CHAPTER 13

Proteome-Based Anti-Tumor Cell Therapy

Andrey S. Bryukhovetskiy[1], Valeriy E. Shevchenko[2], Sergey V. Kovalev[2], Vladimir G. Polyakov[2], Igor S. Bryukhovetskiy[3] and Maria V. Zhukova[4,*]

[1]Federal Research Center for Specialized Types of Medical Assistance and Medical Technologies of FMBA of Russia, 28 Orekhoviy blvd. 115682 Moscow, Russia; NeuroVita Clinic of Restorative and Interventional Neurology and Therapy, 23 Kashirskoe shosse, 115478, Moscow, Russia; [2]FGBU Blokhin Russian Cancer Research Center of RAMN,24 Kashirskoe shosse, 115478, Moscow, Russia; [3]School of Biomedicine, Far Eastern Federal University, 8 Sukhanova St. 690091, Vladivostok, Russia and [4]NeuroVita Clinic of Restorative and Interventional Neurology and Therapy,23 Kashirskoe shosse, 115478, Moscow, Russia

Abstract: The chapter is dedicated to the issues of clinical oncoproteomics, its role and place in contemporary medicine in general, and in oncology in particular. We attempted to systemize the views of different researchers and detect information and significance of the evidence of clinical oncoproteomics for the development of novel methods of diagnostics and cancer therapy using cell, genome and post-genome technologies. The chapter summarizes contemporary ideas of tumor carcinogenesis centrally laying emphasis on the theoretical, methodological and technic the aspects of the novel medical approach of personalized proteome-based cell therapy of neoplasms. Experience of mapping, proteome and transcriptome profiling of postnatal regional progenitors and cancer stem cells in different cancer cases, methods of bioinformatical processing and mathematical modeling of the results have been elucidated. The basic mathematical tool kit to detect targets for the regulation of proliferative and reproductive functions of cancer stem cell in the tumor and the way to obtain target individually tailored anti-cancer proteome-modified cell preparations have been highlighted. Further, the mechanisms, limitations and prospects of application of these biotechnologies based on clinical oncoproteomics in the target therapy of cancer and recent patents have also been discussed.

Keywords: Cancer cells, cancer immune therapy, cancer stem cells, cancer, cell

***Corresponding author Maria V. Zhukova:** NeuroVita Clinic of Restorative and Interventional Neurology and Therapy, 23 Kashirskoe shosse, 115478, Moscow, Russia; Tel: +74993249339; Fax: +74959801373; E-mail: neurovitaclinic@gmail.com

Atta-ur-Rahman and Khurshid Zaman (Eds)

signaling, chemical induction, clinical proteomics, glioblastoma, intracellular signal transduction pathways, multipotent mesenchymal stromal cells, neoplasm, neural stem cells, personalized cancer therapy, proteome mapping, proteome, proteomics, stem cells, target therapy, tumor, U87 glioblastoma.

INTRODUCTION

Until now, the systemic solution for the therapy of cancer and malignant tumors has neither been found in theory, nor in practice. Despite constantly increasing number of carcinogenesis concepts, development of novel anti-cancer medical technologies and pharmaceuticals, no fundamental breakthrough in understanding of carcinogenesis and cancer therapy has been observed. In our opinion, inefficiency of cancer and malignant tumors therapy is conditioned by a wrong paradigm and incorrect methodological approach to the development of anti-cancer strategies. The latest therapies of cancer become dated before they shape into pharmaceuticals and accomplished treatment strategies and exhibit such significant methodological flaws which cross their advantages, that they appear anti-evolutionary, anti-adaptive and outdated in their nature. All previously known conventional as well as brand-new therapies of cancer have one thing in common, and that is unfaltering and firm intention of all researchers, molecular biologists, surgeons, oncologists, chemotherapists, radiologists and pharmacologists to eliminate all cancer cells (CCs). The goal of any contemporary cancer therapy is complete elimination of CCs in any possible way: to poison CCs with chemotherapy (cytotoxic methods), to arrest their growth or destroy internal structure of CCs (cytostatic methods), or to surgically remove CCs from the organism (cytoreductive method).

The methodological approach of clinical oncoproteomics that we propose in the chapter basically permits new technological solution in the tumor therapy (cytoregulatory therapy) that relies on the novel paradigm of regulation of the number of CCs and tumor growth control. A new paradigm will imply refusal from unreasonably high doses of toxic chemicals and radiation, individualization of anti-cancer therapy and transfer of lethal uncontrolled neoplastic process into chronic non-lethal disease which does not affect the life quality of the patient and requires follow-up and, if necessary, repeated courses of the personalized cell therapy developed for the patient.

In spite of continuous search and detection of new targets for CCs elimination by anti-cancer therapy, still no convincing results of targeted therapy have been received. We presume that contemporary target therapy in its current content will always lag behind the adaptive processes of intracellular molecular reorganizations of the proteins in CCs, as it is directed at the targets on the signal transduction pathways, cell cycle or effector functions of CCs, which are extremely volatile and modify at every new stage of cell adaptation to constantly changing aggressive environment, microenvironment and treatment. This evolutionary adaptive advantage of a somatic cell is extremely important for its survival and is realized by a somatic neoplastic cell even in the case of irreversible mutations of its genome, distortions of proteome and disturbance of secretome and metabolome. Low efficiency of cancer and tumor therapy presented as CCs elimination is a naturally determined and consistent outcome of such methodological approaches to the therapy. Hence, the paradigm of anti-cancer treatment aimed only at CCs elimination seems weak and requires revision. We published in the Russian journal in 2010, a new alternative concept of cancer and neoplasms therapy that could be named a cytoregulatory therapy (CRT) related to cell transplantation and tissue engineering [1]. The key idea of the therapy is to learn to control the number of CCs and manipulate tumor growth using autologous stem and progenitor cells with remodeled proteome profile. The approach can be rationalized by the fact that up to 500 000 ($5x10^5$) CCs circulate in every healthy human, while in pre-cancer, their amount rises up to 1 billion (10^9), and cancer is characterized by over 1 billion CCs [2]. Hence, restoration of quantitative balance and regulation of proliferative activity of cell systems in the organism of a cancer patient is the way to the control of tumor growth and compensation of misbalance of tumor tissue homeostasis. At the first stage (2005-2010) of tests in animals, we applied the technology of chemical induction using different inductors of apoptosis (viscumin and ricin) and demonstrated technical possibility to use the CRT methodology for the therapy of neoplasms [3, 4]. However, experimental use of the CRT in the models of different tumors showed that regulation of CCs and cancer stem cells (CSCs) must be extremely accurate and precise. It demands knowledge of individual features, genomics and proteomics of CCs and CSCs of specific tumor and SCs of the patient. We focused on mapping, profiling and cataloguing for the analysis of proteins of CCs,

CSCs and native tissue-specific stem cells of a cancer patient accurate analysis. We established that the proteins of CSCs and proteins of hematopoietic SCs (HSCs), neural SCs (NSCs) and multipotent mesenchymal stromal cells (MMSCs) isolated from the patients with breast cancer, lung cancer and glioblastoma have a small number of proteins in common, and they can become the key to regulation and control of CCs growth. If they are affected by the autologous healthy SCs and progenitor cells with individually remodeled proteome, it would provide an opportunity to control the number of CCs and their effector functions (angiogenesis, proliferation, migration etc.). Relying on a new concept of carcinogenesis as speciation [5], we concluded that the application of post-genome technologies in the analysis of CCs and CSCs permits detection of "Achilles heel" in their proteome presented as similarity matrix of the proteins. More specifically, we detected a continuously existing group of proteome targets that were applicable for the target therapy of CCs and CSCs by healthy SCs with the same specific proteins. For this, the proteome profile (PP) (namely, cell proteins) of healthy SCs can be remodeled by targeted chemical induction, changing their transcriptome by low-molecular substances or affecting cell proteins with appropriate microRNA up- or down-regulating gene expression of these cell proteins. We proposed several methods of individual remodeling of SC proteome (avoiding gene engineering) with further biocomputation of regulatory PP and biological modeling of personalized therapy of tumors *in vitro* and *in vivo*.

For illustration in the chapter, we demonstrate the main stages of the development of proteome-based technology of tumor therapy on the model of well-known line of glioblastoma multiforme.

Contemporary achievements of medicine have not dramatically changed the efficiency of glioblastoma (GB) therapy. Even if the patients comply with standard protocols of complex treatment, their survival hardly exceeds two years, making this cancer one of the most unfavorable. Transplantation of different lines of stem cells can be one of the most promising methods to improve the survival rates in glioblastoma cases [4].

The goal of research that became the cornerstone for CRT was proteome mapping of U87 gliomasphere line lysates, comparing them to cell proteins of the tissue-

specific NSCs and MMSCs of a neurooncological patient (standard control) to detect specific neoplasm proteomic structure of a cancer stem cell (CSC), diagnostics and analysis of carcinogenesis unaffected intracellular signal transduction pathways (ISTPs) in CSCs, as well as the search for membrane proteins that can provide the ISTPs CSC targets for regulation of CSC effector functions in the cell therapy of tumors. The human U87 glioblastoma cells were chosen as a stable, standard and ubiquitously available cell line.

Available data analysis showed that two-dimensional gel electrophoresis [6-10] or liquid chromatography [11-15] combined with mass-spectrometry was employed for NSCs, MSSCs and U87 glioblastoma CSCs proteome mapping most frequently. Gel electrophoresis is more suitable to detect differentially expressed proteins, than to map proteins, so we focused on the combination of high performance liquid chromatography and mass spectrometry HPLC-MS. Application of two-dimensional chromatography permits the identification of about 1000 proteins in a sample (1002 in operation [10], 867 in operation [13]), thus, we chose the method of two-dimensional division of tryptic peptides in cation-exchange and reverse phase columns. To evaluate protein expression, we used label free method as it did not require additional stages of peptide labeling, and was proven to reveal correct quantitative results for stem cells [13, 14].

MATERIALS AND METHODS

Reagents:We used deionized MilliQ water from Millipore Simplicity 185 ultrapure water system (Millipore, Billerica, MA, USA). Mammalian Cell Lysis Kit (containing Sodium Lauryl sulfate (SDS); Deoxycholic acid (DOC); Igepal and protease inhibitor cocktail), tris(hydroxymethyl)aminomethane (Tris), Bradford assay kit, 2.2.2-trifluoroethanol (Reagent Plus), iodoacetomide, and trypsin (from porcine pancreas, proteomics grade, Bioreagent, dimethylated) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Sodium chloride NaCl (purum), potassium chloride KCl (purum) and hydrochloric acid HCl (puriss) were purchased from Chimmed (Moscow, Russia). Ammonium bicarbonate NH_4HCO_3 (Ultra), tris(2-carboxyethyl)phosphine hydrochloride (CCEP) (purum), and trifluoroacetic acid (for protein sequence analysis) were purchased from Fluka (Buchs, Germany). We used acetonitrile (ACN) (HPLC-gradient grade, PAI-

ACS) from Panreac Quimica SA (Barcelona, Spain) and formic acid (FA) (Suprapur) from Merck (Darmstadt, Germany).

Bone Marrow Sampling. After a neurooncological patient (male, 42; NeuroVita Clinic, Moscow, Russia) signed the informed consent (Protocol# 17 of Ethical Committee Meeting, Federal Research Center for Specialized Types of Medical Assistance and Medical Technologies of FMBA of Russia, Moscow, Russia), the bone marrow was taken from the pelvic or breast bone in the medical treatment room under local anesthesia (or in the operating room under general anesthesia) in sterile conditions. The optimal volume of bone marrow for mesenchymal cells isolation was 5ml. Aspirated bone marrow was placed into Vacuette sterile tube (Vacutest kima s.r.l., Arzergrande (PD), Italy) with disodium ethylenediaminetetraacetic acid (Na_2EDTA).

Isolation and Culturing of MMSC. MMSCs were isolated from bone marrow according to the previously described methods [16]. The bone marrow sample was resuspended in Roswell Park Memorial Institute (RPMI)-1640 medium containing 10% of fetal bovine serum (FBS) and 1% penicillin-streptomycin solution and cultured in T150 culture flasks (Z707554 SIGMA TPP) at 37°C in 5% CO_2 atmosphere. Four to five days later, the medium with non-adherent cells was replaced by a fresh one. Adhered cells were cultured till 80% confluence and then passaged at 1:3. The medium was changed every three days. MMSCs were characterized by surface antigen expression: cluster of differentiation 29 positive, $CD44^+$, $CD73^+$, $CD90^+$, $CD34^-$evaluated in flow cytometry.

Isolation and Culturing of Brain Tumor Stem Cells. Brain TSCswere isolated from human U87 glioblastoma cell culture (male, 44) by the method described previously [17]. U87 glioblastoma cells were provided by the Department of Biosynthesis of Nucleic Acids, Institute of Molecular Biology and Genetics, National Academy of Sciences of Ukraine, Kyiv, Ukraine. The cells were cultured in low glucose DMEM (Gibco, Life Technologies, Moscow, Russia) with 10% FBS at 37°C in wet atmosphere with 5% CO_2. Adherent cells were cultured till 80% confluence and passaged at 1:3. To obtain tumor spheres, the tumor cells were resuspended in neurosphere medium (Dulbecco's modified Eagle's medium [DMEM] /F12 (Sigma Aldrich, St. Louis, MO, USA); L-glutamine (Life Tech.,

USA); B27(Life Tech., USA); basic fibroblast growth factor [bFGF] (Sigma Aldrich, St. Louis, MO, USA), 20ng/ml; epidermal growth factor [EGF] (Sigma Aldrich, St. Louis, MO, USA), 20ng/ml; penicillin/streptomycin, 100U/ml; heparin, 5μg/ml (OAO Sintez; Kurgan, Russia)) and cultured in T75 flasks (Z7077546 SIGMA TPP) at 37°C, in 5% CO_2. New growth factors were added every 3 days. Once a sufficient cell quantity was reached, CD133+ cells were immunosorted by anti-CD133 coated magnetic beads (Miltenyi Biotec, Bergisch Gladbach, Germany). After that the CD133+ cells were cultured in the medium applied earlier; their purity was evaluated in flow cytometry for CD133 antibody (Miltenyi Biotec, Bergisch Gladbach, Germany) and it exceeded 90%.

Isolation of Neural Stem and Progenitor Cells (NSPC) of a Human. After signing of the informed consent, NSPCs were isolated from the patient's olfactory epithelium of the upper nasal cavity according to the method described previously (male, 42; *NeuroVita* Clinic, Moscow, Russia) [18], and cultured in DMEM medium with 10% FBS and a growth factors cocktail until cytospheres developed. Cytosphere cells were characterized by nestin, thymocyte antigen 1(Thy1; CD90), heavy neurofilament 200kDa (NF200) and glial firbrillary acidic protein (GFAP) expression in immunocytochemical analysis. The olfactory sheath was sampled and resuspended and NSCs were isolated from the cell culture of olfactory sheath by CD133-positive immunoseparation by magnetic beads. The lysates for proteome research were prepared from cell cultures.

Proteome Research. Three samples of the cells were cryopreserved in PBS. After defreezing, the cells were lysed with Mammalian Cell Lysis Kit according to the manufacturer's guidelines.

Method of Lysing. One milliliter of lysing buffer was prepared. For that the following mixture was prepared from the kit contents: 200μl of buffer (250mM Tris-HCl, pH 7.5, 5mM EDTA); 200μl 750mM NaCl; 200μl 0.5% SDS (Lauryl sulfate); 200μl 2.5% DOC (Deoxycholic acid); 200μl 5% Igepal; 10μl of protease inhibitor cocktail. All procedures were performed at 4°C. One milliliter of lysing buffer was added to the cells, and the cells were incubated for 15 min in a cooled Eppendorf Thermomixer Comfort shaker (Hamburg, Germany) and centrifuged in a cooled Eppendorf Centrifuge 5415F for 1 hour. Supernatant was removed for

further research. After lysis, the amount of total proteins in lysates was: NSCs (Sample01) - 2032 + 85µg/ml; U87 glioblastoma CSCs (Sample02) - 2150 + 360µg/ml; MMSCs (Sample03) - 3198 + 281µg/ml. The received samples were purified from low-molecular components by Agilent 5K MWCO 4ml Spin Concentrators for Proteins (Wilmington, Germany).

Purification from Low-Molecular Components. The sample was put into the concentrator and centrifuged at 2000g until 200µl of fluid remained. Then 4ml of MilliQ water was added and centrifuged again. The samples were washed three times and 200µl of the sample was left. Additionally, the sample was washed from the concentrator twice with 200µl of MilliQ water. The obtained 600µl of the samples were used for further research and total protein was measured by Bradford assay kit. The amount of lysate containing 300µg of proteins was calculated and dried at 60°C in a CentriVap vacuum evaporator (Labconco, Kansas City, MO, USA).

Tryptic Cleavage. For trypsinolysis, 150µl of Sample 01 and Sample 02 and 100µl of Sample 03 were taken. Dried lysates were added to 25µl of 2.2.2-trifluoroethanol, 25µl of 100mM water solution of NH_4HCO_3 and 2µl of fresh 50mM aqueous solution of trichloroethylphosphate (CCEP). This reaction mixture was exposed for 1 hour at 60°C, and then cooled at 25°C; 1µl of fresh 84mM aqueous iodoacetomide was added and exposed for 30 minutes at 25°C; then, 100µl of 100mM of NH_4HCO_3 solution, 300µl of water and trypsin solution in 1mM of hydrochloric acid (trypsin concentration 100ng/µl, trypsin: protein ratio 1:50 by weight) and exposed for 18 hours at 37°. Trypsin was added 1:50 by weight, i.e. Sample 01 - 305µg protein, 61µl trypsin solution; Sample 02 - 323µg protein, 64.5µl trypsin solution; Sample 03 - 320µg protein, 64µl trypsin solution. Three microliters of solutions were analyzed by mass spectrometry for completion of trypsinolysis. Completion of trypsinolysis was controlled by the peaks of tryptic peptides and by the areas of peaks with m/z 842.51 Da and 421.76 Da. When the reaction was over, the content of the tubes was stripped to dryness at 60°C in the CentriVap.

Separation of tryptic peptides. Tryptic peptides (50µg) were dissolved in 60µl of A (30% acetonitrile, 70% water, 0.1% FA, pH 2.7) and separated by a Dionex

Ultimate 3000 chromatographic system (Amsterdam, Netherlands), equipped with a fraction collector at strong cation exchange (SCX) column MIC-10-CP. The volume of injected probe was 20µl, the flow of solvent 30µl/min, column temperature 25°C and detection by UV absorption at wave length of 214nm. Solvents: Mobile A phase - 30% acetonitrile (ACN), 70% water, 0.1% formic acid (FA), mobile B phase - mobile A phase + 500mM KCl. Gradient: 0-10min - 0% B, 10-100min - 0-30% B, 100-114min - 30-100% B, 114-119 min - 100% B, then column equilibration for 35 minutes at A phase. Twenty fractions were gathered from 2 to 122 minutes with 6 minutes intervals. The fractions were stripped off to 100µl at 60°C in the Eppendorf Concentrator 5301 centrifuge evaporator.

Mass-Spectrometry. Tryptic peptides were analyzed in nano flow chromatograph Dionex Ultimate 3000 in combination with mass-spectrometer LTQ Orbitrap XL (ThermoFisher Scientific, San Jose, CA, USA) with NanoSpray ionization ion source. Peptides were separated in Acclaim C18 PepMap100 column (75µm x 150mm, grain size 3µm, Dionex), equipped with trap column (Acclaim C18 Pepmap 100, 500µm x 5 mm, grain size 5µm, Dionex). The sample (20µl) was loaded in trap column for 1 min in 99% water/1% ACN/ 0.1% FA, then washed for 4 minutes with 0.05% solution of trifluoroacetic acid in water, and equilibrated for 1min with 99% water/1% ACN/ 0.1% FA, 20µl/min flow. Chromatography: 0.3µl/min flow, mobile A phase - 98% water/2% ACN/ 0.1% FA, mobile B phase -20% water/80% ACN/0, 08% FA. Gradient: 0-6 min - 0% B, 6-120 min - 0-50% B, 120-150 min -50-100% B, 150-165 min -100% B, 165-170 min - 100-0% B, complete analysis time 175 min. Mass-spectra were registered in the positive ions mode in m/z 300-2000 Da range, needle voltage - 1.7kV, source temperature 200°C, capillary voltage 43V, to lens potential 165 V. MS spectra were registered in orbital trap in Fourier Transform (FT) mode (resolution 60000, number of accumulated ions 1·106, maximal accumulation time - 700ms, 1 microscan), tandem (MS/MS) spectra were obtained by collision induced dissociation (CID) in a linear trap (Enhanced scanning mode, number of accumulated ions 50000, maximal accumulation time- 500ms, 3 microscans, collision energy 35% from maximal). MS/MS spectra were registered for the 7 most intensive ions. Dynamic exclusion turned on after registration of 1 mass spectrum, with the exclusion time being 1 min. Ions with charge over +1 underwent secondary fragmentation. Data

analysis. To identify proteins, the mass spectra were converted into mgf files by Proteome Discoverer 1.0 (ThermoFisher Scientific) software, pattern WF_Spectrum_Export_MGF set by default except for mass range (from 300 to 10000Da) and retention time (0-180 minutes). Protein search was done on the local server with Mascot Server 2.3.02 software (Matrix Science, London, UK). Search parameters: database National Center for Biotechnology Information non-redundant GenBank (NCBInr, version dated 25 January, 2012), species - Homo Sapiens, enzyme - trypsin, number of missed cleavages 2, fixed modifications - carbamidomethylation of cysteines, variable modifications N-terminus protein acetylation and oxidized methionine, other modifications were checked with error tolerant search. Accuracy of parent ion mass was 10ppm, fragments 0.8Da; instrument type Ion Trap. Identified proteins were sorted by MudPIT score, presented peptide at significance level $p < 0.05$. Received lists of identified proteins and chromatic mass specters were uploaded to Skyline 1.2.0.3 (University of Washington, https://skyline.gs.washington.edu/labkey/project/home/software/Skyline), and received peak peptide areas in every probe. Then the areas of all peaks of every protein identified peptide were summed and normalized according to total area of all identified peaks in the probe.

RESULTS

Data processing by Mascot Server software permitted the identification of 1664 proteins in all probes. Further processing by Skyline software detected the following: in Sample 01 - 1447 proteins by 11176 peptides (range of protein molecular weight from 3.53 to 3908.10kDa); in Sample 02 - 1225 proteins by 13674 peptides (range of protein molecular weight from 4.60 to 3908.10kDa); in Sample03 - 842 proteins by 10932 peptides (range of protein molecular weight from 5.02 to 1017.07kDa). Dynamic range of identified proteins of 7 orders (from $4.8 \cdot 10^{-7}$% to 5.3%), permits identification of regulatory low copy proteins, such as interleukins 25 and 36, growth factor receptors etc. Also specific markers of mesenchymal (CD44, integrins α-V and β-1) and neural progenitor (nestin) stem cells were present. In each of the three samples, 606 proteins had been identified (common core for all cells).

Proteome maps of NSC, MMSC and U87 GB CSC are shown in the research reports at the webpage www.neurovita.ru [19-21]. The data for proteome mapping (PM) of the proteins of different types have undergone comparative bioinformatical analysis. First, rough computing analysis of PM proteins from compared groups was performed and the stem cell (SC) proteins of low functional significance were excluded. The proteins identified only in one cell type were excluded from the examined SC proteomes. Out of 1664 proteins we obtained comparison group A (NSCs and CSCs; 1052 proteins) [19] and comparison group B (MMSCs and CSCs; 607 proteins) [19]. The results of comparison of group A (63.2% identical proteins) demonstrated an affinity of morphofunctional phenotypes of NSCs and gliomaspheres as with cells that had neural-like differentiation and localization in the nervous system. While comparison of group B showed only 607 (36.47%) identical proteins in the analyzed MMSCs and CSCs. Although the features of stemness are characteristic for MMSCs and CSCs and they are not so different phenotypically, their multipotent differentiation condition showed considerable differences in the proteome profile of these cells.

We excluded all proteins with normalized spectral intensity (NSI) values that deviated less than twice from the groups. We presumed that only significantly different NSI values represent basic regulatory tendencies. Therefore, only 637 proteins were left in group A (38.28%), and 425 proteins in group B (25.54%).

A summarizing table of the group A and group B proteins (503 proteins) was compiled [19] and showed the proteins with doubled NSI and greater-fold changed NSI in either group. The proteins from the table were annotated and further processed; the results were presented in an additional file “Annotation of NSC, MMSC and CSC proteins” [19]. Using the data of the survey [14] and databases, we excluded all known proteins involved in GB carcinogenesis. These are three proteins: molecule CD44 (Indian blood group), isocitrate dehydrogenase 1 (nicotinamide adenine dinucleotide phosphate positive ($NADP^+$) soluble and vimentin; and they were also excluded from further study.

The next stage of bioinformatical analysis was the distribution of the proteins into functionally significant clusters for intracellular and intercellular regulation, protein clusters-targets.

The first cluster of the proteins "Receptor targets" was set: we isolated proteins located on membranes of NSCs, MMSCs and CSCs (Table **1**), totaling 105 proteins (6.31%), from the remaining proteins of proteomic profiles of NSC and CSC as well as MMSC and CSC. For that, we selected the proteins with GO: 0005886 plasma membrane code in annotation. The distribution of the proteins depending on molecular functions, biological processes and signaling pathways was presented graphically (Figs. (**1**-**3**)).

Table 1: Cluster of Membrane Proteins.

NSC		MMSC		CSC		Protein
NSI, %	**NSC/CSC**	**NSI, %**	**MMSC/CSC**	**NSC, %**		
0.099	0.67	0.029	0.20	0.15	1	A kinase (PRKA) anchor protein 12
0.029	7.82	0.042	11.3	0.0037	1	ADP-ribosylation factor 1
0.027	9.39	0.013	4.63	0.0029	1	ADP-ribosylation factor 4
0.030	2.50	0.0010	0.085	0.012	1	ADP-ribosylation factor 5
0.20	10.7	0.64	34.6	0.019	1	ARP3 actin-related protein 3 homolog (yeast)
0.014	0.25	0.0018	0.032	0.057	1	ATP synthase, H^+ transporting, mitochondrial F1 complex, O subunit
0.19	0.58	0.037	0.38	0.17	1	ATP synthase, H^+ transporting, mitochondrial F1 complex, alpha subunit 1, cardiac muscle
0.13	0.45	0.086	0.055	0.23	1	ATP synthase, H^+ transporting, mitochondrial F1 complex, beta polypeptide
0.0068	0.17	0.0058	0.15	0.040	1	ATPase, H^+ transporting, lysosomal 31kDa, V1 subunit E1
0.19	1.13	0.037	0.22	0.17	1	ATPase, Na^+/K^+ transporting, alpha 1 polypeptide
0.0075	0.42	0.0090	0.50	0.018	1	CD59 molecule, complement regulatory protein
0.092	1.10	0.013	0.15	0.084	1	IQ motif containing GTPase activating protein 1
0.13	37.6	0.0060	1.77	0.0034	1	LIM and SH3 protein 1
0.00054	4.06	0.00097	7.32	0.00013	1	LIM domain containing preferred translocation partner in lipoma
0.035	0.77	0.018	0.41	0.045	1	RAB10, member RAS oncogene family
0.041	0.51	0.013	0.17	0.080	1	RAB1B, member RAS oncogene family
0.0024	0.079	0.0076	0.25	0.030	1	RAP1B, member of RAS oncogene family
0.019	0.38	0.064	1.29	0.049	1	Rho GDP dissociation inhibitor (GDI) alpha
0.0026	0.16	0.00031	0.019	0.016	1	Rho GTPase activating protein 1
0.0073	11.1	0.00059	0.91	0.00066	1	VAMP (vesicle-associated membrane protein)-associated protein A, 33kDa
0.0093	14.1	0.011	16.8	0.00066	1	VAMP (vesicle-associated membrane protein)-associated protein B and C

Table 1: contd…

1.04	4.52	1.21	5.26	0.23	1	Actinin, alpha 1
0.046	7.52	0.0031	0.50	0.0061	1	Acyl-Coenzyme A binding domain containing 3
0.0086	156	7.52E-05	1.36	5.52E-05	1	Adaptor-related protein complex 1, beta 1 subunit
0.19	159	0.080	68.9	0.0012	1	Adaptor-related protein complex 2, beta 1 subunit
0.022	2.54	0.0027	0.32	0.0088	1	Adenylate kinase 1
0.0041	0.34	0.0093	0.77	0.012	1	Adhesion regulating molecule 1
0.0018	0.065	0.00024	0.0086	0.028	1	Aldo-keto reductase family 1, member A1 (aldehyde reductase)
0.29	0.26	0.48	0.43	1.12	1	Annexin A1
0.90	1.28	1.43	2.03	0.70	1	Annexin A2 pseudogene 3; annexin A2; annexin A2 pseudogene 1
0.10	5.69	0.094	5.33	0.018	1	Annexin A6
0.016	0.45	0.0095	0.27	0.035	1	Archain 1
0.027	0.35	0.12	1.49	0.077	1	Brain abundant, membrane attached signal protein 1
0.00095	0.16	0.0030	0.51	0.0059	1	Calcium/calmodulin-dependent protein kinase II delta
0.093	1.79	0.20	5.10	0.087	1	Calmodulin 3 (phosphorylase kinase, delta); calmodulin 2 (phosphorylase kinase, delta); calmodulin 1 (phosphorylase kinase, delta)
0.060	0.42	0.030	0.21	0.14	1	Calpain 2, (m/II) large subunit
0.12	4.49	0.0067	0.25	0.027	1	Calpain, small subunit 1
0.033	4.63	0.0093	1.02	0.0016	1	Cathepsin B
0.012	0.42	0.016	0.54	0.029	1	Cell division cycle 42 (GTP binding protein, 25kDa); cell division cycle 42 pseudogene 2
0.0066	0.24	0.0020	0.074	0.027	1	Chaperonin containing CCP1, subunit 3 (gamma)
0.059	0.48	0.084	0.68	0.12	1	Chloride intracellular channel 1
0.048	3.56	0.057	4.21	0.013	1	Chloride intracellular channel 4
0.24	1.11	0.049	0.22	0.22	1	Clathrin, heavy chain (Hc)
0.040	3.17	0.0072	0.58	0.013	1	Collagen, type VI, alpha 3
0.33	7.33	0.060	1.35	0.045	1	Cytochrome b5 reductase 3
3.23E-05	0.53	0.0012	20.4	6.09E-05	1	Dihydrolipoamide S-succinyltransferase (E2 component of 2-oxo-glutarate complex); dihydrolipoamide S-succinyltransferase pseudogene (E2 component of 2-oxo-glutarate complex)
0.016	0.16	0.064	0.61	0.11	1	Early endosome antigen 1
0.65	0.45	0.99	0.69	1.45	1	Enolase 1, (alpha)
0.14	2.51	0.35	6.41	0.054	1	Eukaryotic translation initiation factor 5A; eukaryotic translation initiation factor 5A-like 1

Table 1: contd…

0.00072	0.83	0.00023	0.26	0.00087	1	Family with sequence similarity 120A
0.028	25.6	0.0011	1.01	0.0011	1	Fascin homolog 1, actin-bundling protein (Strongylocentrotus purpuratus)
0.021	0.69	0.012	0.38	0.030	1	Ferritin, heavy polypeptide 1; ferritin, heavy polypeptide-like 16; similar to ferritin, heavy polypeptide 1; ferritin, heavy polypeptide-like 3 pseudogene
0.43	1.08	0.18	0.46	0.40	1	Filamin C, gamma (actin binding protein 280)
0.0025	0.83	0.0093	3.08	0.0030	1	Flotillin 2
0.21	1.03	0.011	0.053	0.20	1	Glucose-6-phosphate dehydrogenase
0.00090	0.18	0.0013	0.27	0.0049	1	Guanine nucleotide binding protein (G protein), alpha activating activity polypeptide, olfactory type
0.00096	0.19	0.0037	0.73	0.0050	1	Guanine nucleotide binding protein (G protein), beta polypeptide 1
0.046	0.31	0.29	1.93	0.15	1	Heat shock 27kDa protein-like 2 pseudogene; heat shock 27kDa protein 1
0.072	0.11	0.016	0.024	0.67	1	Heat shock 60kDa protein 1 (chaperonin) pseudogene 5; heat shock 60kDa protein 1 (chaperonin) pseudogene 6; heat shock 60kDa protein 1 (chaperonin) pseudogene 1; heat shock 60kDa protein 1 (chaperonin) pseudogene 4; heat shock 60kDa protein 1 (chaperonin)
0.028	2.76	0.022	2.18	0.010	1	High density lipoprotein binding protein
0.10	6.52	0.020	1.29	0.016	1	Hypothetical protein LOC100129652; ezrin
0.27	4.25	0.0020	0.083	0.12	1	Integrin, beta 1 (fibronectin receptor, beta polypeptide, antigen CD29 includes MDF2, MSK12)
0.24	2.04	0.12	1.05	0.12	1	Keratin 1
0.089	4.70	0.0028	0.15	0.019	1	Keratin 8 pseudogene 9; similar to keratin 8; keratin 8
0.0044	0.10	0.00098	0.023	0.043	1	Kinectin 1 (kinesin receptor)
0.31	0.72	0.090	0.21	0.42	1	Lamin A/C
0.061	4.77	0.091	7.12	0.013	1	Lymphocyte cytosolic protein 1 (L-plastin)
0.0089	0.50	0.002	0.12	0.018	1	Lysosomal-associated membrane protein 1
0.00012	0.45	0.00024	0.92	0.00026	1	Major histocompatibility complex, class I, C; major histocompatibility complex, class I, B
0.049	2.56	0.0013	0.067	0.019	1	Metadherin
0.18	0.68	0.023	0.089	0.26	1	Microtubule-associated protein 1B
0.019	5.73	0.019	5.84	0.0033	1	Microtubule-associated protein, RP/EB family, member 1
0.34	1.28	0.095	0.36	0.26	1	Moesin
0.0047	0.95	0.012	2.35	0.0050	1	Myosin IC

Table 1: contd…

0.28	0.23	0.18	0.14	1.24	1	Myosin, heavy chain 9, non-muscle
0.012	3.46	0.0013	0.37	0.0035	1	Parvin, alpha
0.027	0.33	0.049	0.58	0.084	1	Phosphatidylethanolamine binding protein 1
0.16	25.3	0.022	3.65	0.0061	1	Polymerase I and traNSCript release factor
0.018	1.54	0.0048	0.41	0.012	1	Protein kinase, cAMP-dependent, regulatory, type II, alpha
0.095	6.65	0.019	1.32	0.014	1	Radixin
0.15	0.37	0.088	0.21	0.41	1	Reticulon 4
0.031	0.84	0.010	0.27	0.037	1	Ribosomal protein S3 pseudogene 3; ribosomal protein S3
0.060	0.45	0.0073	0.054	0.13	1	Ribosomal protein SA pseudogene 9; ribosomal protein SA pseudogene 8; ribosomal protein SA pseudogene 58; ribosomal protein SA pseudogene 19; ribosomal protein SA pseudogene 18; ribosomal protein SA; ribosomal protein SA pseudogene 15; ribosomal protein SA pseudogene 61; ribosomal protein SA pseudogene 29; ribosomal protein SA pseudogene 12
0.012	0.57	0.0030	0.15	0.020	1	Scavenger receptor class B, member 2
0.037	0.25	0.020	0.14	0.15	1	Septin 2
0.016	8.95	0.0017	0.94	0.0018	1	Septin 9
0.046	0.67	0.0025	0.037	0.068	1	Solute carrier family 25 (mitochondrial carrier adenine nucleotide translocator), member 4
0.39	1.49	0.0025	0.0096	0.26	1	Solute carrier family 25 (mitochondrial carrier; adenine nucleotide translocator), member 5; solute carrier family 25 (mitochondrial carrier; adenine nucleotide translocator), member 5 pseudogene 8
0.019	2.07	0.024	2.64	0.0092	1	Solute carrier family 25 (mitochondrial carrier; phosphate carrier), member 3
0.061	1.28	0.017	0.35	0.048	1	Spectrin, alpha, non-erythrocytic 1 (alpha-fodrin)
0.055	8.45	0.035	5.35	0.0065	1	Spectrin, beta, non-erythrocytic 1
0.0042	0.87	0.00020	0.041	0.0048	1	Stomatin
0.024	0.29	0.058	0.69	0.084	1	Superoxide dismutase 1, soluble
0.37	0.73	1.29	2.54	0.51	1	Talin 1
0.0029	11.9	0.00025	1.03	0.00025	1	Tight junction protein 1 (zona occludens 1)
0.054	0.69	0.40	5.14	0.078	1	Transgelin 2
0.022	3.86	0.019	3.34	0.0058	1	Transglutaminase 2 (C polypeptide, protein-glutamine-gamma-glutamyltransferase)
0.0036	0.32	0.00086	0.076	0.011	1	Tripartite motif-containing 25
0.29	3.41	1.58	18.4	0.086	1	Tropomyosin 1 (alpha)
0.62	2.56	0.33	1.36	0.24	1	Tubulin, beta 3; melanocortin 1 receptor (alpha melanocyte stimulating hormone receptor)

Table 1: contd…

0.0024	0.14	0.0024	0.14	0.017	1	Ubiquilin 2
0.046	14.8	0.026	8.37	0.0031	1	Vasodilator-stimulated phosphoprotein
0.14	1.17	0.64	5.17	0.12	1	Vinculin
0.030	0.43	0.0053	0.077	0.069	1	Voltage-dependent anion channel 1; similar to voltage-dependent anion channel 1
0.0059	0.62	0.043	4.48	0.0096	1	Zyxin

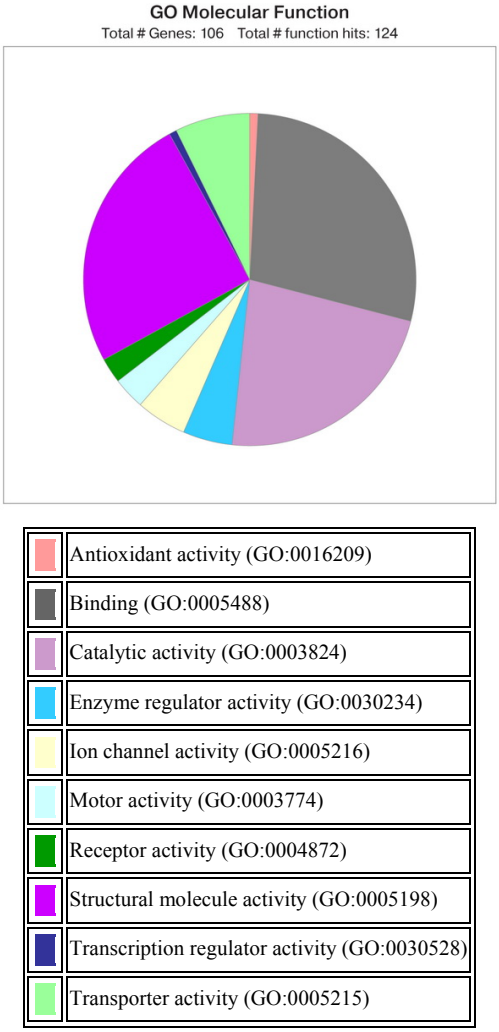
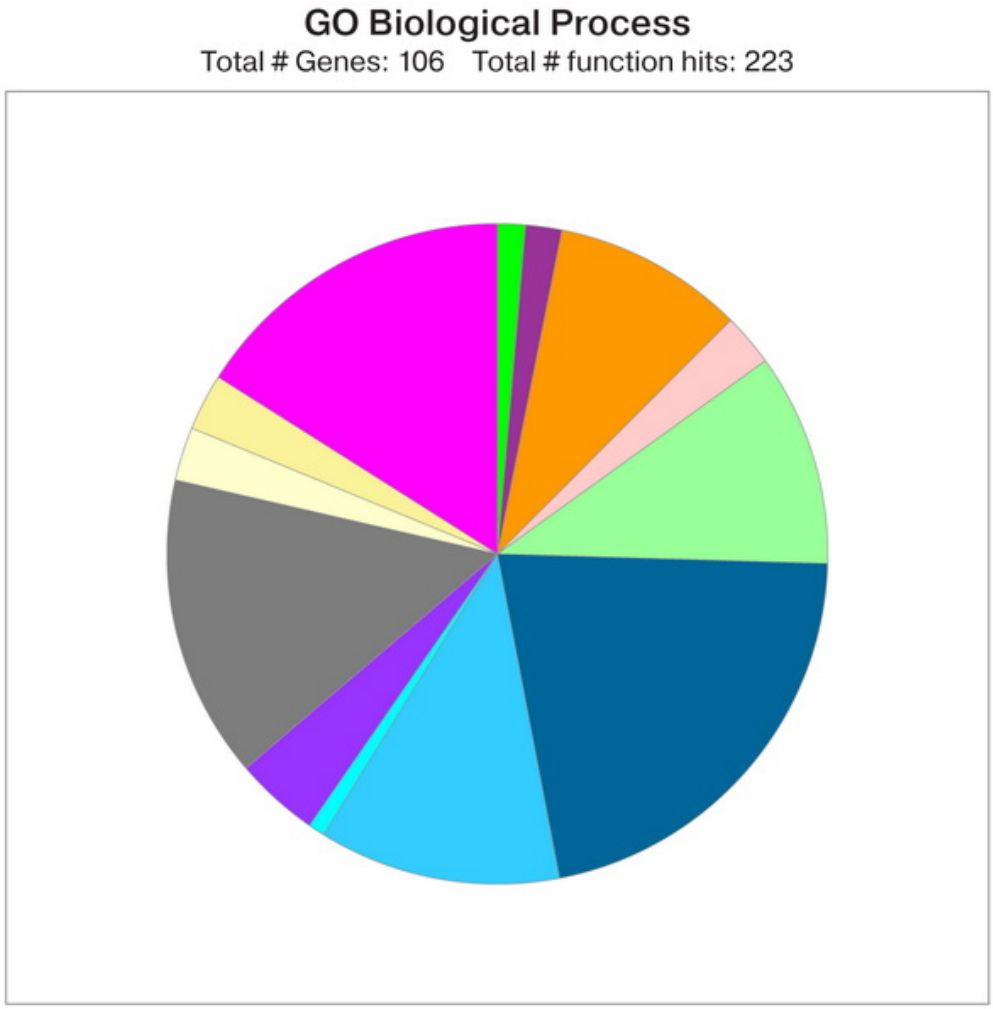
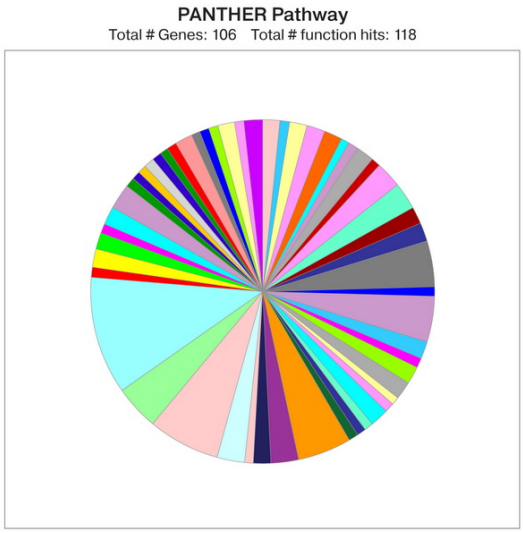


Figure 1: Molecular functions of membrane proteins observed in neural stem cells (NSCs), multipotent mesenchymal stromal cells (MMSCs) and U87 glioblastoma Cancer stem cells (CSCs).



	Apoptosis (GO:0006915)		Cell adhesion (GO:0007155)
	Cell communication (GO:0007154)		Cell cycle (GO:0007049)
	Cellular component organization (GO:0016043)		Cellular process (GO:0009987)
	Developmental process (GO:0032502)		Generation of precursor metabolites and energy (GO:0006091)
	Homeostatic process (GO:0042592)		Immune system process (GO:0002376)
	Metabolic Process (Go:0008152)		Response To Stimulus (Go:0050896)
	System process (Go:0003008)		Transport (Go:0006810)

Figure 2: Biological processes in which membrane proteins of NSCs, MMSCs and U87 glioblastoma CSCs participate.



	5HT1 Type receptor mediated signaling pathway (P04373)		5HT2 Type receptor mediated signaling pathway (P04374)		5HT4 Type receptor mediated signaling pathway (P04376)
	ATP synthesis (P02721)		Angiogenesis (P00005)		Angiotensin II-stimulated signaling through G proteins and beta-arrestin (P05911)
	Axon guidance mediated by Slit/Robo (P00008)		Axon guidance mediated by netrin (P00009)		B cell activation (P00010)
	Beta1 adrenergic receptor signaling pathway (P04377)		Beta 2 adrenergic receptor signaling pathway (P04378)		Beta 3 adrenergic receptor signaling pathway (P04379)
	Cortocotropin releasing factor receptor signaling pathway (P04380)		Cytoskeletal regulation by Rho GTPase (P00016)		*De novo* purine biosynthesis (P02738)
	Dopamine receptor mediated signaling pathway (P05912)		EGF receptor signaling pathway (P00018)		Endogenous_cannabinoid_signaling (P05730)
	Endothelin signaling pathway (P00019)		Enkephalin release (P05913)		FAS signaling pathway (P00020)
	FGF signaling pathway (P00021)		GABA-B_receptor_II_signaling (P05731)		General transcription by RNA polymerase I (P00022)
	Glycolysis (P00024)		Hedgehog signaling pathway (P00025)		Heterotrimeric G-protein signaling pathway-Gi alpha and Gs alpha mediated pathway (P00026)
	Heterotrimeric G-protein signaling pathway-Gq alpha and Go alpha mediated		Heterotrimeric G-protein signaling pathway-rod outer segment phototransduction		Histamine H1 receptor mediated signaling pathway (P04385)

	pathway (P00027)		(P00028)		
	Histamine H2 receptor mediated signaling pathway (P04386)		Huntington disease (P00029)		Inflammation mediated by chemokine and cytokine signaling pathway (P00031)
	Integrin signalling pathway (P00034)		Ionotropic glutamate receptor pathway (P00037)		Metabotropic glutamate receptor group II pathway (P00040)
	Metabotropic glutamate receptor group III pathway (P00039)		Muscarinic acetylcholine receptor 1 and 3 signaling pathway (P00042)		Muscarinic acetylcholine receptor 2 and 4 signaling pathway (P00043)
	Nicotinic acetylcholine receptor signaling pathway (P00044)		Opioid prodynorphin pathway (P05916)		Opioid proenkephalin pathway (P05915)
	Opioid proopiomelanocortin pathway (P05917)		Oxytocin receptor mediated signaling pathway (P04391)		PDGF signaling pathway (P00047)
	PI3 kinase pathway (P00048)		Ras Pathway (P04393)		T cell activation (P00053)
	TGF-beta signaling pathway (P00052)		Thyrotropin-releasing hormone receptor signaling pathway (P04394)		Transcription regulation by bZIP transcription factor (P00055)
	VEGF signaling pathway (P00056)		Wnt signaling pathway (P00057)		p38 MAPKpathway (P05918)

Figure 3: Signal pathways of membrane proteins expressed in NSCs, MMSCs and U87 glioblastoma CSCs.

The second cluster "Nuclear targets" was set: we isolated protein groups located in the nuclei of the remaining proteins of proteomic profiles of NSCs and CSCs as well as MMSCs and CSCs predominantly, totaling 146 proteins (8.77%, Table **2**). For that the proteins with GO: 0005634 nucleus codes were selected. The distribution of the proteins depending on molecular functions, biological processes and signaling pathways is presented graphically (Figs. (**4-6**)).

Table 2: Nucleus Proteins Expresses in NSCs, MMSCs and U87 Glioblastoma CSCs.

NSC		MMSC		CSC		Protein
NSI, %	**NSC/CSC**	**NSI, %**	**MMSC/CSC**	**NSI, %**		
0.10	0.67	0.029	0.20	0.15	1	A kinase (PRKA) anchor protein 12
0.36	2.35	0.36	2.35	0.15	1	AHNAK nucleoprotein
0.0029	0.33	7.81E-05	0.0088	0.0088	1	CSE1 chromosome segregation 1-like (yeast)
0.089	9.38	0.00090	0.28	0.0026	1	DEAD (Asp-Glu-Ala-Asp) box polypeptide 17

Table 2: contd…

0.12	34.0	0.0037	0.35	0.013	1	DEAD (Asp-Glu-Ala-Asp) box polypeptide 5
0.030	7.64	0.0011	0.29	0.0039	1	FK506 binding protein 3, 25kDa
0.0091	9.12	0.0011	1.15	0.0010	1	GTPase activating protein (SH3 domain) binding protein 2
0.57	0.30	0.30	0.011	0.27	1	H2A histone family, member Z
0.050	22.0	0.020	8.72	0.0023	1	HECT, UBA and WWE domain containing 1
0.29	1.10	0.0075	0.15	0.33	1	IQ motif containing GTPase activating protein 1
0.00054	4.06	0.00097	7.32	0.00013	1	LIM domain containing preferred translocation partner in lipoma
0.012	0.052	0.027	1.36	0.036	1	PDZ and LIM domain 1
0.019	0.049	0.16	0.75	0.080	1	Parkinson disease (autosomal recessive, early onset) 7
0.012	1.56	0.0023	0.29	0.0079	1	RAD23 homolog B (*Saccharomyces cerevisiae*)
0.018	0.84	0.00065	0.030	0.022	1	RAN, member RAS oncogene family
0.022	0.16	0.051	0.38	0.14	1	S100 calcium binding protein A11; S100 calcium binding protein A11 pseudogene
0.0037	1.25	0.00064	0.22	0.0030	1	S100 calcium binding protein A6
5.6E-06	0.0017	0.0078	2.42	0.0032	1	SEC13 homolog (*S. cerevisiae*)
0.0035	0.48	0.023	3.11	0.0073	1	SH3 domain binding glutamic acid-rich protein like
0.024	0.87	0.0027	0.096	0.028	1	SH3 domain binding glutamic acid-rich protein like 3
0.040	1.27	0.0045	0.14	0.032	1	Sjogren syndrome antigen B (autoantigen La)
0.0056	5.38	0.00054	0.52	0.0010	1	Tax1 (human T-cell leukemia virus type I) binding protein 3
0.058	2.12	6.0E-05	0.0022	0.027	1	X-ray repair complementing defective repair in Chinese hamster cells 5 (double-strand-break rejoining)
0.0061	1.60	0.021	5.44	0.0038	1	YY1 associated protein 1; gon-4-like (C. elegans)
1.82	1.52	3.96	3.30	1.20	1	Actin, beta
1.04	4.53	1.21	5.26	0.23	1	Actinin, alpha 1
0.62	2.13	0.69	2.37	0.29	1	Actinin, alpha 4
0.10	2.54	0.029	0.32	0.15	1	Adenylate kinase 1
0.0041	0.34	0.0093	0.77	0.012	1	Adhesion regulating molecule 1
0.095	0.26	0.15	0.43	0.025	1	Annexin A1
0.90	3.75	1.43	5.84	0.70	1	Annexin A11
0.022	10.0	0.0027	38.5	0.0086	1	Apoptosis-inducing factor, mitochondrion-associated, 1
0.00095	0.16	0.0030	0.51	0.0059	1	Calcium/calmodulin-dependent protein kinase II delta
0.046	1.79	0.15	5.10	0.024	1	Calmodulin 3 (phosphorylase kinase, delta); calmodulin 2 (phosphorylase kinase, delta); calmodulin 1 (phosphorylase kinase, delta)
0.12	4.49	0.0067	0.25	0.027	1	Calpain, small subunit 1
0.047	0.83	0.00038	0.0069	0.056	1	Capping protein (actin filament), gelsolin-like
0.14	2.81	0.081	1.65	0.049	1	Chaperonin containing CCP1, subunit 2 (beta)
0.0066	0.24	0.0020	0.074	0.027	1	Chaperonin containing CCP1, subunit 3 (gamma)

Table 2: contd…

0.059	0.48	0.084	0.68	0.12	1	Chloride intracellular channel 1
0.048	3.56	0.057	4.21	0.013	1	Chloride intracellular channel 4
0.0069	0.44	0.0020	0.13	0.016	1	Chromatin modifying protein 5
0.0087	4.94	0.062	35.5	0.0018	1	Cofilin 2 (muscle)
0.012	0.27	0.022	0.48	0.045	1	Cold shock domain protein A; cold shock domain protein A pseudogene 1
0.0080	0.20	0.0039	0.037	0.033	1	Cullin-associated and neddylation-dissociated 1
0.033	0.036	0.0093	0.010	0.0016	1	Cystatin B (stefin B)
0.011	5.43	0.00098	0.48	0.0020	1	Cytidine monophosphate *N*-acetylneuraminic acid synthetase
0.0074	26.7	0.00097	3.50	0.00028	1	Emerin
0.65	0.45	0.99	0.69	1.45	1	Enolase 1, (alpha)
0.033	0.23	0.047	0.32	0.15	1	Estrogen-related receptor alpha
0.026	0.18	0.022	0.15	0.14	1	Eukaryotic translation initiation factor 4A, isoform 3
0.14	2.51	0.35	6.41	0.054	1	Eukaryotic translation initiation factor 5A; eukaryotic translation initiation factor 5A-like 1
0.046	0.31	0.29	1.93	0.15	1	Heat shock 27kDa protein-like 2 pseudogene; heat shock 27kDa protein 1
0.072	0.11	0.016	0.024	0.67	1	Heat shock 60kDa protein 1 (chaperonin) pseudogene 5; heat shock 60kDa protein 1 (chaperonin) pseudogene 6; heat shock 60kDa protein 1 (chaperonin) pseudogene 1; heat shock 60kDa protein 1 (chaperonin) pseudogene 4; heat shock 60kDa protein 1 (chaperonin)
0.0052	0.18	2.02E-06	7.11E-05	0.028	1	Heat shock 70kDa protein 4
0.12	0.67	0.00097	0.0054	0.18	1	Heterogeneous nuclear ribonucleoprotein A/B
0.050	0.64	0.032	0.41	0.079	1	Heterogeneous nuclear ribonucleoprotein A2/B1
0.051	26.6	0.00043	0.23	0.0019	1	Heterogeneous nuclear ribonucleoprotein C (C1/C2)
0.040	0.37	0.0034	0.031	0.11	1	Heterogeneous nuclear ribonucleoprotein D-like
0.0078	0.46	0.014	0.82	0.017	1	Heterogeneous nuclear ribonucleoprotein F
0.0076	0.16	4.89E-05	0.0010	0.047	1	Heterogeneous nuclear ribonucleoprotein R
0.028	2.76	0.022	2.18	0.010	1	High density lipoprotein binding protein
0.017	1.47	1.19E-05	0.0010	0.012	1	High mobility group AT-hook 2
0.052	0.31	0.0074	0.012	0.095	1	Histone cluster 1, H1c
0.040	0.32	0.0016	0.093	0.13	1	Histone cluster 1, H2aa
0.04	0.45	0.011	0.92	0.082	1	Histone cluster 1, H2bn
0.065	0.32	0.019	0.093	0.20	1	Histone cluster 1, H3j; histone cluster 1, H3i; histone cluster 1, H3h; histone cluster 1, H3g; histone cluster 1, H3f; histone cluster 1, H3e; histone cluster 1, H3d; histone cluster 1, H3c; histone cluster 1, H3b; histone cluster 1, H3a; histone cluster 1, H2ad; histone cluster 2, H3a; histone cluster 2, H3c; histone cluster 2, H3d

Table 2: contd…

0.0032	0.38	0.0026	0.31	0.0084	1	Huntingtin interacting protein 1
0.056	1.31	0.019	0.44	0.043	1	Hypothetical LOC100130009; high mobility group AT-hook 1
0.62	0.31	0.30	0.15	2.02	1	Hypothetical gene supported by AF216292; NM_005347; heat shock 70kDa protein 5 (glucose-regulated protein, 78kDa)
0.073	0.64	0.0077	0.068	0.11	1	Hypothetical gene supported by BC000665; t-complex 1
0.011	1316	3.85E-05	4.74	8.14E-06	1	Hypothetical protein FLJ11822; aminopeptidase puromycin sensitive
0.29	0.88	0.0075	0.022	0.33	1	Importin 5
0.044	3.53	0.0011	0.084	0.013	1	Interleukin enhancer binding factor 3, 90kDa
0.022	0.35	0.0030	0.049	0.061	1	Karyopherin (importin) beta 1
0.0037	0.47	0.0051	0.66	0.0077	1	Karyopherin alpha 4 (importin alpha 3)
0.31	0.72	0.091	0.21	0.42	1	Lamin A/C
0.028	3.03	0.018	2.01	0.0091	1	Leucine rich repeat (in FLII) interacting protein 1
0.32	2.25	0.015	0.10	0.14	1	Major vault protein
0.0078	1.05	0.0010	0.14	0.0074	1	Matrin 3
0.049	2.56	0.0013	0.067	0.019	1	Metadherin
0.34	1.28	0.095	0.36	0.27	1	Moesin
0.0047	0.95	0.012	2.35	0.0050	1	Myosin IC
0.28	0.23	0.18	0.14	1.24	1	Myosin, heavy chain 9, non-muscle
0.013	0.27	0.014	0.30	0.048	1	Nascent polypeptide-associated complex alpha subunit
0.076	0.57	0.0052	0.039	0.13	1	Non-metastatic cells 1, protein (NM23A) expressed in; NME1-NME2 readthrough traNSCript; non-metastatic cells 2, protein (NM23B) expressed in
0.029	0.48	0.00063	0.010	0.062	1	Nucleolin
0.051	0.17	0.032	0.11	0.30	1	Nucleophosmin 1 (nucleolar phosphoprotein B23, numatrin) pseudogene 21; hypothetical LOC100131044; similar to nucleophosmin 1; nucleophosmin (nucleolar phosphoprotein B23, numatrin)
0.0095	3.46	0.0013	0.37	0.0035	1	Parvin, alpha
0.11	2.57	0.045	1.03	0.044	1	Peroxiredoxin 6
0.040	0.23	0.016	0.091	0.18	1	Poly(rC) binding protein 1
0.055	0.47	0.0047	0.040	0.12	1	Poly(rC) binding protein 2
0.15	25.3	0.022	3.65	0.0061	1	Polymerase I and traNSCript release factor
0.036	0.88	0.015	0.37	0.041	1	Polypyrimidine tract binding protein 1
0.010	12.5	0.024	29.3	0.00083	1	Progesterone receptor membrane component 1
0.015	0.65	0.0083	0.36	0.023	1	Prohibitin 2
0.0049	0.42	0.00022	0.019	0.012	1	Prostaglandin E synthase 3 (cytosolic)
0.0025	0.16	0.00044	0.028	0.016	1	Proteasome (prosome, macropain) 26S subunit, ATPase, 1; similar to protease (prosome, macropain) 26S subunit, ATPase 1

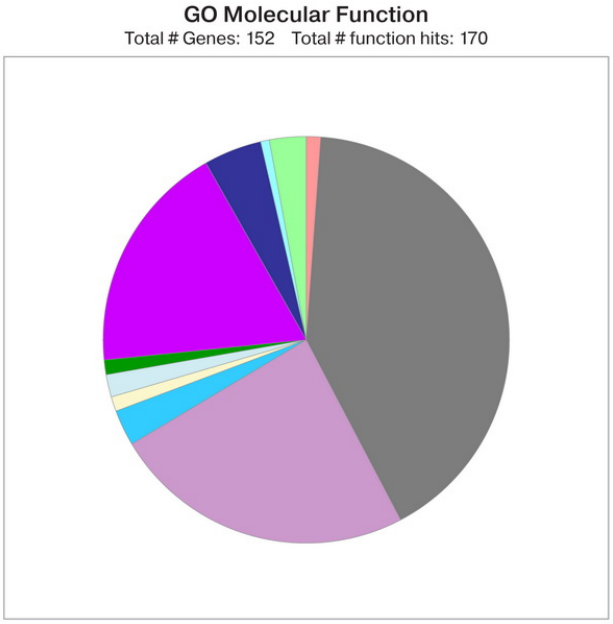
Table 2: contd…

0.013	0.26	0.0019	0.037	0.050	1	Proteasome (prosome, macropain) 26S subunit, ATPase, 6
0.013	0.57	0.0012	0.056	0.022	1	Proteasome (prosome, macropain) subunit, alpha type, 1
0.0090	7.27	0.0092	7.51	0.0012	1	Proteasome (prosome, macropain) subunit, alpha type, 5
0.013	0.73	0.0022	0.13	0.017	1	Proteasome (prosome, macropain) subunit, beta type, 2
0.034	0.47	0.078	1.08	0.073	1	Protein phosphatase 1, catalytic subunit, alpha isoform
0.015	2.08	0.030	4.33	0.0098	1	Protein phosphatase 2 (formerly 2A), regulatory subunit A, alpha isoform
0.0062	0.41	0.0048	0.32	0.015	1	Reticulon 3
0.15	0.37	0.088	0.21	0.41	1	Reticulon 4
0.018	0.94	0.0076	0.39	0.019	1	Ribosomal protein L23 pseudogene 6; ribosomal protein L23
0.0054	0.39	0.0047	0.35	0.014	1	Ribosomal protein L35; ribosomal protein L35 pseudogene 1; ribosomal protein L35 pseudogene 2
0.0080	0.70	0.00095	0.083	0.011	1	Ribosomal protein L3; similar to 60S ribosomal protein L3 (L4)
0.095	8.98	0.0072	0.68	0.011	1	Ribosomal protein L5 pseudogene 34; ribosomal protein L5 pseudogene 1; ribosomal protein L5
0.018	0.53	0.0058	0.17	0.035	1	Ribosomal protein L9; ribosomal protein L9 pseudogene 25
0.0084	0.95	0.0019	0.22	0.0088	1	Ribosomal protein S13 pseudogene 8; ribosomal protein S13; ribosomal protein S13 pseudogene 2
0.024	2.32	0.13	12.8	0.010	1	Ribosomal protein S14
0.0010	0.025	0.00044	0.011	0.040	1	Ribosomal protein S15 pseudogene 5; ribosomal protein S15
0.025	0.53	0.0039	0.083	0.048	1	Ribosomal protein S25 pseudogene 8; ribosomal protein S25
0.031	0.84	0.010	0.27	0.037	1	Ribosomal protein S3 pseudogene 3; ribosomal protein S3
0.019	1.35	0.033	2.37	0.014	1	Ribosomal protein S3A pseudogene 5; ribosomal protein S3a pseudogene 47; ribosomal protein S3a pseudogene 49; ribosomal protein S3A; hypothetical LOC100131699; hypothetical LOC100130107
0.060	0.45	0.0073	0.054	0.13	1	Ribosomal protein SA pseudogene 9; ribosomal protein SA pseudogene 8; ribosomal protein SA pseudogene 58; ribosomal protein SA pseudogene 19; ribosomal protein SA pseudogene 18; ribosomal protein SA; ribosomal protein SA pseudogene 15; ribosomal protein SA pseudogene 61; ribosomal protein SA pseudogene 29; ribosomal protein SA pseudogene 12
0.037	0.25	0.020	0.14	0.15	1	Septin 2
0.0040	0.38	1.16E-05	0.0011	0.010	1	Similar to 26S protease regulatory subunit 6B (MIP224) (MB67-interacting protein) (TAT-binding protein 7) (TBP-7); proteasome (prosome, macropain) 26S subunit, ATPase, 4

Table 2: contd…

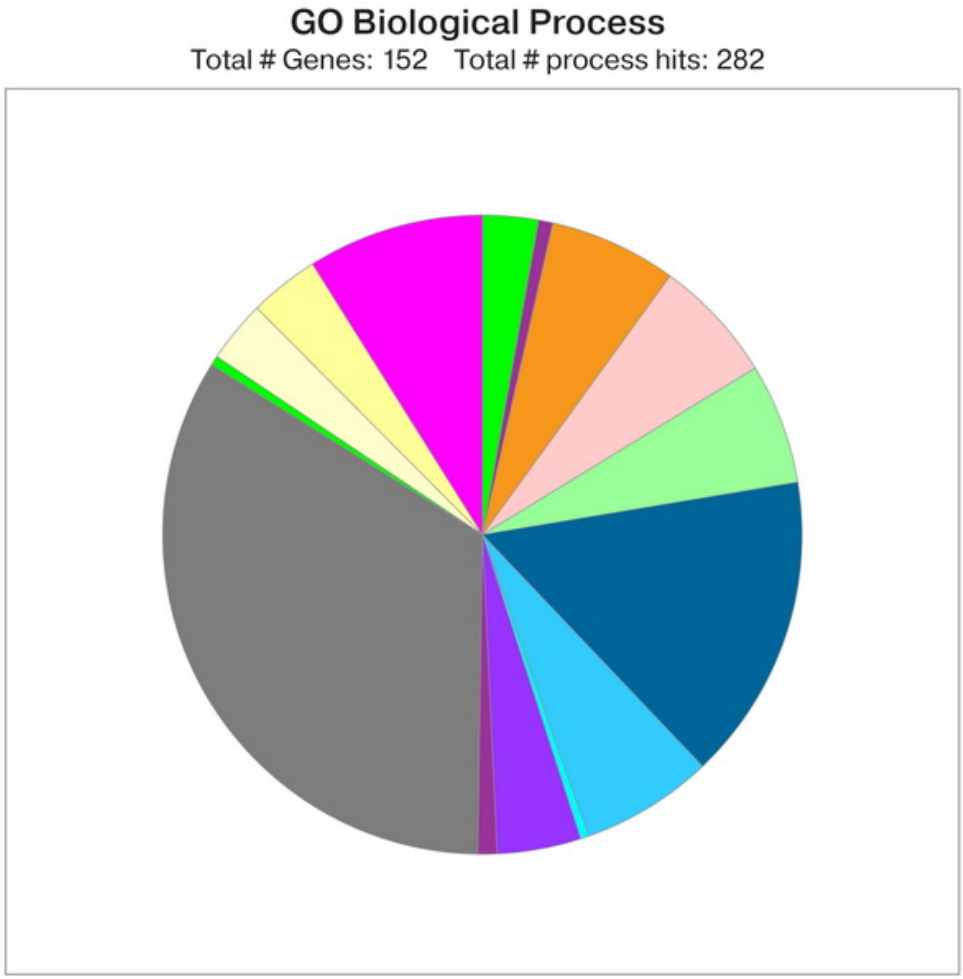
0.67	0.56	0.35	0.30	1.186	1	Similar to Pyruvate kinase, isozymes M1/M2 (Pyruvate kinase muscle isozyme) (Cytosolic thyroid hormone-binding protein) (CTHBP) (THBP1); pyruvate kinase, muscle
0.058	1.72	0.0030	0.090	0.034	1	Similar to chaperonin containing CCP1, subunit 8 (theta); chaperonin containing CCP1, subunit 8 (theta)
0.10	0.29	0.0018	0.0049	0.36	1	Similar to protein kinase, DNA-activated, catalytic polypeptide; protein kinase, DNA-activated, catalytic polypeptide
0.0061	6.15	0.0031	3.16	0.00099	1	Small nuclear ribonucleoprotein D1 polypeptide 16kDa; hypothetical protein LOC100129492
0.042	8.60	0.035	7.09	0.0049	1	Spectrin, beta, non-erythrocytic 1
0.013	0.29	0.014	0.33	0.044	1	Splicing factor, arginine/serine-rich 7, 35kDa
0.00027	0.79	0.0013	3.87	0.00034	1	Squamous cell carcinoma antigen recognized by T cells
0.016	0.64	0.0012	0.050	0.024	1	Staphylococcal nuclease and tudor domain containing 1
0.043	0.26	0.013	0.078	0.17	1	Stress-induced-phosphoprotein 1
0.024	0.29	0.058	0.69	0.084	1	Superoxide dismutase 1, soluble
0.046	0.37	0.0098	0.078	0.13	1	Synaptotagmin binding, cytoplasmic RNA interacting protein
0.0030	11.9	0.00025	1.03	0.00025	1	Tight junction protein 1 (zona occludens 1)
0.054	0.69	0.40	5.14	0.078	1	Transgelin 2
0.0026	54.6	0.0063	130	4.84E-05	1	Translocase of inner mitochondrial membrane 50 homolog (*Saccharomyces cerevisiae*)
0.030	5.29	0.00070	0.12	0.0056	1	Transmembrane protein 43
0.0036	0.32	0.00086	0.076	0.011	1	Tripartite motif-containing 25
0.12	0.35	0.12	0.33	0.36	1	Tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, beta polypeptide
0.21	0.40	0.31	0.54	0.57	1	Tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, zeta polypeptide
0.035	0.55	0.00090	0.0093	0.054	1	Tyrosyl-tRNA synthetase
0.0024	0.14	0.0024	0.14	0.017	1	Ubiquilin 2
0.53	0.49	0.12	0.11	1.09	1	Ubiquitin C
0.077	0.17	0.11	0.24	0.42	1	Ubiquitin carboxyl-terminal esterase L1 (ubiquitin thiolesterase)
0.18	8.06	0.0013	0.059	0.022	1	Ubiquitin-fold modifier 1
0.068	0.37	0.022	0.12	0.18	1	Valosin-containing protein
0.021	1.17	0.0089	5.17	0.075	1	Vesicle amine transport protein 1 homolog (T. californica)
0.030	6.75	0.025	5.66	0.0044	1	Zinc finger protein 91 homolog (mouse); ZFP91-CNTF read through traNSCript; ciliary neurotrophic factor
0.0059	0.62	0.043	4.48	0.0096	1	Zyxin

In conclusion, the third cluster "Proteins of signal induction and intercellular regulation" was set: we selected the proteins expressed by NSCs and MMSCs, and compiled the table of NSC and MMSC expressed proteins (Table **3**), totaling 33 proteins (1.98%). For that the proteins with the word "extracellular" in the code were selected: GO: 0005576 extracellular region, GO: 0005578 proteinaceous extracellular matrix, GO:0005615 extracellular space, GO:0031012 extracellular matrix, GO:0044420 extracellular matrix part. The distribution of the proteins depending on molecular functions, biological processes and signaling pathways is presented graphically (Figs. (**7-9**)).



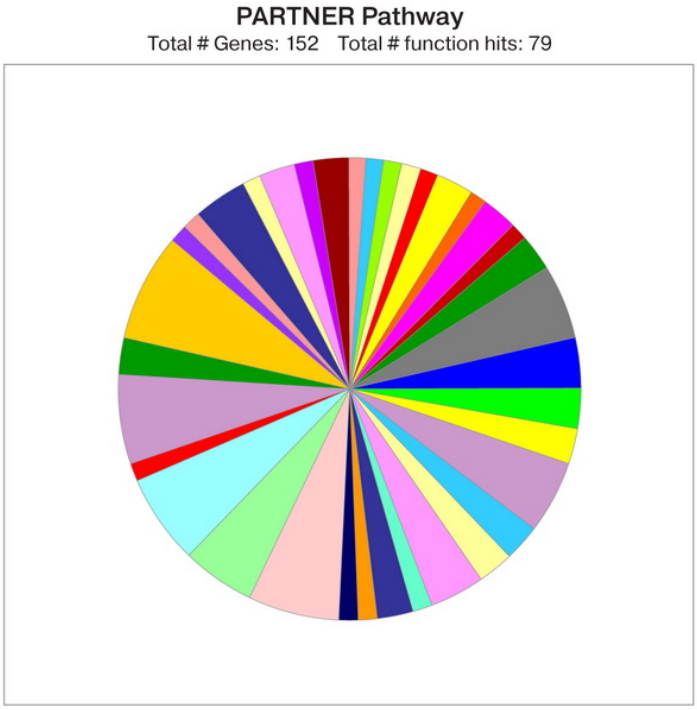
	Antioxidant activity (GO:0016209)		Binding (GO:0005488)
	Catalytic activity (GO:0003824)		Enzyme regulator activity (GO:0030234)
	Ion channel activity (GO:0005216)		Motor activity (GO:0003774)
	Receptor activity (GO:0004872)		Structural molecule activity (GO:0005198)
	Transcription regulator activity (GO:0030528)		Translation regulator activity (GO:0045182)
	Transporter activity (GO:0005215)		

Figure 4: Molecular functions of nuclear proteins expressed in NSCs, MMSCs and U87 glioblastoma CSCs.



	Apoptosis (GO:0006915)		Cell adhesion (GO:0007155)		Cellular process (GO:0009987)
	Cell communication (GO:0007154)		Cell cycle (GO:0007049)		Generation of precursor metabolites and energy (GO:0006091)
	Cellular component organization (GO:0016043)		Metabolic process (GO:0008152)		Localization (GO:0051179)
	Developmental process (GO:0032502)		Response to stimulus (GO:0050896)		Reproduction (GO:0000003)
	Immune system process (GO:0002376)		Transport (GO:0006810)		System process (GO:0003008)

Figure 5: Biological processes that involve nucleus proteins expressed in NSCs, MMSCs and U87 glioblastoma CSCs.



	5HT1 type receptor mediated signaling pathway (P04373)		5HT2 type receptor mediated signaling pathway (P04374)		5HT3 type receptor mediated signaling pathway (P04375)
	5HT4 type receptor mediated signaling pathway (P04376)		Adrenaline and noradrenaline biosynthesis (P00001)		Alzheimer disease-presenilin pathway (P00004)
	Angiogenesis (P00005)		Apoptosis signaling pathway (P00006)		B Cell activation (P00010)
	Cadherin signaling pathway (P00012)		Cytoskeletal regulation by Rho GTPase (P00016)		*De novo* purine biosynthesis (P02738)
	De novo pyrimidine deoxyribonucleotide biosynthesis (P02739)		*De novo* pyrmidine ribonucleotides biosythesis (P02740)		Dopamine receptor mediated signaling pathway (P05912)
	EGF receptor signaling pathway (P00018)		FAS signaling pathway (P00020)		FGF signaling pathway (P00021)
	General transcription by RNA polymerase I (P00022)		Glycolysis (P00024)		Heterotrimeric G-protein signaling pathway-Gi alpha and Gs alpha mediated pathway (P00026)
	Heterotrimeric G-protein signaling pathway-rod outer segment phototransduction (P00028)		Huntington disease (P00029)		Inflammation mediated by chemokine and cytokine signaling pathway (P00031)

	Integrin signalling pathway (P00034)		Ionotropic glutamate receptor pathway (P00037)		Nicotinic acetylcholine receptor signaling pathway (P00044)
	PI3 kinase pathway (P00048)		Parkinson disease (P00049)		Pyruvate metabolism (P02772)
	T cell activation (P00053)		Ubiquitin proteasome pathway (P00060)		VEGF signaling pathway (P00056)
	Wnt signaling pathway (P00057)		p38 MAPK pathway (P05918)		p53 pathway (P00059)

Figure 6: Signal pathways of nuclear proteins expressed in NSCs, MMSCs and U87 glioblastoma CSCs.

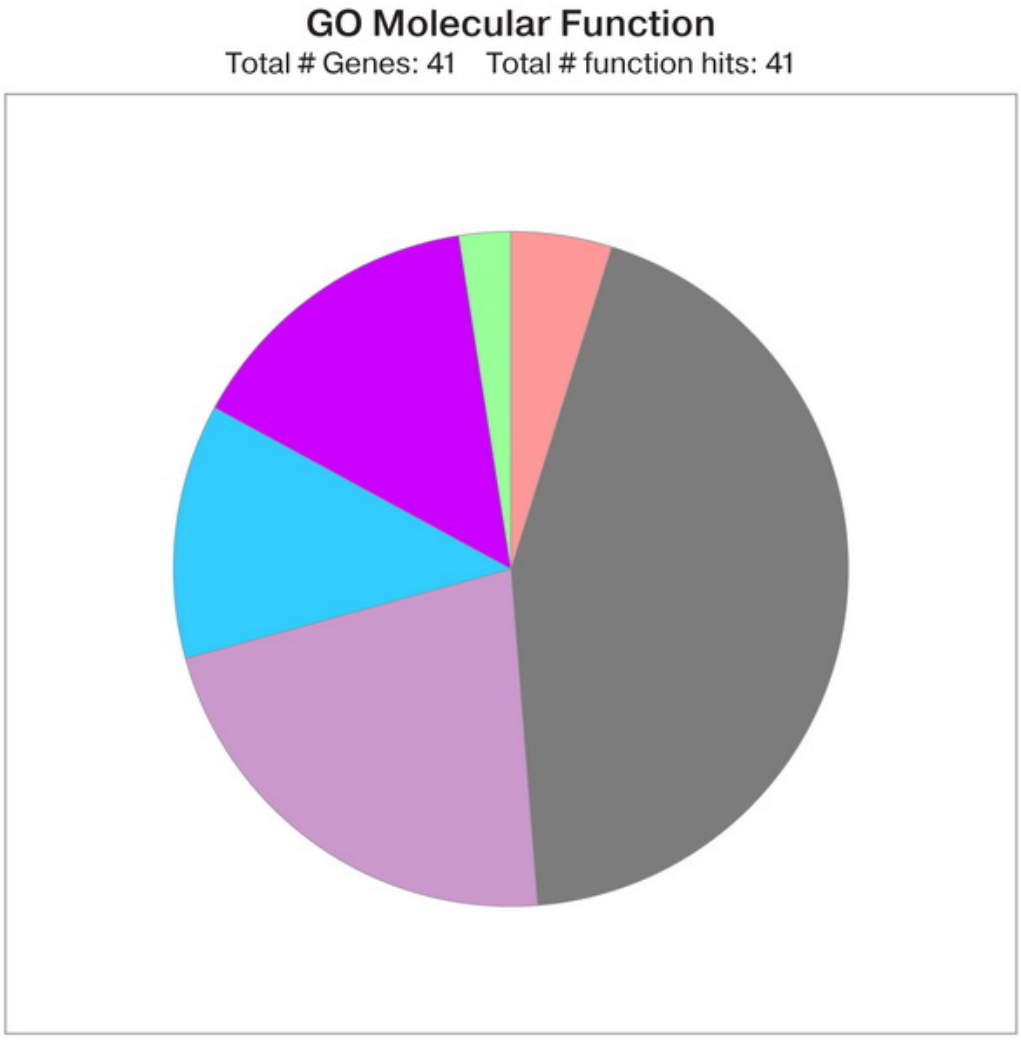
Table 3: Proteins Expressed in NSCs, MMSCs and U87 Glioblastoma CSCs.

NSC		MMSC		CSC		Protein
NSI, %	**NSC/CSC**	**NSI, %**	**MMSC/CSC**	**NSI, %**		
0.0075	0.42	0.0090	0.50	0.018	1	CD59 molecule, complement regulatory protein
0.0044	0.25	0.017	0.98	0.017	1	Niemann-Pick disease, type C2
1.04	4.53	1.21	5.26	0.23	1	Actinin, alpha 1
0.62	2.13	0.69	2.37	0.29	1	Actinin, alpha 4
0.010	0.061	0.010	0.060	0.17	1	ALBUMIN
0.41	0.55	0.32	0.43	0.75	1	Aldolase A, fructose-bisphosphate
0.90	1.28	1.43	2.03	0.70	1	Annexin A2 pseudogene 3; annexin A2; annexin A2 pseudogene 1
0.042	1.79	0.12	5.10	0.024	1	Calmodulin 3 (phosphorylase kinase, delta); calmodulin 2 (phosphorylase kinase, delta); calmodulin 1 (phosphorylase kinase, delta)
0.048	0.80	0.13	2.12	0.060	1	Calumenin
0.042	21.0	0.0093	5.86	0.0091	1	Cathepsin B
0.045	0.27	0.058	0.34	0.17	1	Cathepsin D
0.040	3.17	0.0072	0.58	0.013	1	Collagen, type VI, alpha 3
0.013	2.01	0.0016	0.25	0.0066	1	Dermcidin
0.11	1.32	0.17	2.05	0.084	1	Gelsolin (amyloidosis, Finnish type)
0.064	0.96	0.027	0.41	0.067	1	Glucose phosphate isomerase
0.0015	2.32	0.014	22.1	0.00064	1	Granulin
0.072	0.12	0.016	0.026	0.61	1	Heat shock 60kDa protein 1 (chaperonin) pseudogene 5; heat shock 60kDa protein 1 (chaperonin) pseudogene 6; heat shock 60kDa protein 1 (chaperonin) pseudogene 1; heat shock 60kDa protein 1 (chaperonin) pseudogene 4; heat shock 60kDa protein 1 (chaperonin)
0.028	2.76	0.022	2.18	0.010	1	High density lipoprotein binding protein

Table 3: contd…

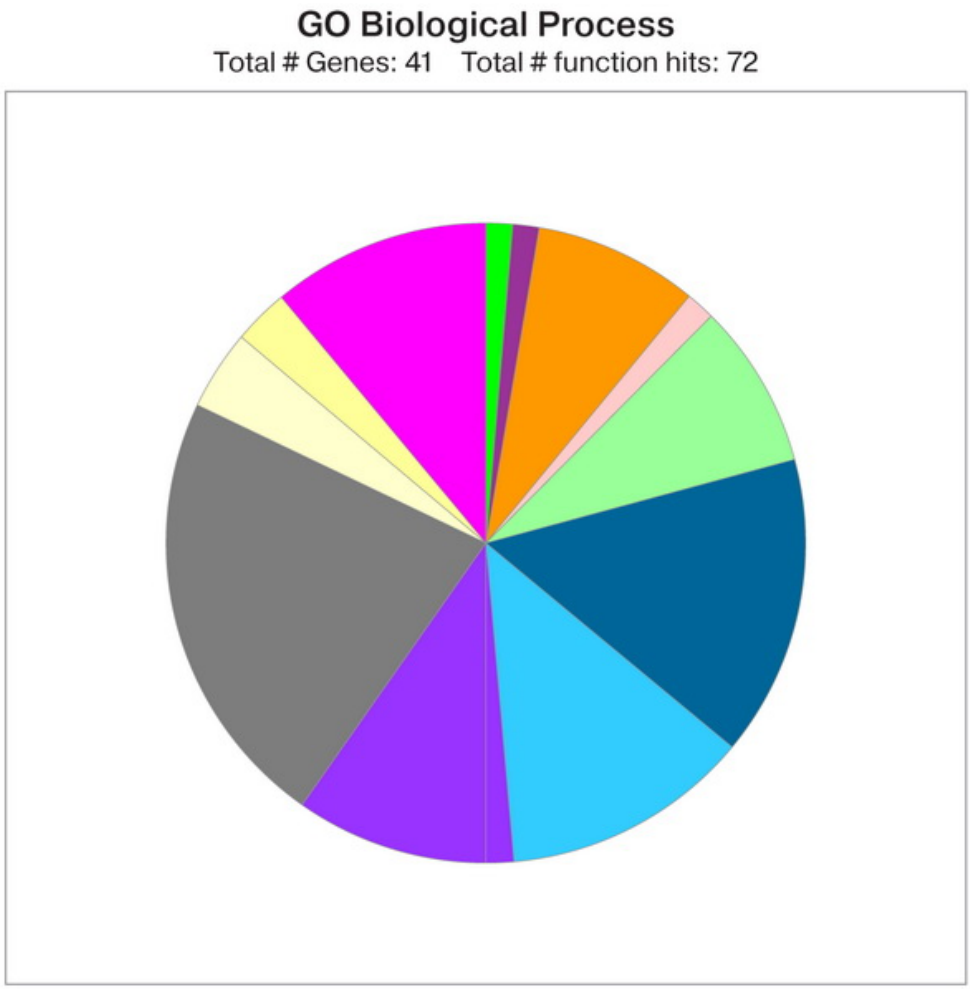
0.0065	1.18	2.42E-06	0.00044	0.0055	1	Hydroxysteroid (17-beta) dehydrogenase 12
0.068	0.43	0.13	0.85	0.16	1	Macrophage migration inhibitory factor (glycosylation-inhibiting factor)
0.00012	0.45	0.00024	0.92	0.00026	1	Major histocompatibility complex, class I, C; major histocompatibility complex, class I, B
0.039	1.40	0.00057	0.19	0.030	1	Peroxiredoxin 4
0.027	0.33	0.049	0.58	0.084	1	Phosphatidylethanolamine binding protein 1
0.080	0.77	0.018	0.17	0.10	1	Plasminogen
0.089	0.49	0.059	0.32	0.18	1	Prosaposin
0.0062	0.41	0.0048	0.32	0.015	1	Reticulon 3
0.22	0.85	0.57	2.20	0.26	1	Ribonuclease/angiogenin inhibitor 1
0.022	3.14	0.025	3.47	0.0071	1	Serpin peptidase inhibitor, clade E (nexin, plasminogen activator inhibitor type 1), member 2
0.024	0.29	0.058	0.69	0.084	1	Superoxide dismutase 1, soluble
0.37	0.73	1.29	2.54	0.51	1	Talin 1
0.035	0.65	0.00090	0.016	0.054	1	Tyrosyl-tRNA synthetase
0.14	1.17	0.64	5.17	0.12	1	Vinculin
0.030	6.75	0.025	5.66	0.0044	1	Zinc finger protein 91 homolog (mouse); ZFP91-CNTF readthrough traNSCript; ciliary neurotrophic factor

Proteome similarity matrix (PSM) for group A and B has been defined. The third stage of bioinformatical processing of protein proteome mapping was named the "trenchant" bioinformatical analysis of group A PSM and group B PSM using proteome information databases and exclusion of PSM proteome profile of the proteins involved in GB carcinogenesis and tumor metabolism. As seen from the tables, the lists of the proteins contain proteins involved in the carcinogenesis of other neoplasms, but not found in GB carcinogenesis. Searching protein annotations for key words: mitochondrion, mitochondrial, metabolic, metabolism, Warburg electron transport, we excluded mitochondrial proteins and NSC and CSC metabolome-associated proteins from group A PSM and group B PSM [18]: the proteins that provide quick adenosine triphosphate (ATP) production to support energy, quick macromolecules synthesis, respiratory chain proteins, Warburg effect proteins and other metabolome proteins. As a result, 34 nuclear, 53 membrane and 10 extracellular proteins remained in the groups.



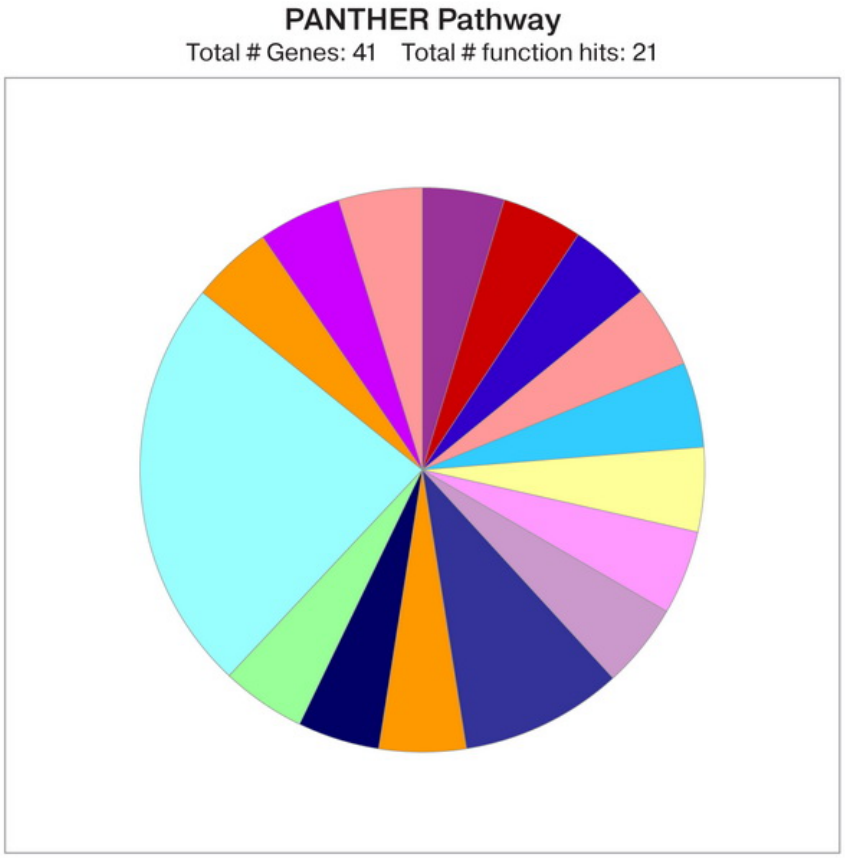
	Antioxidant activity (GO:0016209)
	Binding (GO:0005488)
	Catalytic activity (GO:0003824)
	Enzyme regulator activity (GO:0030234)
	Structural molecule activity (GO:0005198)
	Transporter activity (GO:0005215)

Figure 7: Molecular functions of the proteins expressed in NSCs, MMSCs and U87 glioblastoma CSCs.



	Apoptosis (GO:0006915)		Cell adhesion (GO:0007155)
	Cell communication (GO:0007154)		Cell cycle (GO:0007049)
	Cellular component organization (GO:0016043)		Cellular process (GO:0009987)
	Developmental process (GO:0032502)		Homeostatic process (GO:0042592)
	Immune system process (GO:0002376)		Metabolic process (GO:0008152)
	Response to stimulus (GO:0050896)		System process (GO:0003008)
	Transport (GO:0006810)		

Figure 8: Biological processes that involve proteins expressed in NSCs, MMSCs and U87 glioblastoma CSCs.



	Androgen/estrogene/progesterone biosynthesis (P02727)		B cell activation (P00010)
	Blood coagulation (P00011)		DNA replication (P00017)
	EGF receptor signaling pathway (P00018)		FAS signaling pathway (P00020)
	FGF signaling pathway (P00021)		Fructose galactose metabolism (P02744)
	Glycolysis (P00024)		Heterotrimeric G-protein signaling pathway-Gi alpha and Gs alpha mediated pathway (P00026)
	Heterotrimeric G-protein signaling pathway-rod outer segment phototransduction (P00028)		Inflammation mediated by chemokine and cytokine signaling pathway (P00031)
	Integrin signalling pathway (P00034)		Pentose phosphate pathway (P02762)
	Plasminogen activating cascade (P00050)		T cell activation (P00053)

Figure 9: Signal pathways of secreted proteins of U87 glioblastoma TSCs.

We excluded proteins associated with GB typically disordered signal transduction pathways from group A PSM and group B PSM. These proteins were excluded previously.

We excluded proteins of cell carcass (cytoskeletal proteins, endoplasmic reticulum proteins) from group A PSM and group B PSM through the search for key words in protein annotations: cytoskeleton, cytoskeletal, and reticulum. As a result 19 nuclear, 20 membrane and 5 extracellular proteins remained in the groups (Tables **4**-**6**).

Table 4: Membrane Proteins Shared by NSCs, MMSCs and CSCs, Remaining after "Trenchant" Analysis.

NSC		MMSC		CSC		Gene	Protein
NSI, %	NSC/CSC	NSI, %	MMSC/CSC	NSI, %			
0.029	7.82	0.042	11.3	0.0037	1	ARF1	ADP-ribosylation factor 1
0.027	9.39	0.013	4.63	0.0029	1	ARF4	ADP-ribosylation factor 4
0.027	2.50	0.0010	0.085	0.012	1	ARF5	ADP-ribosylation factor 5
0.0075	0.42	0.0090	0.50	0.018	1	CD59	CD59 molecule, complement regulatory protein
0.035	0.77	0.018	0.41	0.045	1	RAB10	RAB10, member RAS oncogene family
0.041	0.51	0.013	0.17	0.080	1	RAB1B	RAB1B, member RAS oncogene family
0.0024	0.079	0.0076	0.25	0.030	1	RAP1B	RAP1B, member of RAS oncogene family
0.0026	0.16	0.00031	0.019	0.016	1	ARHGAP1	Rho GTPase activating protein 1
0.0086	156	7.52E-05	1.36	5.52E-05	1	AP1B1	Adaptor-related protein complex 1, beta 1 subunit
0.19	159	0.080	68.9	0.0012	1	AP2B1	Adaptor-related protein complex 2, beta 1 subunit
0.10	5.69	0.094	5.32	0.018	1	ANXA6	Annexin A6
0.016	0.45	0.0095	0.27	0.035	1	ARCN1	Archain 1
0.059	0.48	0.084	0.68	0.12	1	CLIC1	Chloride intracellular channel 1
0.040	3.17	0.0072	0.58	0.013	1	COL6A3	Collagen, type VI, alpha 3
0.016	0.16	0.064	0.61	0.11	1	EEA1	Early endosome antigen 1
0.00072	0.83	0.00023	0.26	0.00087	1	FAM120A	Family with sequence similarity 120A
0.0025	0.83	0.0093	3.08	0.0030	1	FLOT2	Flotillin 2
0.15	0.37	0.088	0.21	0.41	1	RTN4	Reticulon 4
0.012	0.57	0.0030	0.15	0.020	1	SCARB2	Scavenger receptor class B, member 2
0.0029	11.9	0.00025	1.03	0.00025	1	TJP1	Tight junction protein 1 (zona occludens 1)

Table 5: Membrane Proteins Shared by NSCs, MMSCs and CSCs Remaining After "Trenchant" Analysis.

NSC		MMSC		CSC		Gene	Protein
NSI, %	**NSC/CSC**	**NSI, %**	**MMSC/CSC**	**NSI, %**			
0.029	7.82	0.042	11.3	0.0037	1	ARF1	ADP-ribosylation factor 1
0.027	9.39	0.013	4.63	0.0029	1	ARF4	ADP-ribosylation factor 4
0.027	2.50	0.0010	0.085	0.012	1	ARF5	ADP-ribosylation factor 5
0.0075	0.42	0.0090	0.50	0.018	1	CD59	CD59 molecule, complement regulatory protein
0.035	0.77	0.018	0.41	0.045	1	RAB10	RAB10, member RAS oncogene family
0.041	0.51	0.013	0.17	0.080	1	RAB1B	RAB1B, member RAS oncogene family
0.0024	0.079	0.0076	0.25	0.030	1	RAP1B	RAP1B, member of RAS oncogene family
0.0026	0.16	0.00031	0.019	0.016	1	ARHGAP1	Rho GTPase activating protein 1
0.0086	156	7.52E-05	1.36	5.52E-05	1	AP1B1	Adaptor-related protein complex 1, beta 1 subunit
0.19	159	0.080	68.9	0.0012	1	AP2B1	Adaptor-related protein complex 2, beta 1 subunit
0.10	5.69	0.094	5.32	0.018	1	ANXA6	Annexin A6
0.016	0.45	0.0095	0.27	0.035	1	ARCN1	Archain 1
0.059	0.48	0.084	0.68	0.12	1	CLIC1	Chloride intracellular channel 1
0.040	3.17	0.0072	0.58	0.013	1	COL6A3	Collagen, type VI, alpha 3
0.016	0.16	0.064	0.61	0.11	1	EEA1	Early endosome antigen 1
0.00072	0.83	0.00023	0.26	0.00087	1	FAM120A	Family with sequence similarity 120A
0.0025	0.83	0.0093	3.08	0.0030	1	FLOT2	Flotillin 2
0.15	0.37	0.088	0.21	0.41	1	RTN4	Reticulon 4
0.012	0.57	0.0030	0.15	0.020	1	SCARB2	Scavenger receptor class B, member 2
0.0029	11.9	0.00025	1.03	0.00025	1	TJP1	Tight junction protein 1 (zona occludens 1)

Table 6: Extracellular Proteins of Signal Transduction Shared by NSCs, MMSCs and CSC Remaining After "Trenchant" Analysis.

NSC		MMSC		CSC		Gene	Protein
NSI, %	NSC/CSC	NSI, %	MMSC/CSC	NSI, %			
0.0075	0.42	0.0090	0.50	0.018	1	CD59	CD59 molecule, complement regulatory protein
0.048	0.80	0.13	2.13	0.060	1	CALU	Calumenin
0.040	3.17	0.0072	0.58	0.013	1	COL6A3	Collagen, type VI, alpha 3
0.013	2.01	0.0016	0.25	0.0066	1	DCD	Dermcidin
0.0062	0.41	0.0048	0.32	0.015	1	RTN3	Reticulon 3

Protein signaling pathways were annotated for the proteins from Tables 4, 5 and 6 using the data from Biocarta (http://www.biocarta.com/), KEGG_PATHWAY (http://www.genome.jp/kegg/), PANTHER_PATHWAY (http://www.pantherdb.org/) and Reactome (http://www.reactome.org/). Protein-protein interactions were annotated using Biomolecular Interaction Network Database (BIND: http://bind.ca), Database of Interacting proteins (DIP: http://dip.doe-mbi.ucla.edu/), Molecular Interaction Database (MINT, http://mint.bio.uniroma2.it/mint/), NCICB_CAPATHWAY_interaction (http://cgap.nci.nih.gov/ Pathways/), and Reactome_Interaction (http://www.reactome.org/).

Identified signaling pathways free from carcinogenesis in U87 glioblastoma CSC (CD133+), are presented in Tables **7-9**.

Table 7: Signaling Pathways of Extracellular Proteins.

Gene	BIOCARTA	KEGG_PATHWAY	PANTHER_PATHWAY	REACTOME_PATHWAY
CD59		hsa04610:Complement and coagulation cascades,hsa04640:Hematopoietic cell lineage		
CALU				REACT_604:Hemostasis
COL6A3		hsa04510:Focal adhesion,hsa04512:ECM-receptor interaction	P00031:Inflammation mediated by chemokine and cytokine signaling pathway,P00034:Integrin signaling pathway	REACT_16888:Signaling by PDGF,REACT_18266:Axon guidance
DCD				
RTN3				

Table 8: Signaling Pathways of Membrane Proteins.

Gene	BIOCARTA	KEGG_PATHWAY	PANTHER_PATHWAY	REACTOME_PATHWAY
ARF1	h_arap Pathway:ADP-Ribosylation Factor, h_ptdinsPathway:Phosphoinositides and their downstream targets	hsa05110:*Vibrio cholerae* infection	P00029:Huntington disease, P00034:Integrin signaling pathway	REACT_11123:Membrane Trafficking, REACT_6185:HIV Infection
ARF4			P00029:Huntington disease, P00034:Integrin signaling pathway	
ARF5			P00029:Huntington disease, P00034:Integrin signaling pathway	
CD59		hsa04610:Complement and coagulation cascades, hsa04640:Hematopoietic cell lineage		
RAB10			P00052:TGF-beta signaling pathway	
RAB1B				
RAP1B	h_ephA4Pathway:Eph Kinases and ephrins support platelet aggregation, h_metPathway:Signaling of Hepatocyte Growth Factor Receptor	hsa04010:MAPK signaling pathway,hsa04062:Chemokine signaling pathway,hsa04510:Focal adhesion,hsa04670:Leukocyte transendothelial migration,hsa04720:Long-term potentiation,hsa04722:Neurotrophin signaling pathway,hsa05211:Renal cell carcinoma	P00026:Heterotrimeric G-protein signaling pathway-Gi alpha and Gs alpha mediated pathway,P00027:Heterotrimeric G-protein signaling pathway-Gq alpha and Go alpha mediated pathway,P00034:Integrin signaling pathway	REACT_13552:Integrin cell surface interactions, REACT_15380:Diabetes pathways, REACT_604:Hemostasis
ARHGAP1	h_rhoPathway:Rho cell motility signaling pathway		P00005:Angiogenesis,P00016:Cytoskeletal regulation by Rho GTPase,P00047:PDGF signaling pathway,P00056:VEGF signaling pathway	REACT_11044:Signaling by Rho GTPases
AP1B1		hsa04142:Lysosome		REACT_11123:Membrane Trafficking, REACT_6185:HIV Infection,
AP2B1		hsa04144: Endocytosis,hsa05016:Huntington's disease		REACT_13685:Synaptic Transmission, REACT_6185:HIV Infection

Table 8: contd…

ANXA6				
ARCN1				REACT_11123:Membrane Trafficking
CLIC1			P05912:Dopamine receptor mediated signaling pathway	
COL6A3		hsa04510:Focal adhesion,hsa04512:ECM-receptor interaction	P00031:Inflammation mediated by chemokine and cytokine signaling pathway,P00034:Integrin signaling pathway	REACT_16888:Signaling by PDGF,REACT_18266:Axon guidance
EEA1	h_eea1Pathway:The role of FYVE-finger proteins in vesicle transport, h_ptdinsPathway:Phosphoinositides and their downstream targets.	hsa04144:Endocytosis		
FAM120A				
FLOT2		hsa04910:Insulin signaling pathway		
RTN4				
SCARB2		hsa04142:Lysosome		
TJP1		hsa04520:Adherens junction,hsa04530:Tight junction,hsa04540:Gap junction,hsa05110:*Vibrio cholerae* infection,hsa05120:Epithelial cell signaling in *Helicobacter pylori* infection		

Table 9: Signaling Pathways of Nuclear Proteins.

Gene	BIOCARTA	KEGG_PATHWAY	PANTHER_PATHWAY	REACTOME_PATHWAY
AHNAK				
CSE1L				
G3BP2				REACT_11044:Signaling by Rho GTPases
S100A6				
SH3BGRL				

Table 9: contd…

SH3BGRL3				
TAX1BP3	h_tidPathway :Chaperones modulate interferon Signaling Pathway			
CLIC1			P05912:Dopamine receptor mediated signaling pathway	
HIST1H2BN		hsa05322:Systemic lupus erythematosus		REACT_7970:Telomere Maintenance
KPNB1	h_npcPathway :Mechanism of Protein Import into the Nucleus,h_ranMSpathway:Role of Ran in mitotic spindle regulation			REACT_578:Apoptosis,REACT_6167:Influenza Infection,REACT_6185:HIV Infection
KPNA4				
MVP				
MATR3	h_antisensePathway:RNA polymerase III traNSCription			
PGRMC1				
RTN3				
RTN4				REACT_11061:Signalling by NGF
STIP1		hsa05020:Prion diseases,		
TJP1		hsa04520:Adherens junction,hsa04530:Tight junction,hsa04540:Gap junction,hsa05110:*Vibrio cholerae* infection,hsa05120:Epithelial cell signaling in *Helicobacter pylori* infection		REACT_9480:Gap junction trafficking and regulation
TMEM43				

Comparison of these tables revealed 10 signaling pathways involving two proteins or more (Table **10**), namely: phosphoinositid signaling pathways and their targets, *Vibrio*

cholerae infection pathway, focal adhesion pathway, lysosomal pathway, integrin signaling pathway, Huntington's chorea pathway, *Rho GTPase* signal transfer, HIV infection pathway, membrane transport pathway and hemostasis pathway. The pathways of the proteins of different localization (e.g. nucleus and membrane) are of the principal interest and they are highlighted in yellow in Table **10**.

Table 10: Signaling Pathways Involving Two or More Proteins.

№	Signaling pathway	Proteins
1	h_ptdinsPathway: Phosphoinositides and their downstream targets	ARF1, EEA1
2	hsa05110: *Vibrio cholerae* infection	ARF1, TJP1
3	hsa04510: Focal adhesion	COL6A3, RAP1B
4	hsa04142: Lysosome	AP1B1, SCARB2
5	P00034: Integrin signalling pathway	ARF1, ARF4, ARF5, RAP1B, COL6A3
6	P00029: Huntington disease	ARF1, ARF4, ARF5
7	REACT_11044: Signaling by Rho GTPases	G3BP2, ARHGAP1
8	REACT_6185: HIV Infection	KPNA4, ARF1, ARHGAP1, AP2B1
9	REACT_11123: Membrane Trafficking	ARF1, AP1B1, ARCN1
10	REACT_604: Hemostasis	RAP1B, CALU

DISCUSSION

Having compared proteome profiles of U87 GB CSC proteins with the protein profiles of tissue specific human NSCs and MMSCs that hypothetically present the reference of the species (human), we identified 1664 proteins in the examined lysates, out of which 1052 proteins (63.2%) were identical in NSCs and CSCs, and 607 (36/47%) were identical in MMSCs and CSCs. We supposed that the similarity matrix in CSCs was composed of healthy and species-specific protein cell substrates that remained intact in CSCs. These proteins could serve as the baseline for the development of personalized targeted management of effector functions of CSC unable to respond to the usual signals of intercellular regulation of tissue-specific SCs and regulatory cells of the immune system. Thus, we annotated CSC proteins free from neoplastic transformations of carcinogenesis, and specified membrane, secreted and nuclear proteins using the appropriate databases. These data permitted diagnostics of ten signal transduction pathways

free from carcinogeneic transformation in U87 glioblastoma CSCs. To analyze the opportunity to influence these CSC pathways, we attempted to predict the CSC response to regulatory stimulus. Lysosomal pathways and membrane transport pathways do not transfer the signal to the nucleus, so we excluded them from our further study. HIV and *Vibrio cholerae* infection pathways are characteristic for the cells of the immune system and intestinal epithelium, respectively, and are not known to be associated with malignant degeneration of the cells; hence, they were excluded from further analysis. The hemostasis pathway is typical for platelets and blood coagulation, hence, it was also excluded. Integrin signaling pathway seems to be the most relevant as integrins are markers of stem cells and changes in their regulation can be associated with de-differentiation and cell phenotype change. This pathway mostly coincides with the focal adhesion pathway, which is consequently observed as one pathway.

The integrin/focal adhesion pathway begins from the signal transfer to the cell through various growth factors (EGF, c-fos induced growth factor (FIGF), hepatocyte growth factor (HGF), insulin-like growth factor (IGF1), platelet derived growth factor A (PDGFA), PDGFB, PGF, PDGFC, PDGFD, placental derived growth factor (PGF), vascular endothelial growth factor A (VEGFA), VEGFB, VEGFC) and extracellular matrix proteins (laminins: 1 alpha 1 LAMA1, LAMA2, LAMA3, LAMA4, LAMA5, gamma 3 (LAMC3), beta 4 (LAMB4), LAMB1, LAMB2, LAMB2, LAMC1, LAMC2, collagens: COL1A1, COL1A2, COL2A1, COL3A1, COL4A1, COL4A2, COL4A4, COL4A5, COL4A6, COL5A1, COL5A1, COL5A2, COL6A1, COL6A2, COL6A3, COL11A1, COL11A2, fibronectin FN1, chondroadherin CHAD, cartilage oligomeric matrix protein COMP, tenascins: C (TNC), N (TNN),R (TNR),XB (TNXB), integrin binding sialoprotein IBSP, reelin RELN, secreted phosphoprotein 1 (SPP1), thrombospondins: 1 (THBS1), THBS2, THBS3, THBS4, vitronectin (VTN) and von Willebrand factor (VWF). The signal at the external surface of plasma membrane is transferred due to the integrins: alpha 11 (ITGA11), ITGA6, ITGA1, ITGA2, alpha 2 beta (ITGA2B), ITGA3, ITGA4, ITGA5, ITGA7, ITGA9, alpha V (ITGAV), ITGA10, ITGA8, beta 1 (ITGB1), ITGB3, ITGB4, ITGB5, ITGB6, ITGB7, ITGB8, caveolins: 1 (CAV1), CAV2, CAV3 and tyrosine kinase receptors (epidermal growth factor receptor (EGFR), v-erb-B2 avian

erythroblastic leukemia viral oncogene homolog 2 (ERBB2), fetal liver kinase 1 (FLT1), FLT4, insulin-like growth factor 1 receptor (IGF1R), kinase insert domain receptor (KDR), met proto-oncogene tyrosine kinase (MET), platelet derived growth factor receptor A (PDGFRA), PDGFRB). Accordingly, we received the set of protein targets at the cell membrane, permitting efficient action on the integrins and focal adhesion signal transduction pathways and regulation of reproductive and proliferative functions of CSCs through activation or inhibition of the expression of regulation susceptible CSC genes. This approach radically differs from the current conventional concept of targeted tumor therapy. All contemporary genome, transcriptome and proteome approaches that target the tumor cell, look for oncospecific and carcinodependent target proteins at the cell membrane in the signal transduction pathway or the tumor cell nucleus and target their therapies at them [22-27]. The idea of stem cell reprogramming is not new [28], however, we offer a strategy aimed at the management and control of the proliferative potential of CSCs with no gene engineering. We found what was left intact and species-specific in the CSC proteome, and determined if it was possible to affect the CSC genome that was not responsive to conventional external actions. The contemporary paradigm of all available cancer therapies is directed at complete elimination of all tumor cells or tumor stem cells in every possible way until full recovery of the patient occurs and thus, cannot be efficient by default. Inefficiency of the available paradigm is proven by the absence of functioning technologies of therapeutic assistance in cancer cases [28]. We presume that even theoretically all tumor cells CCs and CSCs cannot be eliminated. CSCs are the way of adaptation and survival for a mutant somatic stem cell [3]. CSCs are similar to a spore in plants permitting survival in unfavorable conditions. Consequently, the available therapeutic paradigm seems to change. The alternative can be provided by the methods aimed at turning the disease into a chronic, therapeutic reduction of CC amount, regulation of CC and control over their number in the body (from 10^7 to 10^3) and, if necessary, management of CSC effector functions [3]. We propose that the goal of anticancer therapy lies in transfer of acute lethal malignant processes into chronic and not lethal processes. The complex proteome-based therapy of tumors must incorporate all available types of cytoreductive, cytotoxic and cytostatic therapy of tumors (surgery, chemo- and radio-therapy and immune therapy) allowing for reduction of CC

number from 10^9 to $5x10^5$, and end in cytoregulatory therapy with autologous tissue-specific regulatory SC managing effector (reproductive and proliferative) functions of CSCs of the tumor.

CURRENT & FUTURE DEVELOPMENTS

The obtained evidence permitted development of novel paradigm of the therapy of glioblastoma that can be further extrapolated to the therapy of any solid tumor. Accordingly, the to-date complex anti-tumor proteome-based therapy (Fig. **10**) should include three compulsory stages: 1) conventional cytoreductive, cytostatic and cytotoxic therapies, 2) adoptive and cytotoxic immune therapy, and 3) cytoregulatory therapy of CSCs. The first stage involves conventional treatment: surgical removal of tumors, chemo- (1-2 line) and radiotherapy (up to 20 Gray). The second stage must consist of adoptive immune therapy (individual anticancer vaccines, cytotoxic lymphocytes, immune drugs and antibodies), which is still considered experimental. The third stage of anti-tumor therapy corrects reproductive, proliferative and metastatic functions of CSCs affecting target proteins of ISTP able to respond. Theoretic and methodological basis for the third stage therapy was provided earlier as the inherent part of the concept of cytoregulatory therapy of neoplasms and published [3]. The international patent application had been filed in 2011 [28].

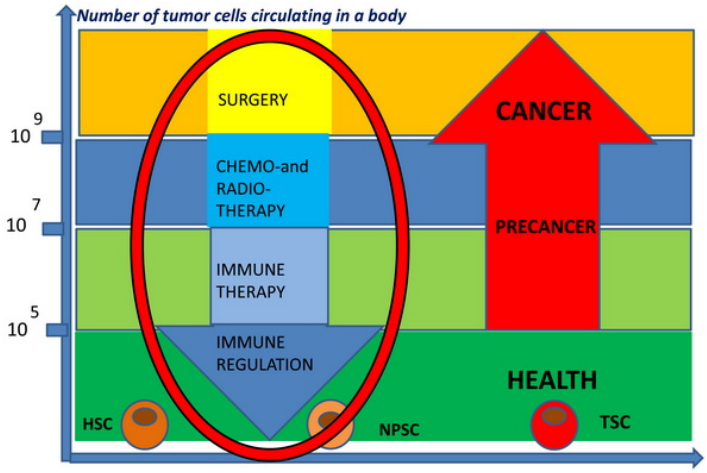


Figure 10: The scheme of complex anti-tumor proteome-based therapy.

After animal tests, our team launched three phase I/IIa clinical trials of the proteome-based anti-tumor therapy. The Ethic Committees and the Scientific Boards of the Blokhin Russian Cancer Research Center and the Federal Clinical Research Center for Specialized Types of Medical Assistance and Medical Technologies of the Medical-Biological Agency of Russia have authorized the clinical trials for the cases of GB and metastases to brain of the lung and breast cancers.

Each trial will include 60 cases (30 cases in control and trial arm) with unresectable relapsed resistant GB, or brain metastases of lung cancer (BMLC), or brain metastases of breast cancer (BMBC). Proteome-based individual immunotherapy of GB/BMLC/BMBC employs autologous cell systems with transcriptome modified profile meant to stimulate individualized adoptive immune response, to toxically affect tumor cells and to regulate CSCs in order to suppress their reproductive and proliferative potential. For GB/BMLC/BMBC immunotherapy, cell systems with specific properties are obtained and applied in three stages: design/analytical, biotechnological and therapeutic. At design/analytical stage, the CCs and CSCs are immunochemically isolated from GB/BMLC/BMBC biopsy sample. The NSCs are isolated from the sample of olfactory sheath of a nose of the same patient. HSCs are isolated from the patient's core needle biopsy sample of bone marrow for the test, and then mobilized from peripheral blood after granulocyte colony-stimulating factor stimulation. The carcinogenesis-free intracellular pathways of signal transduction that are able to respond to targeted regulation by therapeutic cell systems with specific properties, are detected in CSCs using complete transcriptome profiling of gene expression, proteome mapping and profiling of proteins, bioinformatical and mathematical analysis and mathematical modeling of protein profiles of the isolated cells. The targets for regulation of CSCs are detected, as well as protein ligands that are able to regulate reproductive and proliferative properties of CSCs and determine key oncospecific proteins in CSCs and CCs. Using the data of CCs and CSCs proteins proteome profiling and complete transcriptome analysis of gene expression of tissue-specific SCs, the cell preparations to initiate adoptive immune response are prepared: individual antitumor dendritic vaccines loaded with recombinant tumor antigens; cytotoxic lymphocytes as well as proteome-based transcriptome-modified mobilized hematopoietic stem and progenitor cells and NSCs for CSCs immunoregulation.

More detailed descriptions of the trials are available at www.clinicaltrials.gov: NCT01782287 for Proteome-based Immunotherapy of Brain Metastases from Lung Cancer
http://clinicaltrials.gov/ct2/show/NCT01782287?term=NCT01782287&rank=1,
NCT01782274 for Proteome-based Immunotherapy of Brain Metastases from Breast Cancer http://clinicaltrials.gov/ct2/results?term =NCT01782274+&Search=Search and NCT01759810 for Proteome-based Personalized Immunotherapy of Glioblastoma,
http://clinicaltrials.gov/ct2/results?term=NCT01759810+&Search=Search).

In the case, the outcomes of the trials allow for the evaluation of the proposed therapy as efficient, the therapy holds promise to be expanded on other types of cancer and its ubiquitous inclusion into conventional clinical practice, contributing to the improvement of the survival rates and quality of life of the cancer patients.

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CONFLICT OF INTEREST

The authors confirm that this chapter content has no conflict of interest.

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