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Overexpression of genes at different stages of astrosutic glioma development

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Public database of Serial Analysis of Gene Expression (SAGE) was used for identification of potential human astrocytic glioma molecular markers. The comparison of nine glioblastoma SAGE-libraries, eleven anaplastic astrocytoma SAGE-libraries, eight diffusive astrocytoma SAGE-libraries, and five normal human brain SAGE-libraries revealed 57 genes with more than 5-fold increase of expression in astrocytic gliomas (P#0.05) comparing to the normal human brain. Besides the genes expression changes which occur at the early stage of astrocytomas formations and are revealed also during the subsequent stages of progression, some changes are characteristic only of highly malignant stages of tumor development while they are absent in the tumors of low stage of malignancy. The analysis of revealed genes expression can be used for glial tumor molecular classification, diagnosis, prognostic evaluation and determination of potential targets for anticancer therapy.

Key words: *SAGE, differential expression, astrocytic glioma, molecular markers.*

Introduction. Malignant gliomas, the most widely spread primary tumors of a human brain, are characterized by their considerable aggressivity, high invasion, and neurological destruction. The majority of gliomas (up to the half of all intracranial tumors) is comprised of astrocytic gliomas, human brain macroglia neoplasms. The tumors progress through the stages of increasing malignancy, associated with genetic anomalies accumulation on different chromosomes and gene expression changes. WHO [1] classification is based on certain histological tumor cell properties and histological diagnosis is a basic criterion for prognostic evaluation and therapy. The efforts directed towards establishing correlation between a genotype and a phenotype of glial tumors did not come to univalent results. For example, gene p53 mutations (a distinguishing feature of diffusive astrocytomas, chemotherapy stable) and the loss of heterozygosity (LOH) on 1p and 19q chromosomes (distinguishing features of oligodendrogliomas,

which are associated with their sensitivity to chemotherapy and better prognosis) were discovered in astrocytomas, as well as in oligodendrogliomas [2]. The conclusion of another work based on astrocytomas and oligodendrogliomas genetic analysis was that genetic changes (p53 gene mutation and LOH on 1p and 19q chromosomes) cannot be accepted as trustworthy prognostic factors.

With the advent of cDNA-microarrays, oligonucleotide chips, Digital Differential Display (DDD), and Serial Analysis of Gene Expression (SAGE) it became possible to identify genes, whose expression is changing at various diseases. These technologies allow identifying not only single genes but gene clusters that characterize gene expression profiles in norm as well as in disease. The differences in gene expression levels may be used not only for tumor classification and prognosis of medical treatment response but also for multiple markers identification for early cancer diagnostics [4].

Therefore, molecular astrocytoma and oligodendroglioma subtypes of the 2^{nd}-4^{th} malignancy degrees, possessing similar LOH 1p and 19q status, but different in gene expression profiles which correlate with certain clinical features of these tumors, were identified in works on glioma gene expression profiling with differential hybridization cDNA-microarrays[5] and oligonucleotide microarrays[6].

It was also established that the expression of a relatively small quantity of genes was different in malignant and normal tissues. The differences in these genes expression can reflect the important changes in such critical processes as cell proliferation, proteasomal function, energy metabolism and signal transduction. However, considerable heterogeneity of glial tumors and a great number of ways of their development demand further investigation. We have analyzed gene expression in astrocytic gliomas of the 2^{nd}-4^{th} malignancy degrees and normal human brain using SAGE to identify genes that may be used as glial tumors molecular markers. The following results demonstrate that approximately 60 genes are expressed on a significantly higher level in astrocytic gliomas in comparison with normal human brain. Discovered genes are potential markers for glial tumors molecular classification, diagnosis, prognostic evaluation and antitumor therapy.

Materials and Methods. Nine glioblastoma SAGE-libraries (GB, astrocytomas of the 4^{th} degree by WHO classification), eleven anaplastic astrocytoma SAGE-libraries (AA, astrocytomas of the 3^{rd} degree), eight diffusive astrocytoma SAGE-libraries (A, astrocytomas of the 2^{nd} degree), and five normal human brain SAGE-libraries (NHB) were analyzed to compare gene expression using the DGED search program (Digital Gene Expression Displayer) and the SAGE Genie database (http://cgap.nci.nih.gov/SAGE). Taking into consideration the samples combination in some SAGE-libraries (GSM765 is a pool of 5 glioblastomas, and GSM763 is a pool of 2 samples of normal human brain), actually the analysis of 13 GB and 6 NHB samples was carried out (Table 1). The Unigene NCBI database (http://www.ncbi.nlm.nih.gov) was used to search for cDNA clones, which contain coding parts of discovered mRNA. The selected cDNA clones were obtained from the RZPD, Resource Center/Primary Database of the Genome of a Human project, Germany (http://www.rzpd.de).

The surgical samples of glial astrocytomas and normal human brain (histologically normal human brain tissue adjacent to a tumor which is forcedly extracted together with the tumor during the operation) were obtained from the Institute of Neurosurgery named after Romodanov A.P., Academician of Medical Sciences of Ukraine, Kyiv. 35 tumor samples, including 14 glioblastomas, 10 anaplastic astrocytomas, 6 astrocytomas, 2 oligoastrocytomas, 1 anaplastic oligodendroglioma, were analyzed in all.

Total RNA was extracted from tissues, frozen in liquid nitrogen, using the method of phenol extraction from guanidineisothiocyanate solution, as it was described in the previous works [7,8]. RNA (10mkg for a lane) was fractionated in horizontal 1.5% agarous gel in the presence of 2.2M of formaldehyde in borate buffer (0.2mM EDTA, pH 8.0; 30mM boric acid; 3.3mM natrium tetraborate, pH 7.5), then it was transferred to nylon membranes Hybond-N (Amersham Pharmacia Biotech, Austria).

RNA was hybridized for 16 hours at the temperature of 42°C with ^{32}R-marked cDNA samples in the solution of 50% formamide, 5xSSC, 5xDenhardt, 0.1% SDS, 100мg/ml of salmon DNA. The filters were washed 2 times 15 minutes each at the room temperature in the solution of 2xSSC, 0.1% SDS; one time for 15 minutes in the solution of 2xSSC, 0.1% SDS at the temperature of 65°C; finally in the solution of 0.2xSSC, 0.1% SDS for 15 minutes at the temperature of 65°C. The exposure of membranes on the X-ray film was conducted with the usage of the intensifying screen at the temperature of -70°C. The membranes were washed and hybridized repeatedly with the ^{32}R-labelled cDNA of the human в–actin as the control of loading RNA on the agarous gel. Densimetry of hybridizational signals was carried out using the Scion Image 1.62c program.

Results and Discussion. To determine relative levels of genes expression in astrocytic tumors of different malignancy degrees and in NHB with the help of SAGE, we used the public database of CGAP (Cancer Genome Anatomy

Table 1.
SAGE-libraries of astrocytic gliomas analyzed in this work.

Library name	Quantity of "tags" in the library
Normal Human Brain	
Normal human thalamus SAGE library (GSM713: SAGE_Brain_normal_thalamus_B_1)	24015
Normal human brain SAGE library (GSM676: SAGE_Brain_normal_cortex_B_BB542)	94233
Normal human brain SAGE library (GSM763: SAGE_Brain_normal_cortex_B_pool6)	62451
Normal human cerebellum SAGE library (GSM695: SAGE_Brain_normal_cerebellum_B_BB542)	40500
Normal human cerebellum SAGE library (GSM761: SAGE_Brain_normal_cerebellum_B_1)	50385
Total quantity of "tags" in the pool of SAGE-libraries of NHB	**271584**
Diffusive astrocytomas	
Astrocytoma grade II SAGE library (GSM1732: SAGE_Brain_astrocytoma_grade_II_B_H127)	114489
Astrocytoma grade II SAGE library (GSM14739: SAGE_Brain_astrocytoma_grade_II_B_H563)	88568
Astrocytoma grade II SAGE library (GSM14765: SAGE_Brain_astrocytoma_grade_II_B_H530)	102439
Astrocytoma grade II SAGE library (GSM2451: SAGE_Brain_astrocytoma_grade_II_B_H388)	106285
Astrocytoma grade II SAGE library (SAGE_Brain_astrocytoma_grade_II_B_H359)	105764
Astrocytoma grade II SAGE library (GSM14737: SAGE_Brain_astrocytoma_grade_II_B_H518)	116022
Astrocytoma grade II SAGE library (SAGE_Brain_astrocytoma_grade_II_B_H516)	108116
Astrocytoma grade II SAGE library (SAGE_Brain_astrocytoma_grade_II_B_H501)	128309
Total quantity of "tags" in the pool of SAGE-libraries of diffusive astrocytomas	**869992**
Anaplastic astrocytomas	
Astrocytoma grade III SAGE library (GSM14773: SAGE_Brain_astrocytoma_grade_III_B_R140)	118733
Astrocytoma grade III SAGE library (GSM697: SAGE_Brain_astrocytoma_grade_III_B_H1020)	51573
Astrocytoma grade III SAGE library (GSM14763: SAGE_Brain_astrocytoma_grade_III_B_H970)	106982
Astrocytoma grade III SAGE library (GSM14766: SAGE_Brain_astrocytoma_grade_III_B_R927)	107344
Astrocytoma grade III SAGE library (SAGE_Brain_astrocytoma_grade_III_B_407)	108312
Astrocytoma grade III SAGE library (SAGE_Brain_astrocytoma_grade_III_B_343)	100158
Astrocytoma grade III SAGE library (SAGE_Brain_astrocytoma_grade_III_B_439)	107824
Astrocytoma grade III SAGE library (SAGE_Brain_astrocytoma_grade_III_B_828)	99939
Astrocytoma grade III SAGE library (SAGE_Brain_astrocytoma_grade_III_B_584)	103008
Astrocytoma grade III SAGE library (SAGE_Brain_astrocytoma_grade_III_B_H1055)	109886
Astrocytoma grade III SAGE library (SAGE_Brain_astrocytoma_grade_III_B_H272)	96059
Total quantity of "tags" in the pool of SAGE-libraries of anaplastic astrocytomas	**1109818**
Glioblastomas	
Glioblastoma SAGE library (GSM696: SAGE_Brain_glioblastoma_B_H1110)	68986
Glioblastoma SAGE library (GSM765: SAGE_Brain_glioblastoma_B_pooled)	56428
Glioblastoma SAGE library (GSM14768: SAGE_Brain_glioblastoma_B_R336)	102322
Glioblastoma SAGE library (GSM14769: SAGE_Brain_glioblastoma_B_R70)	99099
Glioblastoma SAGE library (GSM14767: SAGE_Brain_glioblastoma_B_H833)	100600
Glioblastoma SAGE library (SAGE_Brain_glioblastoma_B_H1353)	124805
Glioblastoma SAGE library (SAGE_Brain_glioblastoma_B_H1425C)	88990
Glioblastoma SAGE library (SAGE_Brain_glioblastoma_B_H1371)	49338
Glioblastoma SAGE library (SAGE_Brain_glioblastoma_B_R20)("tags")	101053
Total quantity of "tags" in the pool of SAGE-libraries of glioblastomas	**791621**

Table 2
The list of genes with the more than 5-fold distribution of "tags" in SAGE-libraries of glioblastomas (pool A) and NHB (pool B) according to DGED results.

"Tags"	Gene name	graphical symbol	Quantity of libraries with the "tag"		The quantity of the "tag" in the pool		"Tag" surplus in tumor	P**
			A	B	A	B		
TGGGATTCCC	chitinase 3-like 2	CHI3L2	9	0	256	0	NaN*	0.00
CCACAGGGGA	collagen, type III, alpha 1 (Ehlers-Danlos syndrome type IV, autosomal dominant)	COL3A1	8	0	169	0	NaN*	0.00
AGTGGTGGCT	fibromodulin	FMOD	9	0	145	0	NaN*	0.01
TTAAATAGCA	stress-associated endoplasmic reticulum protein 1	SERP1	6	0	140	0	NaN*	0.01
GTCAACAGTA	ATP-binding cassette, sub-family C (CFTR/MRP), member 3	ABCC3	8	0	103	0	NaN*	0.04
TGCTCCTACC	Fc fragment of IgG binding protein	FCGBP	9	2	556	2	95.44	0.00
GTATGGGCCC	chitinase 3-like 1 (cartilage glycoprotein-39)	CHI3L1	9	2	826	4	70.92	0.00
ACCAAAAACC	collagen, type I, alpha 1	COL1A1	8	2	1125	6	64.42	0.01
ACATTCTTTT	glycoprotein (transmembrane) nmb	GPNMB	9	1	361	2	61.95	0.00
AGTACCTTAT	epidermal growth factor receptor (erythroblastic leukemia viral (v-erb-b) oncogene homolog, avian)	EGFR	8	1	163	1	55.93	0.01
GACCACCTTT	microfibrillar-associated protein 2	MFAP2	6	1	153	1	52.50	0.01
ATCCTGAGTT	major histocompatibility complex, class II, DQ beta 1	HLA-DQB1	9	1	125	1	42.89	0.04
GAAATAAAGC	immunoglobulin heavy constant gamma 1 (G1m marker)	IGHG1	7	3	605	5	41.54	0.00
TTTGGTTTTC	collagen, type I, alpha 2	COL1A2	8	2	529	5	36.32	0.00
ATAATAAAGC	retinoic acid receptor responder (tazarotene induced) 2	RARRES2	9	1	205	2	35.17	0.00
CATATCATTA	insulin-like growth factor binding protein 7	IGFBP7	9	5	1189	13	31.42	0.00
TCACCAAAAA	stabilin 1	STAB1	8	1	174	2	29.85	0.01
GCCCTTTCTC	mannose receptor, C type 2	MRC2	9	1	159	2	27.28	0.02
GCCAACAACG	nicotinamide N-methyltransferase	NNMT	9	2	153	2	26.25	0.03
CTTGGGTTTT	insulin-like growth factor 2 (somatomedin A)	IGF2	9	3	289	4	24.80	0.00
GCTGCCCTTG	tubulin alpha 6	TUBA6	9	3	259	4	22.22	0.00
ATCTTGTTAC	fibronectin 1	FN1	9	3	192	3	21.96	0.01
AGAAAGATGT	annexin A1	ANXA1	9	5	437	7	21.43	0.00
TAACTCTCCT	scavenger receptor class A, member 3	SCARA3	9	3	241	4	20.68	0.00
AGAACCTTCC	major histocompatibility complex, class I, A	HLA-A	9	5	395	8	16.95	0.00

"Tags"	Gene name	graphical symbol	Quantity of libraries with the "tag"		The quantity of the "tag" in the pool		"Tag" surplus in tumor	P**
			A	B	A	B		
GCAACAGCAA	Sec61 gamma subunit	SEC61G	9	5	576	12	16.48	0.00
ATCAAGAATC	interferon, gamma-inducible protein 30	IFI30	9	2	185	4	15.87	0.04
GGATATGTGG	early growth response 1	EGR1	9	3	275	6	15.73	0.01
AATAGAAATT	secreted phosphoprotein 1 (osteopontin, bone sialoprotein I, early T-lymphocyte activation 1)	SPP1	8	4	438	11	13.67	0.00
GACTCTTCAG	serine (or cysteine) proteinase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 3	SERPINA3	9	5	1239	32	13.30	0.00
GGGCATCTCT	major histocompatibility complex, class II, DR alpha	HLA-DRA	9	5	814	22	12.71	0.00
AGCAGATCAG	S100 calcium binding protein A10 (annexin II ligand, calpactin I, light polypeptide (p11))	S100A10	9	4	475	13	12.54	0.00
TTCACTGTGA	lectin, galactoside-binding, soluble, 3 (galectin 3)	LGALS3	9	5	386	11	12.04	0.00
TTCTATTTCA	moesin	MSN	9	5	254	8	10.90	0.05
TGCTGACTCC	nestin	NES	9	4	284	9	10.83	0.03
GTTCACATTA	CD74 antigen (invariant polypeptide of major histocompatibility complex, class II antigen-associated)	CD74	9	5	2959	99	10.29	0.00
GTTGTGGTTA	beta-2-microglobulin	B2M	9	5	2830	95	10.25	0.00
CTCTAAGAAG	complement component 1, q subcomponent, alpha polypeptide	C1QA	9	5	882	30	10.10	0.00
TTTGCACCTT	connective tissue growth factor	CTGF	9	3	435	15	9.95	0.01
GGATGTGAAA	CD99 antigen	CD99	9	5	399	14	9.78	0.02
TAATTTTAAC	protein tyrosine phosphatase, receptor-type, Z polypeptide 1	PTPRZ1	9	5	336	12	9.61	0.04
TGGCCCCAGG	apolipoprotein C-I	APOC1	9	3	755	27	9.60	0.00
ATGTGAAGAG	secreted protein, acidic, cysteine-rich (osteonectin)	SPARC	9	5	2717	112	8.35	0.00
ACAAAGCATT	insulin-like growth factor binding protein 5	IGFBP5	9	5	750	36	7.15	0.04

*NaN – "not a number", i.e. absence of gene "tags" in the pool and impossibility of dividing by 0;
**P – possibility

Project). For the previous work the comparison of five SAGE-libraries of GB with two SAGE-libraries of NHB present in this database at that time revealed 117 genes with more than 5-fold expression difference (P#0.05) in GB comparing to NHB.

Four new SAGE-libraries of GB have recently appeared in SAGE Genie database. Besides, the quantity of SAGE-libraries of anaplastic astrocytomas has increased, they are eleven at present, and there are eight SAGE-libraries of diffusive astrocytomas. The comparison of nine

Table 3.
Changes of genes expression at different stages of astrocytic gliomas of human

"Tag"	Gene symbolic notation	The quantity of libraries where "tag" is present				"Tag" quantity in the united pool of "tag" libraries , normalized to 1mln of "tags"				"Tag" surplus in tumour, in comparison with NHB		
		NHB	A	AA	GB	NHB	A	AA	GB	A	AA	GB
TGGCCCCAGG	APOC1	3	8	11	9	99	836	905	951	9.05	9.12	9.60
GTTGTGGTTA	B2M	5	8	11	9	350	2737	2816	3566	8.43	8.08	10.25
GGGCATCTCT	HLA-DRA	5	8	11	9	81	605	874	1026	8.02	10.81	12.71
CATATCATTA	IGFBP7	5	8	11	9	48	1176	1154	1498	26.42	24.16	31.42
ATGTGAAGAG	SPARC	5	8	11	9	412	3852	2815	3423	10.07	6.85	8.35
TAACTCTCCT	SCARA3	3	8	11	9	15	72	108	326	10.38	6.97	20.68
GTTCACATTA	CD74	5	8	11	9	364	1630	2015	3727	4.81	5.54	10.29
TAATTTTAAC	PTPRZ1	5	8	11	9	44	292	312	423	7.10	7.08	9.61
GACTCTTCAG	SERPINA3	5	7	11	9	118	485	758	1561	4.44	6.44	13.30
AGCAGATCAG	S100A10	4	8	11	9	48	171	264	599	3.84	5.52	12.54
CTCTAAGAAG	C1QA	5	8	11	9	110	253	504	1111	2.46	4.57	10.10
GGATGTGAAA	CD99	5	8	11	9	52	275	380	503	5.73	7.39	9.78
TTCACTGTGA	LGALS3	5	8	10	9	40	74	175	486	1.96	4.32	12.04
AGAAAGATGT	ANXA1	5	7	10	9	26	194	256	550	8.07	9.93	21.43
TGGGATTCCC	CHI3L2	0	5	9	9	0	107	133	323	NaN*	NaN*	NaN*
GCTGCCCTTG	TUBA6	3	8	11	9	15	72	108	326	5.23	7.34	22.22
TGCTCCTACC	FCGBP	2	8	11	9	7	49	106	700	7.18	14.44	95.44
ATCTTGTTAC	FN1	3	6	10	9	11	30	116	242	2.91	10.52	21.96
AGAACCTTCC	HLA-A	5	8	11	9	29	224	185	498	8.16	6.30	16.95
TTTGCACCTT	CTGF	3	8	11	9	55	229	241	548	4.45	4.37	9.95
AATAGAAATT	SPP1	4	8	11	8	40	211	644	552	5.59	15.94	13.67
GTATGGGCCC	CHI3L1	2	2	6	9	15	18	280	1040	1.33	19.03	70.92
TCACCAAAAA	STAB1	1	7	11	8	7	46	51	219	6.71	6.97	29.85
GCCCTTTCTC	MRC2	1	7	9	9	7	44	40	200	6.40	5.38	27.28
CCACAGGGGA	COL3A1	0	2	3	8	0	3	5	213	NaN*	NaN*	NaN*
AGTGGTGGCT	FMOD	0	5	5	9	0	9	16	183	NaN*	NaN*	NaN*
GTCAACAGTA	ABCC3	0	3	7	8	0	4	14	130	NaN*	NaN*	NaN*
ATCCTGAGTT	HLA-DQB1	1	7	11	9	4	46	77	158	13.42	21.05	42.89
AGTACCTTAT	EGFR	1	7	10	8	4	44	68	205	12.80	18.35	55.93
TTAAATAGCA	SERP1	0	1	1	6	0	1	1	176	NaN*	NaN*	NaN*
ACCAAAAACC	COL1A1	2	4	2	8	22	4	12	1418	0.21	0.53	64.42
GACCACCTTT	MFAP2	1	2	5	6	4	2	9	193	0.62	2.45	52.50
GAAATAAAGC	IGHG1	3	4	6	7	18	26	13	762	1.50	0.69	41.54
TTTGGTTTTC	COL1A2	2	2	5	8	18	4	11	667	0.25	0.59	36.32
GCCAACAACG	NNMT	2	3	6	9	7	10	23	193	0.94	3.06	26.25
CTTGGGTTTT	IGF2	3	5	3	9	15	74	15	364	5.38	1.04	24.80
GCAACAGCAA	SEC61G	5	8	11	9	44	51	861	726	1.25	19.53	16.48
ATCAAGAATC	IFI30	2	5	9	9	15	142	103	304	1.56	4.04	15.87
TTCTATTTCA	MSN	5	8	10	9	29	128	82	320	4.68	2.78	10.90
ACATTCTTTT	GPNMB	1	5	11	9	7	452	63	455	65.90	7.22	61.95

"Tag"	Gene symbolic notation	The quantity of libraries where "tag" is present				"Tag" quantity in the united pool of "tag" libraries , normalized to 1mln of "tags"				"Tag" surplus in tumour, in comparison with NHB		
		NHB	A	AA	GB	NHB	A	AA	GB	A	AA	GB
ATAATAAAGC	RARRES2	1	8	7	9	7	117	71	258	17.02	9.67	35.17
TGCTGACTCC	NES	4	8	11	9	33	142	116	358	4.61	3.51	10.83
ACAAAGCATT	IGFBP5	5	8	11	9	132	311	270	945	2.52	2.04	7.15
GGATATGTGG	EGR1	3	8	11	9	22	351	177	347	17.07	8.04	15.73

*NaN – "not a number", i.e. absence of gene "tags" in the pool and impossibility of dividing by 0;
**P – possibility

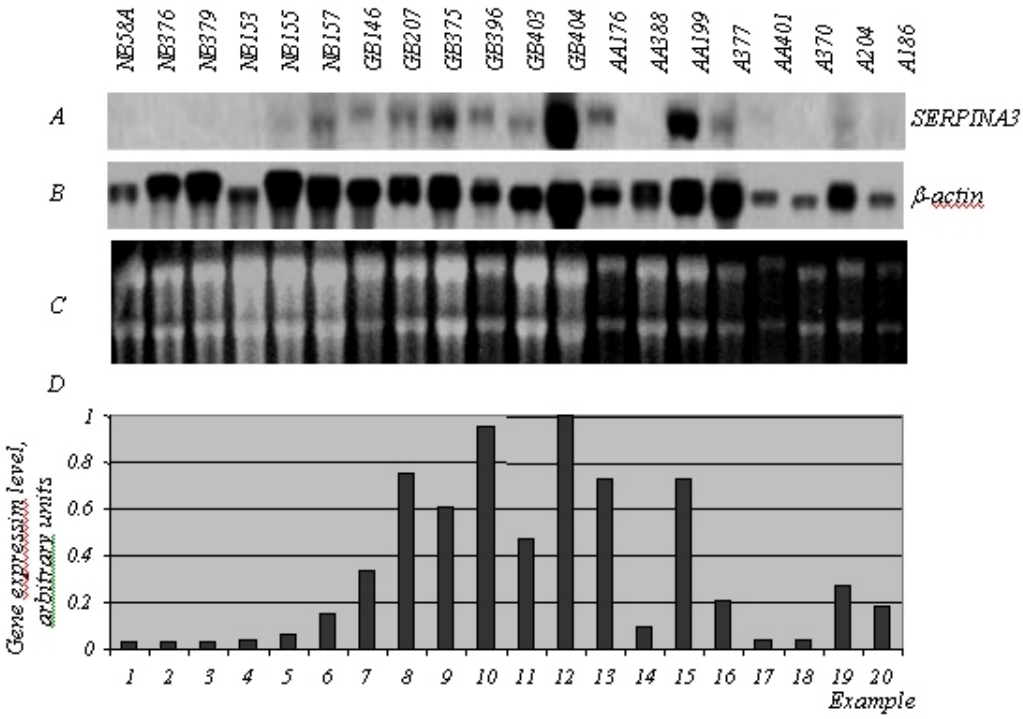

Fig 1. Genes ANXA1, B2M, C1QA, and SEC61G expression analysis in glial tumors. Nothern-hybridization of RNA panel with ^{32}P-marked probe of cDNA ANXA1(A), B2M (B), C1QA(C), SEC61G,(D) and the check sample of cDNA ?-actine(E). Types of tissues and kinds of tumors are marked at blot path, figures in brackets indicate the RNA-sample number. GB – glioblastoma, AA – anaplastic astrocytoma, A – astrocytoma, OA – oligoastrocytoma, AO – anaplastic oligodendroglioma, NB – normal human brain. (G) The picture of agarous gel, stained by bromide etidium. (H) The diagram which shows the relative level of gene expression.

libraries of GB altogether and five libraries of NHB altogether revealed 199 gene "tags" with the 5-fold difference in their distribution in these two pools (P#0.05). Practically all the "tags" correspond to known nucleotide sequences in GenBank, with the exception of two "tags", for which there were no corresponding transcripts found. About 90% of these known nucleotide sequences are well-characterized mRNA, others are not characterized ESTs (expressing sequence "tags"). Nucleotide sequences, which correspond to "tags" with more than 5-fold surplus in glioblastoma group in comparison with NHB group, can be marked as "overexpressed in tumors", while the ones, which correspond to "tags" with more than 5-fold surplus in NHB group in comparison with glioblastoma group, can be marked as "overexpressed in normal human brain".

"Tags" for hypothetical protein genes, unknown cDNA, mitochondrial genes, as well as the ones which are "unreliable", i.e. internal "tags", located inside mRNA in

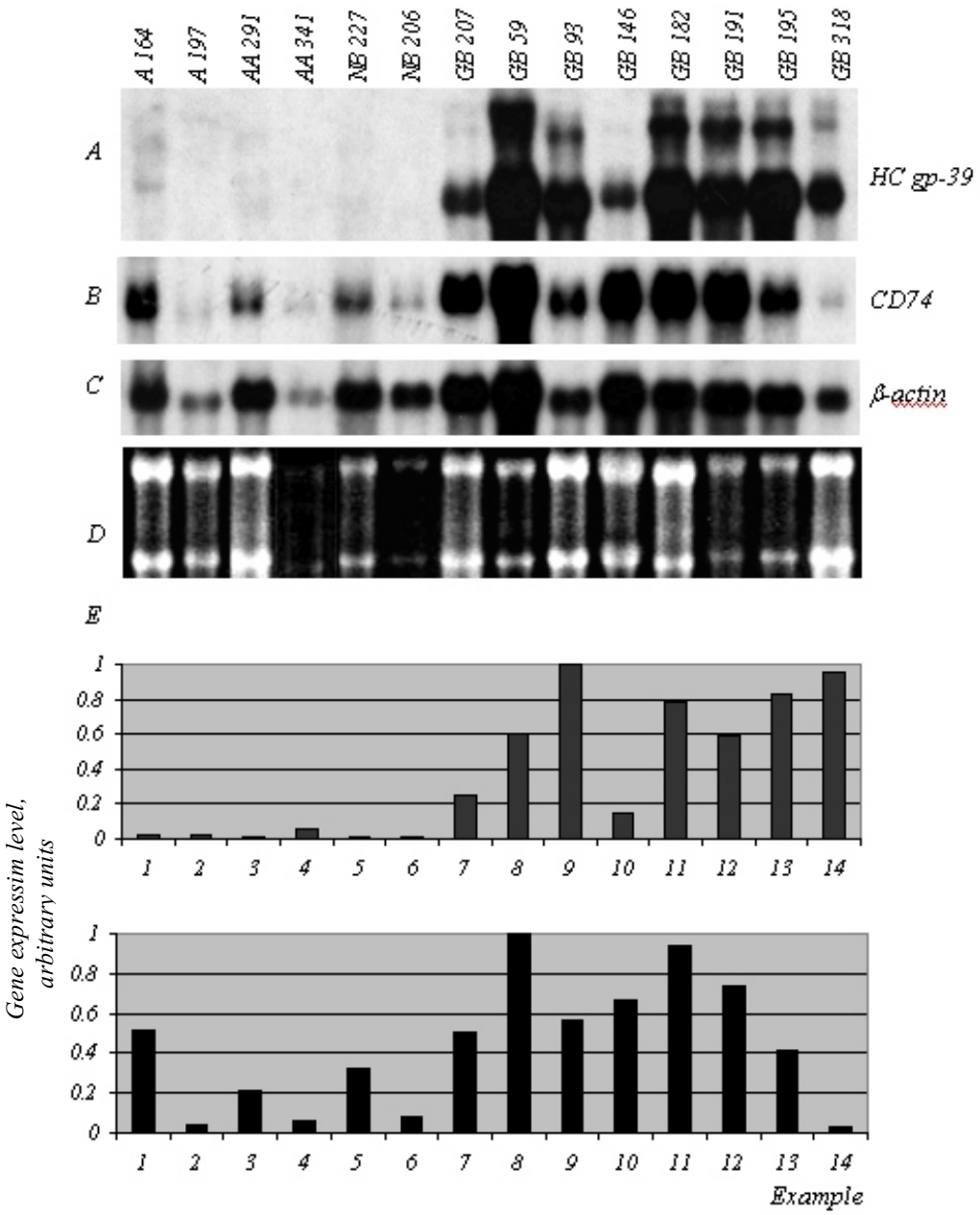

Fig 2. Gene SERPINA3 expression analysis in glial tumors. Nothern-hybridization of RNA panel with ^{32}P-marked probe of cDNA SERPINA3(A), and the check sample of cDNA ?-actine (B). Types of tissues and kinds of tumors are marked at blot path, figures in brackets indicate the RNA-sample number. NB – normal human brain, GB – glioblastoma, AA – anaplastic astrocytoma, A – astrocytoma. (C) The picture of agarous gel, stained by bromide etidium. (D) The diagram which shows the relative level of gene expression.

addition to an "true" not extracted "tag", were from the analysis. Thus, 5-fold expression changes in glioblastomas were revealed for 129 genes in all. 44 out of 129 genes correspond to the criterion of being "overexpressed in tumors". They are presented in Table 2. If the coefficient of expression changes is decreased to less than 5, then the quantity of "overexpressed in tumors" genes will increase, but the genes with greater changes in expression level certainly have greater biological importance. The representatives of this gene group participate in glioma development with great probability [10, 11], so we concentrated our at-

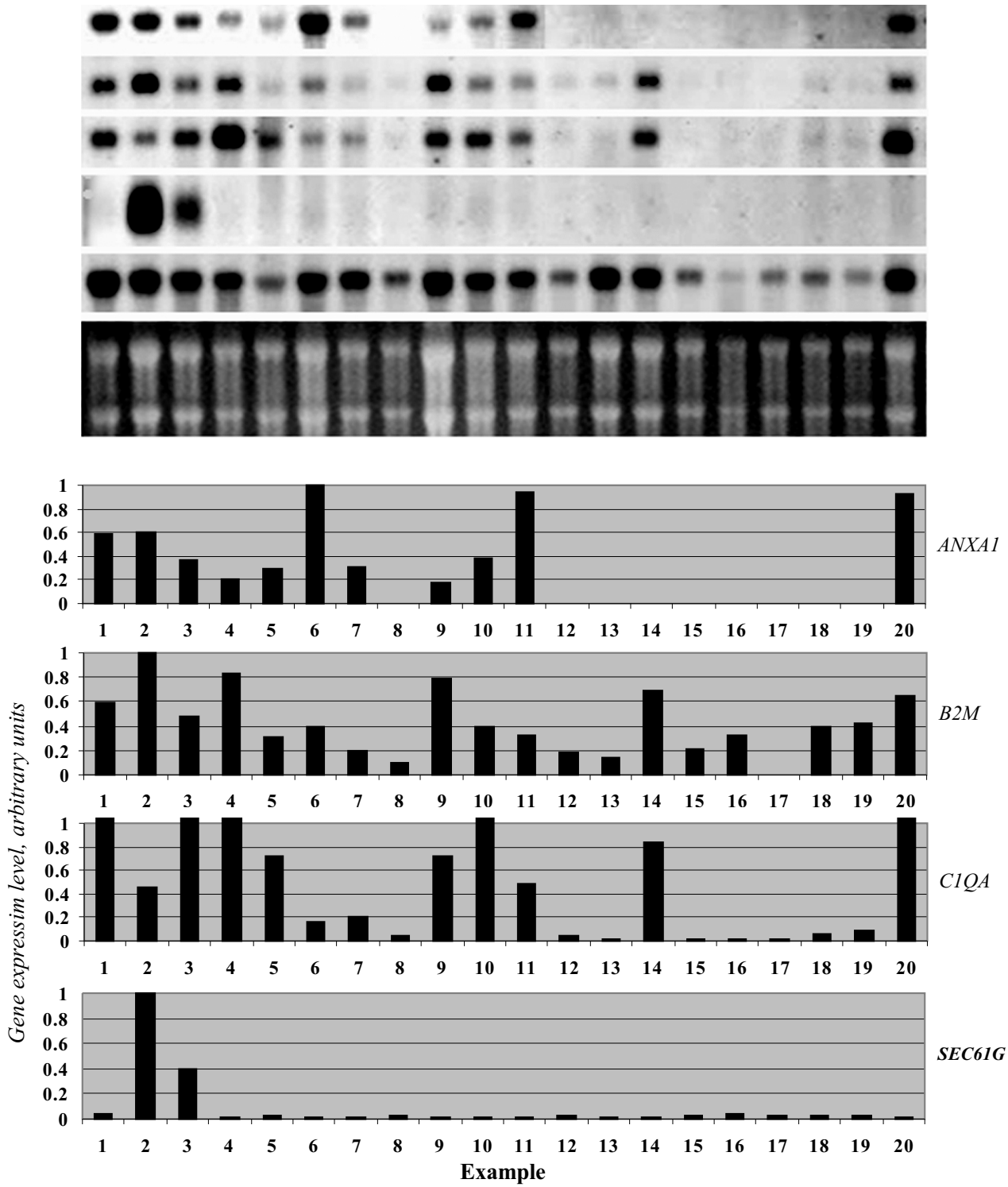

Fig 3. Genes HC gp-39 and CD74 expression analysis in glial tumors. Nothern-hybridization of RNA panel with ^{32}P-marked probe of cDNA HC gp-39 (A), CD74 (B) and the check sample of cDNA в-actine (C). Types of tissues and kinds of tumors are marked at blot path, figures in brackets indicate the RNA-sample number. NB – normal human brain, GB – glioblastoma, AA – anaplastic astrocytoma, A – astrocytoma. (D) The picture of agarous gel, stained by bromide etidium. (E) The diagram which shows the relative level of gene expression.

tention on the research of genes, which are "overexpressed in tumors".

Our research is based on presently existing SAGE-libraries in SAGE Genie database – nine libraries of adult patients' GB, eleven AA libraries, eight libraries of diffue extension of libraries sets can result in some changes in the picture of gene expression. However, the comparison of results, described in this work, to the results, obtained before on five SAGE-libraries of GB and two SAGE-libraries of NHB [9], did not reveal substantial discrepancies in the list of genes, which are differentially expressed.

While comparing eleven SAGE-libraries of AA, taken together, to five SAGE-libraries of NHB at the same analysis conditions (more than 5-fold difference of "tags" distribution and the probability limit P#0.05) the total quantity of "tags" with 5-fold difference of distribution was revealed to be less (118 "tags"), than while comparing glioblastomas and NHB. No corresponding known transcripts were found for 5 "tags", and others are well-characterized mRNA and not characterized ESTs. After removing the "tags", which correspond to hypothetic protein genes, unknown cDNA, mitochondrial genes, as well as unreliable ones, the total quantity of genes, whose expression changes in AA comparing to NHB, was 66 genes, including 18 genes with the increased expression level. While comparing eight SAGE-libraries of diffusive astrocytomas, taken together, to five SAGE-libraries of NHB 5-fold difference was revealed for 83 "tags", 2 of which did not have corresponding known nucleotide sequences. After the corresponding results processing, 42 genes were revealed to have the expression, changed more than 5-fold in diffusive astrocytomas, and 16 with increased expression.

These results indicate that the quantity of genes, whose expression is stimulated in astrocytic gliomas, increases with the malignant progress of tumors. Some expression changes are revealed at the early stage of astrocytoma formation and are observed on a higher level in more malignant astrocytomas (Table 3). The expression of genes APOC1, B2M, HLA-DRA, IGFBP7, and SPARC (marked in bold in Table 3) increases in diffusive astrocytomas and remain at approximately the same level at the following stages of astrocytoma development. The expression changes are distinctive for some genes only for the most malignant form – glioblastoma (marked in italic in Table 3). It is not quite clear why some genes (located in the bottom of Table 3) have a decreased level of expression in anaplastic astrocytomas comparing to diffusive astrocytomas and glioblastomas, where it is much higher than in NHB. Probably, considerably increased expression of these genes is distinctive only for a certain group of tumors and is a reflection of the specific way of their development.

To confirm SAGE results and to estimate the levels of gene expression in independent sets of samples of glial tumors and NHB, we selected eight "overexpressed in tumors" transcripts at random and carried out the analysis of their expression with Nothern-hybridization. The selected genes have a sufficient expression level to be determined by this method and though Nothern-hybridization is less sensitive than reverse transcription – polymerase chain reaction (RT-PCR), it has an advantage in determining not only a relative level of gene transcription but also the amount of possible alternative transcripts.

Generally, the pictures of gene expression were reproduced for the analyzed samples of tumors and NHB. Each of eight selected genes had a considerably higher expression level in GB, than in NHB (Pictures 1-3). It is important to emphasize the variations in the level of gene expression in individual tumor samples, which may be explained by heterogeneity of tumor biological properties, which was stated before [12, 13]. The gene expression varies on a large scale in diffusive and anaplastic astrocytomas for different genes – the levels of gene expression in some tumors are almost as high as in glioblastomas, considerably lower in some tumors, and the gene expression is absent in some, as well as in NHB.

Considering the combinations of expression levels of at least four genes for the tumors of the same malignancy level (Picture 3), one may combine some tumors into separate groups. For example, glioblastomas may be divided into two groups, concerning the expression level of SEC61G gene. Anaplastic astrocytomas AA401 and AA394 have a similar expression profile, AA230 and AA416 also resemble each other. The picture of hybridizational signals for AA199 is similar to glioblastoma GB450, which may testify to a more aggressive character of this anaplastic astrocytoma development. Certainly, the profiles of tumor expression may not be characterized on the basis of expression analysis of four genes, however, the simultaneous analysis of several dozens of genes in a big amount of individual tumors samples must reveal certain molecular variants, which have the same histopathological diagnosis, and combine tumors into separate groups which reflect certain clinical disease peculiarities.

Thus, the malignant progress of astrocytic gliomas is accompanied with expressive changes accumulation. The repetition of these changes in astrocytomas of different levels of anaplasia testifies to the fact that are not accidental, and they probably are a reflection of specific processes in tumor cells. The expression level of the most of "overexpressed in tumor" genes increases gradually at the

astrocytoma progressing and is the highest in GB. There is a limited quantity of genes, whose expression is activated more than 5-fold at all stages of astrocytoma development, or at least at the last two, the most malignant stages of development. These genes may serve for the recognition of the most malignant tumor variants at the comparison of expressive tumor profiles. The expressive gene changes, distinctive only for GB, may be used for the glioblastoma recognition. Generally, the expression analysis of such genes may be used for the recognition of glial tumors with different malignancy degrees, presumably, for early diagnosis, prognostic purposes and anticancer therapy.

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Суперэкспрессия генов на разных стадиях прогрессии астроцитарных глиом

Резюме

Публичная база данных по серийному анализу генной экспрессии (SAGE) была использована для идентификации потенциальны молекулярных маркеров астроцитарных глиом человека. При сравнении девяти SAGE-библиотек глиобластом, одиннадцати SAGE-библиотек анапластических астроцитом, восьми SAGE–библиотек диффузных астроцитом и пяти SAGE-библиотек нормального головного мозга было выявлено 57 генов, уровень экспрессии которых был больше чем в 5 раз выше (P#0.05) в астроцитарных глиомах по сравнению с нормальным головным мозгом. Кроме изменений экспрессии генов, которые происходят на ранней стадии формирования астроцитом и выявляются также на последующих этапах их развития, некоторые изменения характерны лишь для высокозлокачественных стадий развития опухолей и отсутствуют в опухолях низкой степени злокачественности. Анализ экспрессии выявленных генов может быть использован для молекулярной классификации глиальных опухолей, диагностики, прогностической оценки опухолей и определения потенциальных мишеней протиопухолевой терапии.

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