8).	5	9,96E-05	7.2	17881.30	7.82
Protein turnover							
P43686	SW:PRS6_HUMAN	26s protease regulatory subunit 6b (mip224) (mb67 interacting protein) (tat-binding protein-7) (tbp-7).	4	pMism:7.79	5.2	47366.25	5.09
P28838	SW:AMPL_HUMAN	Cytosol aminopeptidase (ec 3.4.11.1) (leucine aminopeptidase).	8	1,93E-09	6.5	52640.08	6.29
O75439	SW:MPPB_HUMAN	Mitochondrial processing protease beta subunit precursor (ec 3.4.24.64)	6	2,18E-05	6.1	54366.14	6.38
P25786	SW:PRC2_HUMAN	Proteasome subunit alpha type 1 (ec 3.4.25.1) (proteasome component c2) (macropain subunit c2) (multicatalytic endopeptidase complex subunit c2) (proteasome nu chain) (30 kDa prosomal protein) (pros-30).	5	3,23E-06	6.5	29555.59	6.15
P25787	SW:PRC3_HUMAN	Proteasome subunit alpha type 2 (ec 3.4.25.1) (proteasome component c3) (macropain subunit c3) (multicatalytic endopeptidase complex subunit c3).	7	3,98E-11	6.9	25767.39	7.12
P25789	SW:PSA4_HUMAN	Proteasome subunit alpha type 4 (ec 3.4.25.1) (proteasome component c9) (macropain subunit c9) (multicatalytic endopeptidase complex subunit c9) (proteasome subunit l).	4	pMism:9.27	7.3	29483.81	7.58
P49721	SW:PSB2_HUMAN	Proteasome subunit beta type 2 (ec 3.4.25.1) (proteasome component c7-I) (macropain subunit c7-I) (multicatalytic endopeptidase complex subunit c7-I).	5	pMism:11.28	6.5	22836.28	6.52
Antioxidant proteins							
Q9y4l1	SW:OXRP_HUMAN	150 kDa oxygen-regulated protein precursor (orp150).	12	pMism:14.80	5.3	111335.39	5.16
P30041	CHR1-AOP2	Antioxidant protein 2 (1-Cys peroxiredoxin) (1-Cys PRX).	4	3,55E-05	6.4	24903.79	6.02
P13804	SW:ETFA_HUMAN	Electron transfer flavoprotein alpha-subunit precursor (alpha-etf).	8	3,22E-11	6.9	35079.57	8.62
P38117	SW:ETFB_HUMAN	Electron transfer flavoprotein beta-subunit (beta-etf).	6	1,05E-05	8.0	27843.61	8.25
P09211	SW:GTP_HUMAN	Glutathione s-transferase p (ec 2.5.1.18) (gst class-pi) (gstp1-1).	5	pMism:11.81	5.6	23224.64	5.44
P30048	SW:TDXM_HUMAN	Mitochondrial thioredoxin-dependent peroxide reductase precursor.	4	7,83E-05	6.1	27692.65	7.68
P49821	SW:NUBM_HUMAN	NADH-ubiquinone oxidoreductase 51 kDa subunit, mitochondrial precursor (ec 1.6.5.3) (ec 1.6.99.3) (complex I-51kd) (ci-51kd).	13	pMism:21.05	7.0	50817.09	8.51
Q06830	SW:PDX1_HUMAN	Peroxisome protein 1 (thioredoxin peroxidase 2)	4	pMism:8.02	7.5/8.0	22110.36	8.27

Table I. *continued*

SW.-Nr	Access. Nr	Protein Name	Matches	Probability	Observed pI	Theoretical MW	Theoretical pI
P32119	SW:PDX2_HUMAN	(thioredoxin-dependent peroxide reductase 2) (proliferation-associated protein pag) (natural killer cell enhancing factor a) (nkef-a). Peroxiredoxin 2 (ec 1.11.1.-) (thioredoxin peroxidase 1) (thioredoxin- dependent peroxide reductase 1) (thiol-specific antioxidant protein) (tsa) (prp) (natural killer cell enhancing factor b) (nkef-b).	4	pMism:10.74	5.6	21891.92	5.66
Q13228	SW:SBP1_HUMAN	Selenium-binding protein 1.	9	7,58E-10	6.2	52312.86	6.13
P04179	SW:SODM_HUMAN	Superoxide dismutase [mn], mitochondrial precursor (ec 1.15.1.1).	4	pMism:7.41	7.0	24722.09	8.35
P22695	CHR16-UCR2	Ubiquinol-cytpchrome c reductase complex core protein 2	4	4,65E-05	7.7	48443.01	8.74
P47985	SW:UCRI_HUMAN	Ubiquinol-cytochrome c reductase iron-sulfur subunit, mitochondrial precursor (ec 1.10.2.2) (rieske iron-sulfur protein) (risp).	4	pMism:9.16	6.7	29651.99	8.55
P31930	SW:UCR1_HUMAN	Ubiquinol-cytochrome-c reductase complex core protein I precursor (ec 1.10.2.2).	7	2,18E-05	5.6	52618.79	5.94
P04040	SW:CATA_HUMAN	Catalase (ec 1.11.1.6).	5	6,05E-05	7.0	59624.98	6.95
Miscellaneous							
P30040	SW:ER29_HUMAN	Endoplasmic reticulum protein erp29 precursor (erp31).	5	1,36E-05	5.9	28993.43	6.77
P30042	SW:ES1_HUMAN	Es1 protein homolog, mitochondrial precursor (protein knp-I) (gt335 protein).	4	pMism:9.49	7.0	28142.38	8.50
O15240	CHR7-O15240	Neuro-endocrine specific protein VGF	6	6,28E-10	4.9	67286.83	4.75
P82979	SW:HCC1_HUMAN	Nuclear protein hcc-1 (hspc316).	4	pMism:8.22	6.5	23670.81	6.10
P06748	SW:NPM_HUMAN	Nucleophosmin (npm) (nucleolar phosphoprotein b23) (numatrin) (nucleolar protein no38).	5	pMism:11.07	4.8	32575.02	4.64
Q9p0i1	HUMANGP: CHR4-Q9P0I1	Nucleoporin p54 protein.	6	pMism:8.75	6.8	55105.12	7.14
P35232	SW:PHB_HUMAN	Prohibitin.	9	6,13E-16	5.5	29804.10	5.57
O14805	CHR1-O14805	RNA-binding protein regulatory subunit.	4	1,38E-06	6.2	19891.05	6.33
P02768	SW:ALBU_HUMAN	serum albumin precursor.	5	3,31E-05	7.7	69366.68	5.92
Q13148	SW:TDBP_HUMAN	Tar dna-binding protein-43 (tdp-43).	4	pMism:7.33	6.0	44739.81	5.85
Q15092	HUMANGP: CHR2-Q15092	Transmembrane protein.	6	pMism:9.13	6.0	83677.91	6.08
O96008	SW:OM40_HUMAN	Probable mitochondrial import receptor subunit tom40 homolog	5	3,51E-07	7.0	37893.10	6.79
O75932	CHR11-O75932-1	SYT interacting protein SIP.	6	6,80E-07	8.0	69491.65	9.68
Hypothetical proteins							
Q96c36	CHR1-AAH20553	Similar to pyrroline 5-carboxylate reductase isoform.	5	3,57E-05	7.8	33637.17	7.66
P42694	SW:Y054_HUMAN	Potential helicase with zinc-finger domain.	4	pMism:7.63	6.7	218971.10	6.98
O60376	CHR9-O60376	P1.11659_4.	6	3,60E-06	5.5	38749.23	6.40
Q9h370	HUMANGP: CHR19-Q9H370	Pro1512.	5	pMism:8.92	6.7	34122.09	5.81
CAD13067 ^a	HUMANGP: CHR3-CAD13067	Unnamed protein product.	8	pMism:13.06	6.5		
Q9BS94	TR_HUM:Q9BS94	Similar to 3-hydroxyisobutyryl-Coenzyme A hydrolase.	5	pMism:8.11	7.0	37380.52	8.76
Q8NBS9	TR_HUM:Q9BVH9	Thioredoxin domain containing protein 5 [Precursor] Thioredoxin-like protein p46 Endoplasmic reticulum protein ERp46.	5	pMism:8.53	5.5	47628.86	5.63

Discussion

A comprehensive map of supratentorial PNET cell line PFSK-1, consisting of 157 proteins from several classes, was established. No specific marker for this cell line, however, could be identified although the hypothetical proteins expressed in PFSK-1 have never been described before in any normal or tumor cell line. The map very much resembles normal non-neuronal and non-glial lineages and, indeed, no specific neuronal or glial structures including neurofilaments, synaptosomal proteins, glial fibrillary acidic protein or CNPase were detectable, proteins that were consistently found in neuronal or glial lineages by proteomic methods (10, 19-23, unpublished results). The map does not resemble those of medulloblastoma cell lines reported recently, either (9). A first clue that may point to the mesenchymal origin of PFSK-1 comes from the expression of vimentin, a class III intermediate filament and the major phosphoprotein found in mesenchymal cells (24) and tumors. FUSE binding protein 1, a helicase and sequence-specific, single-strand binding protein, activates the far upstream element of c-myc and may therefore provide a link to this protooncogene (25). Indeed, Myc plays a major pathogenetic role in a series of tumors (26). The immature nature of the tumor cell line PFSK-1 is proposed by expression of the *potential helicase* P42694, a member of the DNA2/NAM7 helicase family that is highly expressed in embryonic tissues (27). The presence of neuroendocrine protein VGF in PFSK-1, strongly involved in developmental processes, may emphasise the immaturity of the cell line (28-30) but the term "neuroendocrine" must not lead to the interpretation that PFSK-1 may be assigned to a neuronal lineage; VGF is expressed in a series of human tissues although we failed to demonstrate expression in any cell line studied so far, including amnion, fibroblast, kidney epithelial, bronchial epithelial or lymphocytes using comparable methodology.

In cell types given above, mesothelial cells and HCN₂ neurons, we failed to detect SYT interacting protein SIP (Antonson *et al.*, 1998, direct submission to EMBL/GenBank/DBJ databases, 31) but in medulloblastoma cell lines (9) and here in PFSK-1 it was an abundant protein and may therefore be considered a tumor marker candidate.

Metabolic enzymes *similar to pyrroline 5-carboxylate reductase isoform* (32), *similar to 3-hydroxyisobutyryl-CoA hydrolase* (33, 34) and *thioredoxin domain-containing protein 5* have been so far only predicted from nucleic acid sequences from malignant tumors and may reflect isoforms with predominant or specific expression in malignancy, but may not represent tumor markers *per se*, even if never described before in non-malignant cell lineages. However, this finding is too premature to be further addressed in this report.

Predicted *protein P1.11659* contains a prohibitin domain indicating a role for inhibition of DNA synthesis (Lamerdin *et al.*, 1998, direct submission to EMBL/GenBank/DBJ databases) and this protein as well as the following two may be tumor-specific or at least tumor-related structures. Predicted *protein Pro1512* contains a NIF-1 domain representing a minimal protein phosphatase motif. This protein may play a role for signal transduction in terms of phosphorylation/dephosphorylation reactions and may well represent another tumor marker candidate as Pro1512 was never reported in normal or tumor cell lines at the protein level (Zhang *et al.*, 1999, direct submission to EMBL/GenBank/DBJ databases).

Predicted *protein unnamed protein product CAD13067* [Gstaiger *et al.*, 2001; direct submission to EMBL/GenBank/DBJ databases] does not present with any known domain or motif and no putative function can be predicted. Homology searches have not revealed any significant hits and we have therefore decided to study tentative roles and functions of this possible tumor marker candidate in the future.

Methodologically, proteins were unambiguously identified by a protein chemical rather than an immunochemical method. The data are therefore more reliable and independent of antibody specificity and availability. The method, however, has limitations as e.g. very high and very low molecular weight and hydrophobic proteins are hardly detectable and the map demonstrated here consists of hydrophilic structures (10, 35).

In this report we have presented the first hydrophilic protein map of a PNET cell line, we have identified tentative tumor marker candidates and provided some proteins that may point to the mesenchymal origin and immature nature of this tumor. The experienced proteome scientist would consider the PFSK-1 expressional pattern basically comparable to those of normal non-neuronal, non-glial cell lineages.

Acknowledgements

This study was supported by a grant from Oesterreichische Nationalbank, Jubilaeumsfondsprojekt Nr. 9187 to Irene Slavc. We are highly indebted to the Red Bull Company, Salzburg, Austria for their generous support of this study. The authors thank Claudia Avramovic for formatting and editing the manuscript.

References

- 1 Pollak IF: Brain tumors in children. *N Engl J Med* 331: 1500-1507, 1994.
- 2 Hart MN and Earle KM: Primitive neuroectodermal tumors of the brain in children. *Cancer* 32: 890-897, 1973.
- 3 Rorke LB: The cerebellar MB and its relationship to primitive neuroectodermal tumors. *J Neuropathol Exp Neurol* 42: 1-15, 1983.

- 4 Rubinstein LJ: Embryonal central neuroepithelial tumors and their differentiating potential: a cytogenetic view of a complex neuro-oncological problem. *J Neurosurg* 62: 795-805, 1985.
- 5 Reddy AT, Janss AJ, Phillips PC, Weiss HL and Packer RJ: Outcome for children with supratentorial primitive neuroectodermal tumors treated with surgery, radiation, and chemotherapy. *Cancer* 88: 2189-2193, 2000.
- 6 Pomeroy SL, Tamayo P, Gaasenbeek M, Sturla LM, Angelo M, McLaughlin ME, Kim JY, Goumnerova LC, Black PM, Lau C, Allen JC, Zagzag D, Olson JM, Curran T, Wetmore C, Biegel JA, Poggio T, Mukherjee S, Rifkin R, Califano A, Stolovitzky G, Louis DN, Mesirov JP, Lander ES and Golub TR: Prediction of central nervous system embryonal tumour outcome expression. *Nature* 415: 436-442, 2002.
- 7 Fults D, Pedone CA, Morse HG, Rose JW and McKay RD: Establishment and characterization of a human primitive neuroectodermal tumor cell line from the cerebral hemisphere. *J Neuropathol Exp Neurol* 51: 272-280, 1992.
- 8 Jae-Kyung M, Gulesserian T, Fountoulakis M and Lubec G: Deranged hypothetical proteins Rik protein, Nit protein 2 and mitochondrial inner membrane protein, Mitofilin, in fetal Down syndrome brain. *Cell Mol Biol (Noisy-le-grand)* 49: 739-746, 2003.
- 9 Peyrl A, Krapfenbauer K, Slavc I, Yang JW, Strobel T and Lubec G: Protein profiles of medulloblastoma cell lines DAOY and D283: identification of tumor-related proteins and principles. *Proteomics* 3: 1781-1800, 2003.
- 10 Lubec G, Krapfenbauer K and Fountoulakis M: Proteomics in brain research: potentials and limitations. *Progr Neurobiol* 69: 193-211, 2003.
- 11 Engidawork E, Gulesserian T, Fountoulakis M and Lubec G: Expression of hypothetical proteins in human fetal brain: increased expression of hypothetical protein 28.5kDa in Down syndrome, a clue for its tentative role. *Mol Genet Metab* 78: 295-301, 2003.
- 12 Cheon MS, Fountoulakis M, Dierssen M, Ferreres JC and Lubec G: Expression profiles of proteins in fetal brain with Down syndrome. *J Neural Transm Suppl* 61: 311-319, 2001.
- 13 Gulesserian T, Engidawork E, Fountoulakis M and Lubec G: Decreased brain levels of Lupus La protein and increased U5 small ribonucleoprotein-specific 40 kDa protein in fetal Down syndrome. *Cell Mol Biol (Noisy-le-grand)* 49: 733-738, 2003.
- 14 Bradford MM: A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 72: 248-254, 1976.
- 15 Weitzdoerfer R, Fountoulakis M and Lubec G: Reduction of actin-related protein complex 2/3 in fetal Down syndrome brain. *Biochem Biophys Res Commun* 293: 836-841, 2002.
- 16 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.
- 17 Press WM, Teukolsky SA, Vetterling WT and Flannery BP: Nonlinear Models. In: Numerical Recipes in C. The Art of Scientific Computing (Second Edition). Cambridge, Cambridge University Press, 1992, pp. 683-688
- 18 Rockwood AL, VanOrden SL and Smith R: Rapid calculation of isotope distributions. *Anal Chem* 67: 2699-2704, 1995.
- 19 Peyrl A, Krapfenbauer K, Slavc I, Strobel T and Lubec G: Proteomic characterization of the human cortical neuronal cell line HCN-2. *J Chem Neuroanat* 26: 171-178, 2003.
- 20 Fountoulakis M, Schuller E, Hardmeier R, Berndt P and Lubec G: Rat brain proteins: two-dimensional protein database and variations in the expression level. *Electrophoresis* 20: 3572-3579, 1999.
- 21 Karlsson K, Cairns N, Lubec G and Fountoulakis M: Enrichment of human brain proteins by heparin chromatography. *Electrophoresis* 20: 2970-2976, 1999.
- 22 Langen H, Berndt P, Roder D, Cairns N, Lubec G and Fountoulakis M: Two-dimensional map of human brain proteins. *Electrophoresis* 20: 907-916, 1999.
- 23 Vlkolinsky R, Cairns N, Fountoulakis M and Lubec G: Decreased brain levels of 2',3'-cyclic nucleotide-3'-phosphodiesterase in Down syndrome and Alzheimer's disease. *Neurobiol Aging* 22: 547-553, 2001.
- 24 Perreau J, Lilienbaum A, Vasseur M and Paulin D: Nucleotide sequence of the human vimentin gene and regulation of its transcription in tissues and cultured cells. *Gene* 62: 7-16, 1988.
- 25 Duncan R, Bazar L, Michelotti G, Tomonaga T, Krutzsch H, Avigan M and Levens D: A sequence-specific, single-strand binding protein activates the far upstream element of c-myc and defines a new DNA-binding motif. *Genes Dev* 8: 465-480, 1994.
- 26 Raetz EA, Kim MK, Moos P, Carlson M, Bruggers C, Hooper DK, Foot L, Liu T, Seeger R and Carroll WL: Identification of genes that are regulated transcriptionally by Myc in childhood tumors. *Cancer* 98: 841-853, 2003.
- 27 Wagner DS, Gan L and Klein WH: Identification of a differentially expressed RNA helicase by gene trapping. *Biochem Biophys Res Commun* 262: 677-684, 1999.
- 28 Levi A, Eldridge JD and Paterson BM: Molecular cloning of a gene sequence regulated by nerve growth factor. *Science* 229: 393-395, 1985.
- 29 Lombardo A, Rabacchi SA, Cremisi F, Pizzorusso T, Cenni MC, Possenti R, Barsacchi G and Maffei L: A developmentally regulated nerve growth factor-induced gene, VGF, is expressed in geniculocortical afferents during synaptogenesis. *Neuroscience* 65: 997-1008, 1995.
- 30 Snyder SE, Pintar JE and Salton SR: Developmental expression of VGF mRNA in the prenatal and postnatal rat. *J Comp Neurol* 394: 64-90, 1998.
- 31 Brett D, Whitehouse S, Antonson P, Shipley J, Cooper C and Goodwin G: The SYT protein involved in the t(X;18) synovial sarcoma translocation is a transcriptional activator localised in nuclear bodies. *Hum Mol Genet* 6: 1559-1564, 1997.
- 32 Delauney AJ and Verma DP: A soybean gene encoding delta 1-pyrroline-5-carboxylate reductase was isolated by functional complementation in *Escherichia coli* and is found to be osmoregulated. *Mol Gen Genet* 221: 299-305, 1990.
- 33 Minami-Ishii N, Taketani S, Osumi T and Hashimoto T: Molecular cloning and sequence analysis of the cDNA for rat mitochondrial enoyl-CoA hydratase. Structural and evolutionary relationships linked to the bifunctional enzyme of the peroxisomal beta-oxidation system. *Eur J Biochem* 185: 73-78, 1989.
- 34 Muller-Newen G and Stoffel W: Mitochondrial 3-trans-enoyl-CoA isomerase. Purification, cloning, expression, and mitochondrial import of the key enzyme of unsaturated fatty acid beta-oxidation. *Biol Chem Hoppe Seyler* 372: 613-624, 1991.
- 35 Fountoulakis M: Proteomics: current technologies and applications in neurological disorder and toxicology. *Amino Acids* 21: 363-381, 2001.

Received November 3, 2003

Accepted February 18, 2004