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Characterization of genes, down-regulated in human glioma, potential tumour suppressor genes

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Comparison of gene expression profiles in human normal brain and glioblastoma using SAGE database revealed 129 genes with 5-fold difference of expression level in glioblastoma (P 0.05), 85 of them were down-regulated. The number of genes with 5-fold down-regulated expression is less in the diffuse and anaplastic astrocytomas. Five-fold decrease of the expression in the diffuse astrocytoma and nearly the same expression levels in the anaplastic astrocytoma and glioblastoma were revealed for 9 genes only. For overwhelming majority of inactivated genes in the low-grade astrocytoma the expression level decreased progressively in the subsequent stages of malignant progression of astrocytoma. Expression levels of some genes were very low or undetectable in glioblastoma, the most aggressive brain tumour. The decreased expression of selected genes in glioblastoma was confirmed by Northern analysis and RT-PCR. Some genes, described in this work, may encode the tumour suppressors and their decreased expression may play an important role in initiation and progression of human glioma.

Keywords: *glioma, astrocytoma, glioblastoma, down-regulated genes, potential tumour suppressors*

Introduction. Several methods for the identification of differential gene expression have been developed. These methods include two most powerful technologies, hybridization of DNA-microchips and Serial Analysis of Gene Expression (SAGE), which revolutionised biomedical research in determining the roles of genes in various pathologies, particularly during the tumour process. Gene expression patterns and the information on genes, differentially expressed in various tumours, obtained by modern multi-factor analysis, enables the use of the combinations of identified genes for determination of specific tumour type within current tumour category, improving the diagnostics, and determining justified criteria of treatment choice for each patient [1]. The availability of early diagnostic markers would decrease sig-

nificantly the incidence of tumour diseases and the mortality rate. The application of SAGE in the previous publications allowed us to investigate and characterise the genes with significantly increased expression level in glioblastomas [2, 3]. Comparative analysis of SAGE-libraries of astrocytomas of malignancy grades II–IV and human normal brain (NB) revealed the number of genes, the expression of which increases or decreases in tumours cells, to increase in the course of malignant progression. Current work attempts to characterise the genes with decreased level of expression in astrocytic tumours.

Materials and Methods. 9 SAGE libraries of glioblastomas (malignancy grade IV astrocytomas according WHO classification), 11 SAGE libraries of anaplastic astrocytomas (malignancy grade III astrocytomas), 8 SAGE libraries of diffuse astrocytomas (malignancy grade II astrocytomas), and 5 NB SAGE libraries have been analysed in order to compare the expression of genes using Digital Gene Expression Displayer (DGED) software and databases of SAGE Genie web-site (http://cgap.nci.nih.gov/SAGE). Unigene database (National Center of Biotechnological Information, NCBI) was used to search for cDNA clones, containing encoding regions of mRNA. Selected cDNA clones were obtained from Resource Centre/Primary DataBase, RZPD, of *Human Genome Project*, Germany. Surgical samples of glial tumours and NB (histologically normal brain tissue, adjacent to the tumour, compulsorily ablated with the tumour during the operation) were immediately frozen after resection in liquid nitrogen and stored at –70°C.

Total RNA was extracted from frozen tissues by acidic guanidine isothiocyanate-phenol-chloroform solution [4] and analysed by Northern hybridisation, as described in [2, 3]. Densitometric analysis of hybridisation signals was performed using Scion Image 1.62c software.

cDNAs were synthesised with oligo(dT) primer and reverse transcriptase (RT) RevertAid (*Fermentas*, Lithuania) using the same amount of total RNA (10 g for each sample). Semi-quantitative polymerase chain reaction (PCR) was performed according to Rae *et al*. [5] in 20 l of the reaction mixture, containing cDNA, synthesised on 0.5 g of RNA, 2 units of Taq-polymerases 1xPCR buffer, 0.2 mM dNTPs, and 1 primers for corresponding genes:

CPNE6 (for TGTCCCACCTGCACACGTTTG, rev CGTGTCATTCACCACTTGGGG);

DRD1IP (for CAGCAGAATTTCCCTGACCTGG, rev ACGCGCTGGTCACAGGAGCTG);

FAT2 (for GGAAGGAAGGAACTAATTCTTC, rev CTCTACACATGTGTACACACG);

GRIN1 (for TTCGGCATAGGCATGCGCAAAG, rev CACAGACAAGGCGCCCGTTAG);

GRM4 (for GTACACCACTTGCATCGTCTGG, rev ACGCAGGTTCTTGTGGTAGCCT);

SLC12A5 (for CATCAAGGACTCATCAAGGACT, rev AACTGGCACTGAGGAGCTCTGG).

PCR was performed with the following parameters: denaturation at 94°C, 30 sec; annealing at the temperature, corresponding to each of the primer pairs, 1 min; synthesis – 72°C, 1 min (30 cycles) and 72°C, 7 min. The number of cycles was gradually decreased till amplification of PCR-product remained at the level of linear phase (27 cycles). PCR products were detected with ethidium bromide staining after electrophoresis in 2% agarose gel.

Results and Discussion. To compare the relative levels of gene expression in astrocytomas of different malignancy grades and NB by SAGE we used public database of Cancer Genome Anatomy Project. Using DGED option, expression change ratio was set to be 5 for the increase or decrease in the level of expression of each gene, while statistical probability was P 0.05. The comparison of 9 glioblastoma SAGE libraries and 5 NB SAGE libraries revealed 199 gene tags with 5-fold difference in their distribution in these two pools [2]. In case when more than one tag matched one gene, the most probable one was selected. Five-fold changes in expression of 129 genes were detected upon deletion of tags for non-characterised expressed nucleotide sequences, tags without nucleotide sequences in Unigene clusters, tags of mitochondrial genes, and genes encoding hypothetic proteins. 85 genes matched the criteria of overexpressed in NB or of potential tumour-suppressor genes. Table 1, 7th column shows the correlation between the number of tags in all investigated SAGE-libraries of glioblastomas and number of those in all of NB. As it is seen, gene expression decreased more than 10-fold in the vast majority of cases.

Our investigation was based on 9 glioblastoma SAGE-libraries and 5 NB SAGE-libraries present in SAGE Genie database. Certainly, the expansion of the number of libraries may lead to the changes in the pattern of differential expression of genes. However, the comparison of the results obtained in this work with the results of the comparison of 5 glioblastoma SAGE libraries and 2 NB SAGE libraries [6] revealed the majority of 117 genes, identified in the aforementioned work, to be included into the present list of 129 genes [2].

The comparison of the pools of 11 anaplastic astrocytoma SAGE libraries and 8 astrocytoma SAGE libraries with the pool 5 NB SAGE libraries at equal conditions of analysis, revealed the total number of tags with 5-fold distribution difference to be slightly lower than in the case of comparison of glioblastomas and NB – 118 and 83 tags, respectively. The processing of data revealed 66 genes with 5-fold difference of expression level in anaplastic astrocytomas, 48 of which were of decreased expression, and 42 genes with 5-fold difference of expression level in astrocytomas, 26 of which were of decreased level of expression. Therefore, the number of genes with significant decrease of expression level increases throughout astrocytic glioma development.

There are two critical questions in modern investigation – what is the threshold in gene expression after which the changes can be considered significant and trustworthy and what are diagnostic, pathophysiological, and therapeutic consequences of the mentioned changes? Till there is no univalent answer for the latter question, every research group makes its own decision. For example, Lal *et al.* [7] described the genes with more than 5-fold expression with $P<0.001$ analysing the differential expression of genes in glioblastomas and NB by SAGE; Loging *et al.* [1] used the same approach to analyse the genes with more than 10-fold changes in expression; Markert *et al*. [8] and Ljubimova *et al.* [9], as well as some other researchers, discussed the possible role of genes even with less than 2-fold expression change in the initiation and progression of tumours. Definitely, lowering the threshold allows identifying a greater number of differentially expressed genes, although the vast majority of genes with significant changes in expression are possibly more important biologically-wise.

The comparison of lists of genes with 5-fold decreased expression in astrocytomas revealed some expression changes to be at the same level throughout the development of astrocytomas, although significant decrease of expression level of most of the genes occur during the later, the most malignant, stages of tumour development. Thus, the level of expression of *CA11*, *DLG4*, *EEF1A2*, *RPH3A*, *MICAL2*, *EPHB6*, *NRIP3*, and *TTR* (Table 2, group I) decreased in diffuse astrocytomas and remained relatively the same during the next stages of astrocytomas development, yet tags of FAT2 (FAT tumour suppressor homolog 2 (*Drosophila*)) is present in neither one of SAGE-libraries of astrocytomas of malignancy grades II–IV. At the same time, the expression levels of 31 genes (Table 2, group II) decrease more than 5-fold in diffuse astrocytomas, and, furthermore decreases during the following stages of astrocytoma development. Some genes (*CPNE6*, *KCNQ2*, *GALNT9*, *SLC1A6*, *GRM4*, *FSTL5*, *NEUROD1*) terminate their expression in glioblastomas, which is evident from Table 1. The decrease in the level of expression of 35 genes drops down more than 5-fold in glioblastomas only (Table 2, group III). The level of expression of 10 genes (Table 2, group IV) varies at different stages of astrocytoma development – in anaplastic astrocytomas (for *ZIC4* and *SYT5* in glioblastomas as well) level of expression is higher, compared to astrocytomas.

Northern analysis of individual independent tumour samples is the most applicable and the most reliable method to confirm the result of the comparison of gene expression profiles. Northern hybridisation of randomly selected genes generally confirmed the results of SAGE. Fig.1 shows high expression levels of *DNM1* and *NRGN* in the majority of NB samples, however, their mRNA contents in glioblastomas is significantly lower, if any at all. A high content of *MBP* mRNA was observed in NB and was detected to be much lower in astrocytic gliomas of malignancy grades II–IV; in glioblastomas this mRNA was not detected at all (Fig.2). SAGE results for *SNAP25* and *STXBP1* were not confirmed by Northern hybridisation – *SNAP25* is expressed at a high level in both NB and astrocytomas of malignancy grades II–IV, while

Table 1

The list of genes with decreased expression level in glioblastoma, compared to normal human brain (NB)

N°.	Tag	Gene name	Gene symbol	Libraries		Tags		Tag Odds A:B	P
				A	B	A	B		
1	2	3	4	5	6	7	8	9	10
1	TGGGAAGTGG	Maternally expressed 3	*MEG3*	7	5	70	163	0,15	0,04
2	CCCCCAATTC	Vesicle-associated membrane protein 2 (synaptobrevin 2)	*VAMP2*	8	5	63	158	0,14	0,01
3	GTCGCTGAGA	Carbonic anhydrase XI	*CA11*	9	5	48	123	0,13	0,02
4	AAGCATTAAA	Protein kinase, cAMP-dependent, regulatory, type I, beta	*PRKAR1B*	8	5	40	113	0,12	0,01
5	CGGGGAGATG	NDRG family member 2	*NDRG2*	7	5	43	118	0,12	0,01
6	ATTAAAGTCA	Regulating synaptic membrane exocytosis 3	*RIMS3*	7	5	21	65	0,11	0,04
7	GTTTAAAAAG	Stathmin-like 2	*STMN2*	8	5	21	63	0,11	0,05
8	TGGACACTCA	Neurochondrin	*NCDN*	9	5	81	252	0,11	0,00
9	ATCCGTGCCC	Calmodulin 3 (phosphorylase kinase, delta)	*CALM3*	8	5	28	106	0,09	0,00
10	CCCTTCCTTT	ATPase, H^+ transporting, lysosomal 13 kDa, V1 subunit G isoform 2	*ATP6V1G2*	5	5	15	58	0,09	0,01
11	GCCTCAATAA	Mitogen-activated protein kinase 8 interacting protein 2	*MAPK8IP2*	7	5	23	87	0,09	0,00
12	AGTGGAAGGT	Discs, large homolog 4 (Drosophila)	*DLG4*	7	5	17	77	0,08	0,00
13	ATCCCTTCCC	Septin 5	*SEPT5*	7	5	16	69	0,08	0,00
14	CTTCAGGACC	Syntaxin binding protein 1	*STXBP1*	8	5	24	104	0,08	0,00
15	TAATATTAAA	Synaptosomal-associated protein, 25 kDa	*SNAP25*	5	5	25	104	0,08	0,00
16	TCTGCACCTC	Eukaryotic translation elongation factor 1 alpha 2	*EEF1A2*	8	5	44	184	0,08	0,00
17	TGCCCAAATG	Leucine-rich repeat LGI family, member 3	*LGI3*	4	5	10	42	0,08	0,03
18	TGGGACGTGA	EPHB6	*EPHB6*	6	3	9	41	0,08	0,02
19	AAAGGGAATG	Solute carrier family 17 (sodium-dependent inorganic phosphate cotransporter), member 7	*SLC17A7*	2	4	21	100	0,07	0,00
20	CTCTGGCTCT	Secretory carrier membrane protein 5	*SCAMP5*	6	5	10	48	0,07	0,01
21	GAATTTGGGA	Junctophilin 4	*JPH4*	4	5	9	44	0,07	0,01
22	AATAAAGCTA	Synuclein, beta	*SNCB*	6	5	21	128	0,06	0,00
23	AGAATACCTT	Sparc/osteonectin, cwcv and kazal-like domains proteoglycan (testican)	*SPOCK*	2	5	6	34	0,06	0,02
24	GCCTGAGGGC	Phytanoyl-CoA hydroxylase interacting protein	*PHYHIP*	5	5	17	92	0,06	0,00
25	AGTGCCCCTC	ProSAPiP1 protein	*ProSAPiP1*	5	4	8	44	0,06	0,01
26	CCCATTCCTC	Plakophilin 4	*PKP4*	5	5	8	43	0,06	0,01
27	CCGGCCCCTC	Septin 4	*SEPT4*	7	5	27	152	0,06	0,00
28	CGGTTTCCAA	Protein kinase C, zeta	*PRKCZ*	6	5	10	57	0,06	0,00
29	CGTGTCCAGG	Progestin and adipoQ receptor family member VI	*PAQR6*	6	5	15	80	0,06	0,00
30	GTCTCTACGA	Transgelin 3	*TAGLN3*	3	5	6	37	0,06	0,01
31	TCTGTGACCT	Flavoprotein oxidoreductase MICAL2	*MICAL2*	5	4	7	38	0,06	0,02
32	TGTAACAATA	Neuritis with brachial prediliction	*NAPB*	5	5	14	79	0,06	0,00
33	TTCCCGGAAA	Sulfotransferase family 4A, member 1	*SULT4A1*	3	5	6	32	0,06	0,04
34	TTCCGACTGC	Synaptogyrin 3	*SYNGR3*	4	5	5	31	0,06	0,03
35	CACAACCACC	Rabphilin 3A homolog (mouse)	*RPH3A*	5	5	6	41	0,05	0,00
36	CGGCTGCCCA	Synuclein, gamma (breast cancer-specific protein 1)	*SNCG*	6	5	7	48	0,05	0,00
37	GAGGCTGGAA	Glutamate receptor, ionotropic, N-methyl D-aspartate 2C	*GRIN2C*	2	5	4	27	0,05	0,04
38	GCCTGTGGTG	Lymphocyte antigen 6 complex, locus H	*LY6H*	4	3	4	29	0,05	0,02
39	GGGGTGCTGT	Dynamin 1	*DNM1*	8	5	32	223	0,05	0,00
40	TCGGGGCCCC	Complexin 1	*CPLX1*	4	5	17	122	0,05	0,00
41	TCTATTAATA	Myelin basic protein	*MBP*	6	5	41	308	0,05	0,00
42	TGAGGTTATC	Guanine nucleotide binding protein (G protein), gamma 3	*GNG3*	4	5	6	41	0,05	0,00
43	TGGAGTGAAA	SH3-domain GRB2-like 2	*SH3GL2*	6	5	9	59	0,05	0,00
44	TGGCTGGAAG	Phosphoinositide-binding protein PIP3-E	*PIP3-E*	4	5	4	26	0,05	0,05
45	TACCCTCTCA	ATPase, Na^+/K^+ transporting, alpha 3 polypeptide	*ATP1A3*	2	5	5	42	0,,04	0,00
46	AAATAAAGCC	Gamma-aminobutyric acid (GABA) A receptor, delta	*GABRD*	2	5	5	42	0,04	0,00
47	ACAACACTAC	RAB3A, member RAS oncogene family	*RAB3A*	7	5	15	115	0,04	0,00

Table 1 continue

1	2	3	4	5	6	7	8	9	10
48	CAAAAAGTTA	Myelin-associated oligodendrocyte basic protein	*MOBP*	3	4	5	42	0,04	0,00
49	CCAAGGCCCC	Pleckstrin and Sec7 domain containing	*PSD*	2	5	8	65	0,04	0,00
50	CCTGACTCGG	Nuclear receptor interacting protein 3	*NRIP3*	3	5	3	26	0,04	0,03
51	GGCTGGATGG	Cholecystokinin	*CCK*	1	3	3	24	0,04	0,04
52	GTGCGAATCC	Calcium/calmodulin-dependent protein kinase (CaM kinase) II alpha	*CAMK2A*	2	4	9	88	0,04	0,00
53	TGACTGTGCT	Neurogranin (protein kinase C substrate, RC3)	*NRGN*	7	2	61	475	0,04	0,00
54	TGCCGCACGT	Kinesin 2 60/70kDa	*KNS2*	6	5	9	80	0,04	0,00
55	TTAACTTTAT	Reticulon 1	*RTN1*	3	4	7	64	0,04	0,00
56	GCGCTGCATT	Zic family member 4	*ZIC4*	3	2	4	36	0,04	0,00
57	AGACATTGTA	Synaptotagmin XIII	*SYT13*	2	5	2	26	0,03	0,01
58	CTGGATGTTA	Myelin transcription factor 1-like	*MYT1L*	1	5	2	22	0,03	0,04
59	CTGGCCAACC	Synaptotagmin V	*SYT5*	3	4	3	31	0,03	0,01
60	GCTGTTCTTG	Bruno-like 4, RNA binding protein (Drosophila)	*BRUNOL4*	3	4	4	50	0,03	0,00
61	GTAAGTCTCA	Neurofilament, light polypeptide 68 kDa	*NEFL*	2	4	3	39	0,03	0,00
62	GTGCAGTGAA	Synaptophysin	*SYP*	2	5	3	31	0,03	0,01
63	TACAAGGCCA	Dopamine receptor D1 interacting protein	*DRD1IP*	2	5	2	25	0,03	0,02
64	TGGCTGGAGG	Fas apoptotic inhibitory molecule 2	*FAIM2*	5	5	9	98	0,03	0,00
65	TAACCAAGAG	Transthyretin (prealbumin)	*TTR*	2	1	4	47	0,03	0,00
66	CTTATGACAA	Chromogranin B (secretogranin 1)	*CHGB*	2	5	2	36	0,02	0,00
67	TCCATTCAAG	Solute carrier family 12 (potassium-chloride transporter), member 5	*SLC12A5*	1	5	1	19	0,02	0,04
68	TCCGCCCCAG	Calcium binding protein 1 (calbrain)	*CABP1*	3	4	4	64	0,02	0,00
69	TTAGCACTTC	Williams-Beuren syndrome chromosome region 17	*WBSCR17*	1	5	2	39	0,02	0,00
70	AATAAATTGC	Synaptotagmin IV	*SYT4*	1	5	1	50	0,01	0,00
71	ATTGTGTAAT	Protein kinase C and casein kinase substrate in neurons 1	*PACSIN1*	1	5	1	34	0,01	0,00
72	CACAGTTTGC	Neurofilament 3 (150 kDa medium)	*NEF3*	1	4	1	51	0,01	0,00
73	CTGGACAAGG	Parvalbumin	*PVALB*	1	4	1	30	0,01	0,00
74	CTTCAATAGT	Internexin neuronal intermediate filament protein, alpha	*INA*	1	5	1	33	0,01	0,00
75	GCCCCAGCTG	Glutamate receptor, ionotropic, N-methyl-D-aspartate 1	*GRIN1*	1	5	2	61	0,01	0,00
76	GCTCCTGTCT	Protein kinase C, gamma	*PRKCG*	1	4	1	33	0,01	0,00
77	TGGAATGAGC	Creatine kinase, mitochondrial 2 (sarcomeric)	*CKMT2*	1	4	1	30	0,01	0,00
78	CGGCTGCTGG	Copine VI (neuronal)	*CPNE6*	0	5	0	25	0	0,00
79	CTCCAAAGAA	Potassium voltage-gated channel, KQT-like subfamily, member 2	*KCNQ2*	0	4	0	18	0	0,03
80	GACAGCGACA	UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 9 (GalNAc-T9)	*GALNT9*	0	5	0	18	0	0,03
81	GCACAGGAGA	Solute carrier family 1 (high affinity aspartate/glutamate transporter), member 6	*SLC1A6*	0	3	0	18	0	0,03
82	GTTTTGCAAA	Glutamate receptor, metabotropic 4	*GRM4*	0	4	0	29	0	0,00
83	TGGATGCTCT	Follistatin-like 5	*FSTL5*	0	2	0	18	0	0,03
84	CGAGAGGGAG	FAT tumor suppressor homolog 2 (Drosophila)	*FAT2*	0	2	0	34	0	0,00
85	TAAAATGCAG	Neurogenic differentiation 1	*NEUROD 1*	0	2	0	24	0	0,00

STBXP1 mRNA content is very low in both NB and glioblastomas. Most likely, the difference in gene expression levels in individual tumour and NB samples reflects heterogeneity of biological properties of tumours as well as individual polymorphism.

Noteworthy is the fact that the biggest number of genes is expressed in brain compared to any other tissue [10, 11]. And only concerning small number of genes, for instance, *MBP*, *DNM1*, and *NRGN*, the abundance of their mRNA in general pool of mRNA is sufficient for Northern hybridisation analysis. To determine the contents of rare transcripts in tumour cells, we applied semi-quantitative RT-PCR. RT-PCR results for *FAT2* generally correlated with those of SAGE. PCR product was detected in 5 of 7 NB samples and 6 of 14 samples of glioblastomas (Fig.3).

RT-PCR results for *CPNE6* were in very good correlation with those of SAGE, *i.e.* PCR product was dis-

Table 2

Distribution of levels of gene expression at each stage of astrocytoma development

	Tag	Gene name	Gene symbol	Tag gene abundance, compared to NB		
				Astrocytoma	Anaplastic astrocytoma	Glioblastoma
1	2	3	4	5	6	7
		I				
1.	GTCGCTGAGA	Carbonic anhydrase XI	*CA11*	0.16	0.21	0.13
2.	AGTGGAAGGT	Discs, large homolog 4 (Drosophila)	*DLG4*	0.13	0.10	0.08
3.	TCTGCACCTC	Eukaryotic translation elongation factor 1 alpha 2	*EEF1A2*	0.12	0.06	0.08
4.	CACAACCACC	Rabphilin 3A homolog (mouse)	*RPH3A*	0.11	0.08	0.05
5.	TCTGTGACCT	Flavoprotein oxidoreductase MICAL2	*MICAL2*	0.09	0.09	0.06
6.	TGGGACGTGA	EPH receptor B6	*EPHB6*	0.07	0.10	0.08
7.	CCTGACTCGG	Nuclear receptor interacting protein 3	*NRIP3*	0.06	0.03	0.04
8.	TAACCAAGAG	Transthyretin (prealbumin)	*TTR*	0.05	0.02	0.03
9.	CGAGAGGGAG	FAT tumour suppressor homolog 2 (Drosophila)	*FAT2*	0.00	0.00	0.00
		II				
10	GTCTCTACGA	Transgelin 3	*TAGLN3*	0,19	0,18	0,06
11	TGGACACTCA	Neurochondrin	*NCDN*	0,19	0,21	0,11
12	ATCCGTGCCC	Calmodulin 3 (phosphorylase kinase, delta)	*CALM3*	0,17	0,10	0,09
13	AGTGCCCCTC	ProSAPiP1 protein	*ProSAPiP1*	0,16	0,17	0,06
14	TCGGGGCCCC	Complexin 1	*CPLX1*	0,16	0,08	0,05
15	TGCCGCACGT	Kinesin 2 60/70 kDa	*KNS2*	0,16	0,14	0,04
16	CCCATTCCTC	Plakophilin 4	*PKP4*	0,15	0,19	0,06
17	TTCCCGGAAA	Sulfotransferase family 4A, member 1	*SULT4A1*	0,15	0,09	0,06
18	GAGGCTGGAA	Glutamate receptor, ionotropic, N-methyl-D-aspartate 2C	*GRIN2C*	0,15	0,08	0,05
19	TGGCTGGAAG	Phosphoinositide-binding protein PIP3-E	*PIP3-E*	0,17	0,14	0,05
20	ACAACACTAC	RAB3A, member RAS oncogene family	*RAB3A*	0,17	0,17	0,04
21	TGAGGTTATC	Guanine nucleotide binding protein (G protein), gamma 3	*GNG3*	0,14	0,08	0,05
22	CGTGTCCAGG	Progestin and adipoQ receptor family member VI	*PAQR6*	0,14	0,04	0,06
23	CTTCAGGACC	Syntaxin binding protein 1	*STXBP1*	0,16	0,19	0,08
24	TGACTGTGCT	Neurogranin (protein kinase C substrate, RC3)	*NRGN*	0,15	0,27	0,04
25	GGGGTGCTGT	Dynamin 1	*DNM1*	0,14	0,13	0,05
26	CACAGTTTGC	Neurofilament 3 (150kDa medium)	*NEF3*	0,13	0,14	0,01
27	CGGCTGCTGG	Copine VI (neuronal)	*CPNE6*	0,12	0,04	0,00
28	TCCGCCCCAG	Calcium binding protein 1 (calbrain)	*CABP1*	0,12	0,12	0,02
29	CTCCAAAGAA	Potassium voltage-gated channel, KQT-like subfamily, member 2	*KCNQ2*	0,10	0,10	0,00
30	GCTGTTCTTG	Bruno-like 4, RNA binding protein (Drosophila)	*BRUNOL4*	0,11	0,14	0,03
31	TGGCTGGAGG	Fas apoptotic inhibitory molecule 2	*FAIM2*	0,11	0,07	0,03
32	GTGCGAATCC	Calcium/calmodulin-dependent protein kinase (CaM kinase) II alpha	*CAMK2A*	0,11	0,14	0,04
33	GTGCAGTGAA	Synaptophysin	*SYP*	0,09	0,08	0,03
34	TACAAGGCCA	Dopamine receptor D1 interacting protein	*DRD1IP*	0,07	0,06	0,03
35	GCTCCTGTCT	Protein kinase C, gamma	*PRKCG*	0,07	0,03	0,01
36	GCCCCAGCTG	Glutamate receptor, ionotropic, N-methyl D-Aspartate 1	*GRIN1*	0,05	0,03	0,01
37	TGGAATGAGC	Creatine kinase, mitochondrial 2 (sarcomeric)	*CKMT2*	0,05	0,07	0,01
38	TAAAATGCAG	Neurogenic differentiation 1	*NEUROD1*	0,03	0,02	0,00
39	GCACAGGAGA	Solute carrier family 1 (high affinity Aspartate/glutamate transporter), member 6	*SLC1A6*	0,03	0,01	0,00
40	GTTTTGCAAA	Glutamate receptor, metabotropic 4	*GRM4*	0,01	0,02	0,00
		III				
41	CGGGGAGATG	NDRG family member 2	*NDRG2*	0,64	0,34	0,12
42	CTTATGACAA	Chromogranin B (secretogranin 1)	*CHGB*	0,62	0,27	0,02
43	TTAACTTTAT	Reticulon 1	*RTN1*	0,50	0,55	0,04
44	TGGAGTGAAA	SH3-domain GRB2-like 2	*SH3GL2*	0,42	0,46	0,05
45	GGCTGGATGG	Cholecystokinin	*CCK*	0,42	0,37	0,04
46	CCCCCAATTC	Vesicle-associated membrane protein 2 (synaptobrevin 2)	*VAMP2*	0,42	0,33	0,14

Table 2 continue

1	2	3	4	5	6	7
47	CCCTTCCTTT	ATPase, H^+ transporting, lysosomal 13 kDa, VI subunit G isoform 2	*ATP6V1G2*	0,36	0,37	0,09
48	TGGATGCTCT	Follistatin-like 5	*FSTL5*	0,35	0,16	0,00
49	GCCTCAATAA	Mitogen-activated protein kinase 8 interacting protein 2	*MAPK8IP2*	0,34	0,20	0,09
50	TGTAACAATA	Neuritis with brachial prediliction	*NAPB*	0,31	0,26	0,06
51	CGGCTGCCCA	Synuclein, gamma (breast cancer-specific protein 1)	*SNCG*	0,31	0,20	0,05
52	AAAGGGAATG	Solute carrier family 17 (sodium-dependent inorganic phosphate cotransporter), member 7	*SLC17A7*	0,29	0,23	0,07
53	TCTATTAATA	Myelin basic protein	*MBP*	0,29	0,50	0,05
54	AATAAAGCTA	Synuclein, beta	*SNCB*	0,28	0,20	0,06
55	AGAATACCTT	Sparc/osteonectin, cwcv and kazal-like domains proteoglycan (testican)	*SPOCK1*	0,28	0,33	0,06
56	CAAAAAGTTA	Myelin-associated oligodendrocyte basic protein	*MOBP*	0,26	0,18	0,04
57	TTCCGACTGC	Synaptogyrin 3	*SYNGR3*	0,26	0,21	0,06
58	AAATAAAGCC	Gamma-aminobutyric acid (GABA) A receptor, delta	*GABRD*	0,25	0,09	0,04
59	TAATATTAAA	Synaptosomal-associated protein, 25 kDa	*SNAP25*	0,25	0,27	0,08
60	GCCTGAGGGC	Phytanoyl-CoA hydroxylase interacting protein	*PHYHIP*	0,24	0,09	0,06
61	TTAGCACTTC	Williams-Beuren syndrome chromosome region 17	*WBSCR17*	0,24	0,11	0,02
62	CCGGCCCCTC	Septin 4	*SEPT4*	0,24	0,10	0,06
63	GCCTGTGGTG	Lymphocyte antigen 6 complex, locus H	*LY6H*	0,23	0,19	0,05
64	AATAAATTGC	Synaptotagmin IV	*SYT4*	0,22	0,08	0,03
65	TGGGAAGTGG	Maternally expressed 3	*MEG3*	0,22	0,16	0,15
66	ATTGTGTAAT	Protein kinase C and casein kinase substrate in neurons 1	*PACSIN1*	0,21	0,21	0,01
67	TGCCCAAATG	Leucine-rich repeat LGI family, member 3	*LGI3*	0,21	0,31	0,08
68	AAGCATTAAA	Protein kinase, cAMP-dependent, regulatory, type I, beta	*PRKAR1B*	0,21	0,20	0,12
69	ATCCCTTCCC	Septin 5	*SEPT5*	0,21	0,15	0,08
70	ATTAAAGTCA	Regulating synaptic membrane exocytosis 3	*RIMS3*	0,21	0,17	0,11
71	CGGTTTCCAA	Protein kinase C, zeta	*PRKCZ*	0,20	0,11	0,06
72	GAATTTGGGA	Junctophilin 4	*JPH4*	0,20	0,25	0,07
73	CCAAGGCCCC	Pleckstrin and Sec7 domain containing	*PSD*	0,20	0,18	0,04
74	CTGGATGTTA	Myelin transcription factor 1-like	*MYT1L*	0,20	0,17	0,03
75	CTCTGGCTCT	Secretory carrier membrane protein 5 *IV*	*SCAMP5*	0,18	0,20	0,07
76	CTTCAATAGT	Internexin neuronal intermediate filament protein, alpha	*INA*	0,17	0,24	0,01
77	GTTTAAAAAG	Stathmin-like 2	*STMN2*	0,16	0,24	0,11
78	GTAAGTCTCA	Neurofilament, light polypeptide 68 kDa	*NEFL*	0,14	0,34	0,03
79	TCCATTCAAG	Solute carrier family 12, (potassium-chloride transporter) member 5	*SLC12A5*	0,08	0,14	0,02
80	AGACATTGTA	Synaptotagmin XIII	*SYT13*	0,06	0,14	0,03
81	GACAGCGACA	UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 9 (GalNAc-T9)	*GALNT9*	0,05	0,19	0,00
82	TACCCTCTCA	ATPase, Na^+/K^+ transporting, alpha 3 polypeptide	*ATP1A3*	0,04	0,10	0,04
83	CTGGACAAGG	Parvalbumin	*PVALB*	0,01	0,06	0,01
84	GCGCTGCATT	Zic family member 4	*ZIC4*	0,01	0,03	0,04
85	CTGGCCAACC	Synatotagmin 5	*SYT5*	0,00	0,02	0,03

covered in 8 of 9 NB samples and 4 of 10 samples of glioblastomas, while in one glioblastoma only, the level of gene expression was the same as in NB samples and to be significantly lower in other three GB samples (Fig.4). RT-PCR did not confirm the results of SAGE for *GRM4* – PCR product was discovered in 1 of 11 NB samples and 3 of 9 samples of glioblastoma.

The changes in transcription activity of genes, obtained in this sort of comparison, are generally of different genesis, *i.e.* they may cause malignant transformation of cells, they may be the consequences of this transformation or they may not have any direct relation to the latter. Certainly, this sort of grouping is rather subjective, minding that the majority of protein prod-

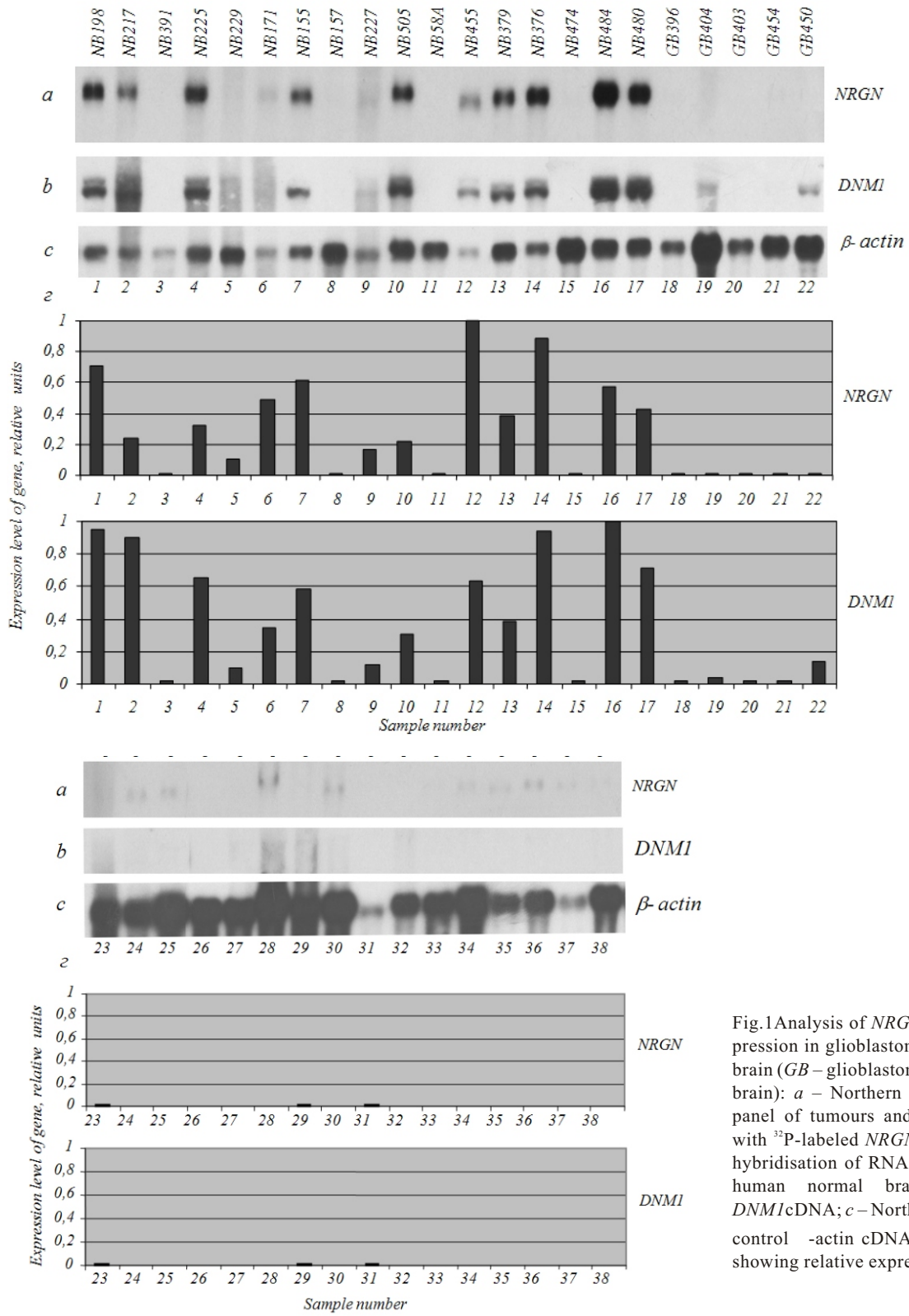

Fig.1Analysis of *NRGN* and *DNM1* gene expression in glioblastomas and human normal brain (*GB* – glioblastoma; *NB* –human normal brain): *a* – Northern hybridisation of RNA panel of tumours and human normal brain with ^{32}P-labeled *NRGN* cDNA; *b* – Northern hybridisation of RNA panel of tumours and human normal brain with ^{32}P-labeled *DNM1*cDNA; *c* – Northern hybridisation with control -actin cDNA probe; *d* – bar graph showing relative expression level of gene

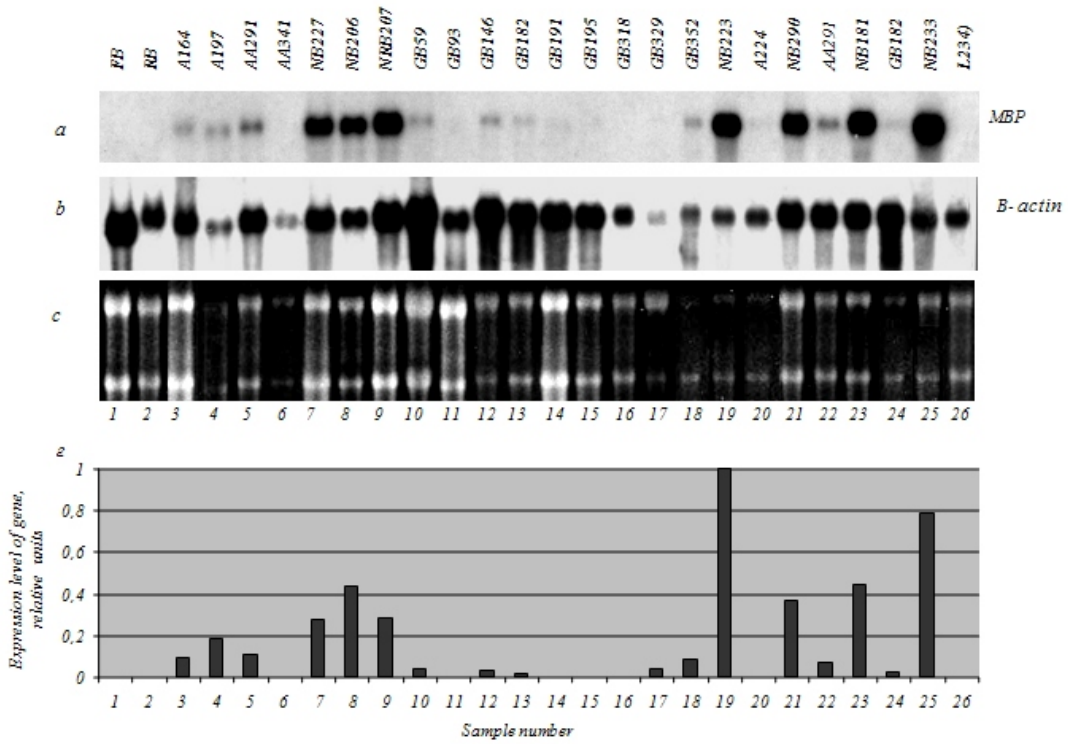

Fig.2 Analysis of *MBP* gene expression in tumours and human normal brain (*GB* – glioblastoma; *RB* – rat brain; *A* – astrocytoma, *AA* – anaplastic astrocytoma; *NB* – normal human brain; *NRB* – neuroblastoma, *GB* – glioblastoma; *L* – lymphoma): *a* – Northern hybridisation of RNA panel of tumours and human normal brain with ^{32}P-labeled *MBP* cDNA; *b* – Northern hybridisation with control -actin cDNA probe; *c* – ethidium bromide stained agarose gel; *d* – diagram of relative level of gene expression

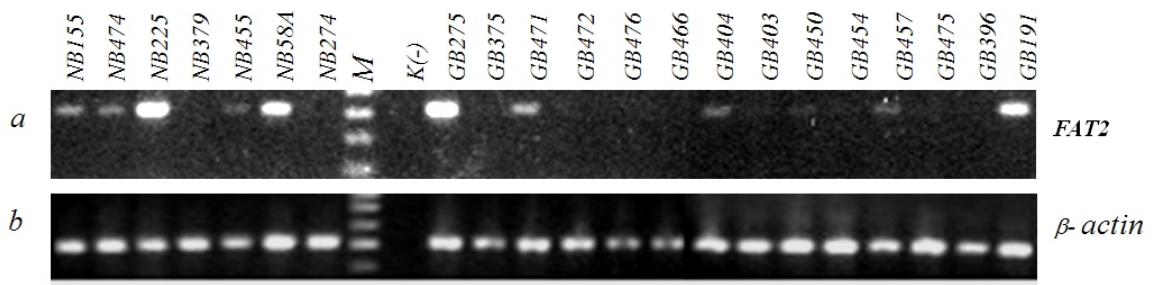

Fig.3 Semi-quantitative PCR-analysis of *FAT2* gene expression in glioblastomas and human normal normal brain (*GB* – glioblastoma; *NB* – normal human brain): *a* – electrophoresis of PCR products with primers for *FAT2*; *b* – electrophoresis of PCR products with primers for beta-actine

ucts of these genes are polyfunctional and the question of which of the functions are involved (if involved at all) in the formation of astrocytomas remains open.

Gene symbol was used to identify genes in CGAP database (http://cgap.nci.nih.gov/SAGE/AnatomicViewer) and to determine potential functions of their protein products in different databases and publications for each gene, which allowed grouping genes with similar functions (Table 3). As it has been expected, the major part of genes with decreased level of expression in gliomas encode the proteins, participating in neurogenesis, synaptic transmission, formation of nerve ensheatment and neurofilament. The decrease in levels of gene expression of the aforementioned functional groups may be trustworthily refer to the third class of expression

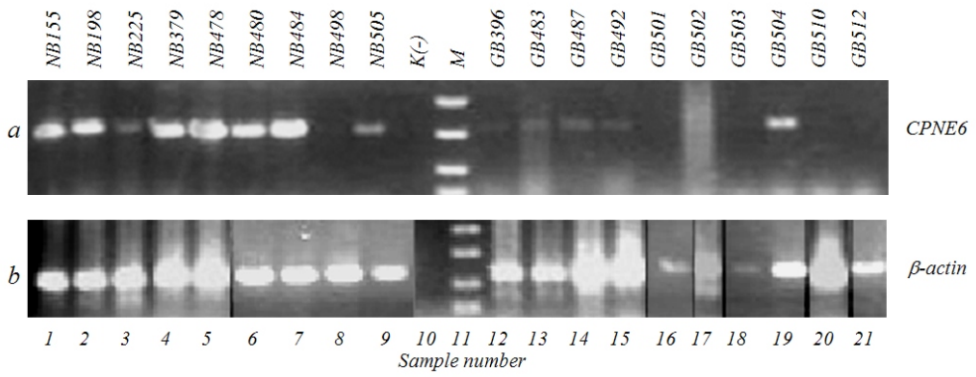

Fig.4 Analysis of *CPNE6* in glioblastomas and normal human brain using with semi-quantitative RT-PCR (*GB* – glioblastoma; *NB* – normal human brain): *a* – electrophoresis of PCR products obtained with primers for *CPNE6*; *b* – electrophoresis of PCR products obtained with primers for -actin

changes, although, there is a possibility of their intermediate participation in tumour development. These genes are expressed predominantly in the neural cells, relative quantity of which decreases in glial tumours. Therefore, the decrease in content of such mRNA possibly reflects the decrease in the percentage of neural cells in astrocytomas. There have been no data of their involvement in the carcinogenesis for more than half of reviewed genes, belonging to these groups. However, some genes may be used for the characterisation of malignant neoplasmas and may have prognostic potential.

A significant number of genes with decreased expression in glioblastoma encode the products, involved in transporter activity and cell signal transduction (Table 3). Molecular distunbances of signal transduction are known to play the important role in the development of glial tumours. Some expression changes, discovered in the present work, were earlier found in human brain cells for the following genes: *CCK*, encoding cholecystokinin (brain/gut peptide); *EPHB6*, encoding ephrin B6 receptor and neurogranin gene *NRGN*, which encodes protein kinase substrate C.

Cholecystokinin is involved in performance of numerous functions in the brain, in particular, feeding behaviour, anxiety and memory. Brain CCK mRNA levels are low before birth, but increase markedly shortly after birth and reach adult like patterns of expression three weeks after birth during the final maturation of the central nervous system. In the adult, several substances induce neuronal CCK mRNA expression via activation of transcription factors binding to regulatory elements in the CCK promoter [13].

Though gastrin, cholecystokinin-related protein, was demonstrated to influence the growth and mobility of glioblastoma cells [14], yet in gliomas there were no evidences of cholecystokinin receptor products [15]. Immunohistochemical analysis revealed the lower content of cholecystokinin in malignant astrocytomas, compared to well-differentiated astrocytomas (malignancy grades I-II) [16]. This protein was detected in neuronal cells, preserved in 7 investigated supra-tentorial anaplastic astrocytomas, however, it was absent in other gliomas – 3 diffuse astrocytomas and 8 glioblastomas [17] as well as in subependymal giant cell astrocytomas [18]. Inactivation of *CCK* expression is likely to take place at the level of transcription or translation, or post-translational processing during malignant progression of gliomas. It is confirmed by our SAGE results – *CCK* gene tags are present in 1 of 9 glioblastoma SAGE-libraries only. *In vivo* experiments show that cholecystokinin may induce signalling processes with assistance of protein kinase C in rat glioma C6 cell line, for which the expression of CCKB-type of receptors has been identified [19]. Cholecystokinin stimulates the growth of C6 cells, activating isoenzymes *1*, *2*, , and of C protein kinase [20].

Ephrin receptors and their ligands play the role of mediators in numerous processes of development, in nervous system, in particular. Based on the structure and sequence relations, ephrins can be classified into class A (EFNA), which are anchored to membrane with

Table 3

Potential suppressor genes of glial tumours, grouped

Gene name	Tag
1	2
Cell cycle regulation	
Septin 5	*SEPT5*
Septin 4	*SEPT4*
Transcription/Translation regulation	
Bruno-like 4, RNA binding protein (Drosophila)	*BRUNOL4*
Eukaryotic translation elongation factor 1 alpha	*EEF1A2*
Myelin transcription factor 1-like	*MYT1L*
Neurogenic differentiation 1	*NEUROD1*
Neurogenesis	
Copine VI (neuronal)	*CPNE6*
Discs, large homolog 4 (Drosophila)	*DLG4*
Lymphocyte antigen 6 complex, locus H	*LY6H*
Myelin-associated olygodendrocyte basic protein	*MOBP*
Neurogranin	*NRGN*
Transgelin 3	*TAGLN3*
Nerve ensheatment	
Myelin basic protein	*MBP*
Myelin-associated olygodendrocyte basic protein	*MOBP*
Neurofilament	
Internexin neuronal intermediate filament protein, alpha	*INA*
Neurofilament 3 (150kDa medium)	*NEF3*
Neurofilament, light polypeptide 68kDa	*NEFL*
Synaptic transmission	
Calcium/calmodulin-dependent protein kinase (CaM kinase) II alpha	*CAMK2A*
Complexin 1	*CPLX1*
Copine VI (neuronal)	*CPNE6*
Discs, large homolog 4 (Drosophila)	*DLG4*
Dopamine receptor D1 interacting protein	*DRD1IP*
Gamma-aminobutyric acid (GABA) A receptor, delta	*GABRD*
Glutamate receptor, ionotropic, N-methyl D-aspartate 1	*GRIN1*
Glutamate receptor, ionotropic, N-methyl D-aspartate 2C	*GRIN2C*
Glutamate receptor, metabotropic 4	*GRM4*
Myelin basic protein	*MBP*
Myelin-associated olygodendrocyte basic protein	*MOBP*
Neuronal pentraxin 1	*NPTX1*
Solute carrier family 1 (high affinity aspartate/glutamate transporter), member 6	*SLC1A6*
Solute carrier family 12, (potassium-chloride transporter) member 5	*SLC12A5*
Synaptosomal-associated protein, 25kDa	*SNAP25*
Synaptogyrin 3	*SYNGR3*
Synaptophysin	*SYP*
Synaptotagmin V	*SYT53*
Synaptotagmin XIII	*SYT13*
Syntaxin binding protein 1	*STXBP1*
Transporter activity	
ATPase, Na+/K+ transporting, alpha 3 polypeptide	*ATP1A3*
ATPase, H+ transporting, lysosomal 13kDa, V1 subunit G isoform 2	*ATP6V1G2*
Potassium voltage-gated channel, KQT-like subfamily, member 2	*KCNQ2*
Solute carrier family 17	*SLC17A7*
Protein kinase C and casein kinase substrate in neurons 1	*PACSIN1*
Synaptotagmin IV	*SYT4*
Synaptotagmin XIII	*SYT13*
Vesicle-associated membrane protein (VAMP)/synaptobrevin	*VAMP2*
Transthyretin (prealbumin)	*TTR*
Phosphoinositide-binding protein PIP3-E	*PIP3-E*
Neuritis with brachial prediliction	*NAPB*
RAB3A, member RAS oncogene family	*RAB3A*
Rabphilin 3A homolog (mouse)	*RPH3A*
Secretory carrier membrane protein 5	*SCAMP5*
Endocytosis	
Dynamin 1	*DNM1*
Protein kinase C and casein kinase substrate in neurons 1	*PACSIN1*
Signal transduction	
Calcium binding protein 1 (calbrain)	*CABP1*
Cholecystokinin	*CCK*
Discs, large homolog 4 (Drosophila)	*DLG4*
EPH (Ephrin) receptor B6	*EPHB6*
Gamma-aminobutyric acid (GABA) A receptor, delta	*GABRD*
Guanine nucleotide binding protein (G protein), gamma 3	*GNG3*
Mitogen-activated protein kinase 8 interacting protein 2	*MAPK8IP2*
Neurogranin	*NRGN*

1	2
Protein kinase C and casein kinase substrate in neurons 1	*PACSIN1*
Protein kinase, cAMP-dependent, regulatory, type I, beta	*PRKAR1B*
Protein kinase C, gamma	*PRKCG*
Protein kinase C, zeta	*PRKCZ*
Plakophilin 4	*PKP4*
Reticulon 1	*RTN1*
SH3-domain GRB2-like 2	*SH3GL2*
Apoptosis	
Fas apoptotic inhibitory	*FAIM2*
Septin 4	*SEPT4*
Neurogranin	*NRGN*
Creatine kinase, mitochondrial 2	*CKMT2*
GTPase activity	
Dynamin 1	*DNM1*
Guanine nucleotide binding protein (G protein), gamma 3	*GNG3*
RAB3A, member RAS oncogene family	*RAB3A*
Septin 5	*SEPT5*
Septin 4	*SEPT4*
Cell adhesion/motility	
FAT tumour suppressor homolog 2 (Drosophila)	*FAT2*
Lipid metabolism	
Synuclein, beta	*SNCB*
Sulfotransferase family 4A, member 1	*SULT4A1*
Cytosceleton related	
Kinesin 2 60/70kDa	*KNS2*
Septin 5	*SEPT5*
Membrane protein	
UDP-N-acetyl-alpha-D-galactosamine : polypeptide N-acetylgalactosaminyl -transferase 9 (GalNAc-T9)	*GALNT9*
Solute carrier family 1 (high affinity aspartate/glutamate transporter), member 6	SLC1A6
Septin 4	*SEPT4*
Hormonal activity	
Chromogranin B (secretogranin 1)	*CHGB*
Transthyretin (prealbumin)	*TTR*
Ion binding	
Follistatin-like 5	*FSTL5*
Parvalbumin	*PVALB*
Unclassified	
Carbonic anhydrase 11	*CA11*
Leucine-rich repeat LGI family, member 3	*LGI3*
Junctophilin 4	*JPH4*
Maternally expressed 3	*MEG3*
Flavoprotein oxidoreductase MICAL2	*MICAL*
Neurochondrin	*NDCN*
NDRG family member 2	*NDRG*
Nuclear receptor interacting protein 3	*NRIP3*
Progestin and adipoQ receptor family member VI	*PAQR6*
Phytanoyl-CoA hydroxylase interacting protein	*PHYHIP*
ProSAPiP1 protein	*ProSaPiP1*
Pleckstrin and Sec7 domain containing	*PSD*
Synuclein, beta	*SNCB*
Synuclein, gamma (breast cancer-specific protein 1)	*SNCG*
Sparc/osteonectin, cwcv and kazal-like domains proteoglycan (testican)	*SPOCK*
Stathmin-like 2	*STMN2*
Sulfotransferase family 4A, member 1	*SULT4A1*
Zic family member 4	*ZIC4*
Williams-Beuren syndrome chromosome region 17	*WBSCR17*

glycosylphosphatidylinositol linkage, and class B (EFNB), which are transmembrane proteins. Ephrin receptors are divided into two groups, on the basis of similarity of amino acid sequences of their extra-cellular domains and affinity in relation towards binding of A and B ephrins. Ephrin receptors present the largest sub-group in the family of tyrosine kinases. Receptors participate in the process of development of nervous system, such as formation of boundaries, vasculogenesis, and cell migration [21]. The expression of one of them, *EphB2*, in gliomas cells was discovered to result in decrease in cell adhesion and increase in cell invasion [22]. Besides, *R-Ras* plays the key role in regulation of integrins activity by this receptor via association and subsequent phosphorylation. Inhibition of expression of endogenic *R-Ras* with small interfering RNA (siRNA) abolished *EphB2* effect on cell adhesion, proliferation and invasion in brain. The degree of *R-Ras* phosphorylation is positively correlated with degree of *EphB2* phosphorylation in glioblastoma cells. These results demonstrate the possibility of therapeutic influencing on signalling pathway *EphB2*/*R-Ras* [22]. Ephrin receptor B6, encoded by *EPHB6*, does not show kinase activity, specific to the majority of tyrosine kinase receptors, and binds B-ephrins. Decreased expression level of *EPHB6* was

discovered in prostate [23], breast [24], and melanoma cancer cells [25]. The reason of inactivation of *EPHB6* was determined to be the increase in the level of methylation of its promoter. Demethylation of this promoter restores the normal level of gene expression [24]. Expression of *EPHB6* is one of favourable factors for prognosis of neuroblastomas [26]. The increase in the level of *EPHB6* transcription was determined in neuroblastoma cell line after transfection with antisense cDNA *MIF* (macrophage migration inhibition factor). The result of this transfection was 80% decrease in cell growth of neuroblastomas, as well as 90% decrease (compared to the control) in tumour growth and the level of metastasis in mice, injected with these transfectants [27].

Human *NRGN* (neurogranin; protein kinase C substrate; *RC3*) is the homologue of neurospecific gene of rat *RC3*/neurogranin. This gene encodes post-synaptic substrate of protein kinase C, which binds calmodulin at the absence of calcium [28]. Neurogranin was discovered to be possible proapoptotic factor, participating in induction of T-cells apoptosis upon elimination of interleukin-2 due to the increase in inner-cellular Ca^{2+} concentration [29]. *NRGN* is identified among genes, which are regulated by gene of early growth response *EGR1* in prostate cancer line cells [30]. The authors suppose that overexpression of *EGR1* may be related to neuroendocrine differentiation, which often accompanies the progression of prostate cancer. The decrease in *NRGN* expression in glioblastoma (along with 44 other genes) was revealed comparing the profiles of gene expression in glioblastoma and NB using cDNA microarrays, containing 25 344 genes, and confirmed by semi-quantitative RT-PCR [31]. Possibly, the decreased *NRGN* expression is associated with inhibition of apoptosis in glioblastomas or is the reflection of decrease in number of neural cells in tumours or their weakened functioning.

Inactivation of ion transport genes in glial tumours has been discovered earlier. Thus, Markert *et al*. [8] determined the decreased expression of several genes, encoding ion transport proteins, particularly subunits GRIN1 and GRIN2C of NMDA receptor, during profiling of gene expression in glioblastomas by the analysis of oligonucleotide microarrays, which is in good correlation with our results.

Disorder in cell cycle regulation is one of attributes of malignant progression of astrocytomas. The disordering is mediated by the inactivation of anti-oncogenes *TP53*, *CDKN2|p16*, and *Rb* on 17p, 9p, and 13q chromosomes respectively [32]. The weakened cell cycle control may be the reason of significant resistance of glioblastomas towards radio and chemotherapies [33]. The group of genes capable of regulating cell cycle includes *SEPT4* and *SEPT5* (Table 3), which encode septin family proteins. Immunohistochemical analysis revealed the absence of SEPT4 (ARTS) protein in normal astrocytes and the increase in its production throughout the malignancy progression [34]. The increase in expression of this gene correlates with the highest degree of apoptosis in tumours. Perhaps the presence of SEPT4 indicates the ability of tumour cells to apoptosis and may be considered as independent prognostic factor. It is also quite possible that the differences in results of protein analysis and SAGE demonstrate the difference in stability and translatability of this mRNA in normal and tumour astrocytes.

FAT2 gene tag (*MEGF1*) is present in neither one of SAGE-libraries of astrocytomas of II-IV malignancy degrees. This protein belongs to the family of cadherins – the group of integral membrane proteins, specific for the presence of cadherin type of repeats. Beside 34 tandem cadherin type repeats, the protein contains 2 EGF-like repeats and one laminin G domain [35, 36]. It is the second identified human homologue of *Drosophila FAT*, which encodes tumour suppressor, important for control of cell proliferation throughout the development of *Drosophila*. *In situ* hybridisation revealed mRNA products in granular cells (small neurons) of inner external germinal layer and migrating granular cells, whereas *FAT2* is not expressed in proliferating cells of outer external germinal layer [37]. Apparently FAT2 protein is responsible for cell adhesion, which controls cell proliferation and plays the important role in the development of brain.

The results, obtained in this work, confirm and supplement the data of other authors, stimulate the search for new therapeutic targets for glial tumours. The genes, expression of which was not detected in glioblastomas at all (*CPNE6*, *KCNQ2*, *GALNT9*, *SLC1A6*, *GRM4*, *FSTL5*, *FAT2*, and *NEUROD1*) are

the most probable candidates for tumour-suppressor genes, among 85 other genes, identified with SAGE.

Therefore, in this work we identified approximately 100 genes with more than 5-fold decreased expression in astrocytic gliomas of malignancy grades II-IV. Some of them may be potential tumour suppressor genes. Abnormalities, resulting in inactivation of tumour suppressor genes, are often linked to the decrease in the level of their expression. In some tumours or cell lines there may be deletions in one or both gene copies, methylation of promoter, mutations of splicing site, nonsense-mutations or combinations of these events, which initiate preliminary termination of translation and destabilise mRNA-transcripts. Such mutations are often connected with complete absence or partial decrease in the level of expression of tumour-suppressor gene [38].

The application of SAGE allows determining similar genes and understanding their functions [39]. However, to determine the decreased levels of gene expression in tumour is the first step at the beginning of a long chain of experiments on the identification of tumour suppressor genes. The second stage includes functional research in order to understand the connection between molecular events and their interactions. Further characterisation will allow using these genes for diagnostics and medical prognostics, antitumour therapy and molecular classification of neoplasms.

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Характеристика генов со сниженной экспрессией в глиомах человека – потенциальных опухолесупрессорных генов

Резюме

В результате сравнения профилей экспрессии генов в нормальном головном мозге и глиобластоме с использованием базы данных серийного анализа генной экспрессии (Serial Analysis of Gene Expression, SAGE) выявлены 129 генов с более чем пятикратным изменением уровня экспрессии (P 0,05) в глиобластоме, из которых 85 генов – со сниженной экспрессией. В диффузной и анапластичной астроцитомах количество генов со сниженной в пять раз экспрессией меньше. Лишь для девяти генов пятикратное снижение экспрессии происходит в диффузных астроцитомах и выявляется приблизительно на таком же уровне в анапластичных астроцитомах и в глиобластомах. Для подавляющего большинства инактивированных генов уровень экспрессии снижается в диффузных астроцитомах и последовательно уменьшается на дальнейших стадиях злокачественной прогрессии астроцитом, причем в глиобластомах – наиболее злокачественных проявлениях глиальных опухолей – экспрессия отдельных генов очень низкая или совсем отсутствует. Нозерн-гибридизация и ОТ-ПЦР (обратная транскрипция–полимеразная цепная реакция) подтвердили сниженную экспрессию в глиобластомах произвольно отобранных генов. Некоторые гены, описанные в этой работе, могут кодировать опухолевые супрессоры и их сниженная экспрессия, очевидно, играет важную роль в инициации и прогрессии глиом человека.

Ключевые слова: глиома, астроцитома, глиобластома, сниженная экспрессия генов, потенциальные опухолесупрессорные гены.

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