Research Article

Overexpression of *YKL-39* Gene in Glial Brain Tumors

Vadym Kavsan,¹ Vladimir Dmitrenko,¹ Oxana Boyko,¹ Valery Filonenko,¹ Stanislav Avdeev,¹ Pavel Areshkov,¹ Andriy Marusyk,¹ Tatiana Malisheva,² Vladimir Rozumenko,² and Yury Zozulya²

¹ Department of Nucleic Acids Biosynthesis, Institute of Molecular Biology and Genetics, 150 Zabolotnogo Street, 03680 Kyiv, Ukraine

² Department of Neurooncology, A.P.Romodanov Institute of Neurosurgery, 32 Manuilsky Street, 04050 Kyiv, Ukraine

Correspondence should be addressed to Vadym Kavsan, kavsan@imbg.org.ua

Received 23 June 2008; Revised 9 September 2008; Accepted 22 September 2008

More than forty genes with considerably increased expression in glioblastoma as compared to normal human brain were identified by SAGE. One of the most prominent among them was *CHI3L2* (*YKL-39*) gene, which encodes 39 kDa chitinase-like protein. Northern blot hybridization confirmed the data of SAGE for the majority of glioblastomas. Anaplastic astrocytomas could be divided on two groups: in one of them the *YKL-39* expression was completely undetectable, but in the other group quite high contents of *YKL-39* mRNA were detected. In this study, preliminary data show that patients with undetectable expression of *YKL-39* in anaplastic astrocytomas did not have recurrent tumors quite long (more than 2-3 years) period of time. *YKL-39* RNA has not been detected in diffuse astrocytomas and in all (but one) samples of normal brain. Increased expression of *YKL-39* gene in glioblastomas was shown also at the protein level. Western blots did not shown simultaneous production of *YKL-39* and *YKL-40*, in spite of having high degree of their sequence identity. Increased expression of *YKL-39* in subsets of patients with glial tumors, reported here for the first time, together with abnormal increase of the *YKL-40* gene expression may be a novel molecular marker for glial tumors.

Copyright © 2008 Vadym Kavsan et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

1. Introduction

Human brain tumors are often characterized by significant aggressiveness, high invasiveness, and neurological destructiveness. Almost half of all the intracranial tumors are gliomas, the majority of which are astrocytic gliomas, they are considered to be one of the most lethal forms of malignant tumors. The tumors progress through series of histopathological stages, associated with accumulation of genetic abnormalities on different chromosomes and with changes in gene expression [1]. One of the specific features of these tumors is high morphologic heterogeneity which occurs to be the result of multiple genetic pathways of their initiation and progression [2]. Histological identification of astrocytomas may be complicated or subjective, resulting in misdiagnosis, especially in cases when the typical picture is absent. Standard treatments of malignant gliomas including surgery, chemo- or radiotherapy are relatively nonspecific and may be applied to all glioma subtypes. However, despite advances in these treatments, the overall prognosis for patients with high-grade brain tumors remains dismal.

Development of molecular biomarkers and molecular therapeutics forms the basis for the personalized cancer medicine in the 21st century. Progress in tumor molecular biology offers the opportunity to direct therapies to specific molecular markers and cellular signaling pathways involved in oncogenesis. This offers the potentials to tailor treatment of tumor subtypes—perhaps with greater efficacy and less toxicity. However, at present there exist only a handful of tumor markers that are being routinely used by physicians. Many other potential markers are still being researched. High heterogeneity and multiple pathways of glioma development challenge discovery of new molecular markers for more detailed and precise tumor subtyping.

In an effort to identify the genes, which might be used as molecular markers for glial tumors, we compared gene expression in glioblastoma, the most common and aggressive malignant brain tumor, and normal adult human brain by Serial Analysis of Gene Expression (SAGE) [3]. Obtained results demonstrated that 44 genes were expressed at the significantly higher level in tumors compared to the normal brain cells. One of such genes overexpressed in glioblastoma

is *CHI3L2* (YKL-39), encoding the secreted chitinase-like protein, which has a molecular weight of 39 kDa and is a member of the 18 glycosyl hydrolase family [4].

The present study was initiated to examine the expression of YKL-39 in independent sets of glioma samples at both RNA and protein levels. However, the use of single clinical factors as markers will be substituted soon by systematic approach, including multiple factors, both molecular biological and genetic. So, the purpose of the research in future is the development of the so-called cDNA-panels, which include a number of genes with significantly changed expression in tumors and which can be used for molecular typing of human brain tumors and determination of certain molecular variants for the tumors of identical histological type.

2. Materials and Methods

2.1. Patients. Sixty one patients with different brain tumors (28 glioblastomas, 16 anaplastic astrocytomas, 7 diffuse astrocytomas, 3 oligodendrogliomas, 3 anaplastic oligoastrocytomas, 1 sarcomatous meningioma, 1 metastatic cancer, 1 neuroblastoma, and 1 angioblastoma), classified on the basis of review of haematoxylin and eosin-stained sections of surgical specimens according to WHO criteria [5], were enrolled in this study. 12 surgical specimens of histologically normal brain tissue adjacent to tumors were used as a source of normal adult human brain RNA and protein. All patients were being treated at the hospital of A.P. Romodanov Institute of Neurosurgery (Kiev, Ukraine). The study protocol was approved by the human ethics review committee of both institutions, and signed consent forms from patients were obtained.

2.2. Serial Analysis of Gene Expression (SAGE). Comparison of gene expression in glioblastoma with that of normal human brain was provided by accessing SAGE NCBI web site <http://www.ncbi.nlm.nih.gov/SAGE/> and using the search tool of Digital Gene Expression Displayer (DGED) [6, 7].

2.3. Preparation of YKL-39 cDNA and Construction of Recombinant Plasmid. The tissue samples were stored at -70°C until analysis. RNA was extracted from frozen samples as described in our previous works [3, 8] according to the acid-guanidinium thiocyanate-phenol-chloroform method [9], and then converted to cDNA by reverse transcription and oligo(dT) priming. The DNA fragment encoding YKL-39 was amplified by a polymerase chain reaction (PCR) from the cDNA sample using forward primer YKL39expfor (5'-ATCATATGGGAGCAACCACCATG-3') and reverse primer YKL39exprev (5'-ATCTCGAGCAGGGAGCCAAGGCTTC-TCTT-3') with additional nucleotides on 5' end for generation of restriction sites CATATG (Nde I) and CTCGAG (Xho I). PCR was performed under the following conditions: 30 cycles at 94°C for 1 minute, 56°C for 1 minute, and 72°C for 1.5 minutes. The amplified DNA was then cloned in a plasmid vector pGemT-Easy (Promega, USA).

2.4. Northern Blot Hybridization. Total RNA was fractionated by electrophoresis ($10\text{ }\mu\text{g}$ per lane) in a 1.5% agarose gel containing 2.2 M formaldehyde in borate buffer (0.2 mM EDTA, pH 8.0/30 mM boric acid/3, 3 mM sodium tetraborate, pH 7.5), and then transferred to a Hybond-N nylon membrane (Amersham Pharmacia Biotech, Austria). YKL-39 PCR-product synthesized on the template of recombinant plasmid pET-24a(+)-YKL-39 by random priming in presence of [α - ^{32}P] dCTP (Amersham Biosciences, Austria) was used as a hybridization probe. Membranes were incubated with ^{32}P -labeled cDNA probe in 50% formamide/5xSSC/5xDenhardt's solution/0.1% SDS/100 mg/ml salmon sperm DNA at 42°C overnight. Extensive washing was performed: twice with 2xSSC/0.1% SDS for 15 minutes at room temperature, once with 2 x SSC/0.1% SDS for 30 minutes at 65°C , and finally with 0.2xSSC/0.1% SDS for 30 minutes at 65°C . Subsequently, the membranes were exposed to radiographic film with an intensifying screen at -70°C . The membranes were stripped and rehybridized with a ^{32}P -labelled human β -actin cDNA probe as a control of RNA gel loading. Hybridization bands were normalized to β -actin and compared by densitometry of hybridization signals using the Scion Image 1.62c program.

2.5. Preparation of Recombinant Protein YKL-39 and Polyclonal Antibody. After restriction of pGemT-Easy-YKL-39, the Nde I/Xho I fragment was subcloned in expression vector pET-24a(+) (Novagen, Germany). The map of recombinant plasmid pET-24a(+)-YKL-39, nucleotide, and protein sequence of YKL-39 are presented in Figure 1. The nucleotide sequence was checked by the dideoxy method (3130 Genetic Analyzer, Applied Biosystems, USA). *E. coli* (BL21, DE3) cells transformed by recombinant plasmid pET-24a(+)-YKL-39 were grown at 37°C to optical density 0.5–0.7 (600 nm) and synthesis of recombinant protein was induced during 3 hours by 1 mM IPTG. Affinity purification of YKL-39, containing His₆ at the C-terminus, was carried out on Ni-NTA agarose (Ni²⁺-charged nitriloacetic acid-modified agarose) under denaturing conditions in the presence of 8 M urea according to manufacturer's protocol (Qiagen, USA). Protein products were analyzed by 12% SDS-PAGE and Coomassie Brilliant Blue R-250 staining.

Rabbit antiserum was raised against *Escherichia coli*-expressed His₆-fusion protein YKL-39 as described earlier [10] with some modifications. Male rabbits (2–3 kg) were inoculated 10 times subcutaneously along spinal cord with purified recombinant YKL-39-His₆ protein (30 μg each time) emulsified in PBS with 50% Freund's complete adjuvant (Sigma, St. Louis, Mo, USA). Two more immunizations were repeated in eight and four week intervals by the same antigen quantities in the presence of 50% Freund's incomplete adjuvant. Five days after the final boost, blood was collected by cardiac puncture. Serum was obtained after overnight incubation of blood at 4°C and centrifugation at 1000 g for 15 minutes. YKL-39 antibody titer in serum was determined by enzyme-linked immunosorbent assay (ELISA).

FIGURE 1: Recombinant plasmid pET-24a(+)-YKL-39, nucleotide, and amino-acid sequences of YKL-39 (GenBank Ac N: NM.004000). Oligonucleotide PCR primers are shown by arrows.

FIGURE 2: Analysis of YKL-39 gene expression in glioblastoma. (A) Northern blot hybridization of ^{32}P -YKL-39 cDNA probe with RNA panel. (B) Northern blot hybridization of ^{32}P - β actin cDNA probe with RNA panel. (C) Ethidium bromide-stained agarose gel. (D) Bar graph showing relative expression level of YKL-39. Tissue and tumor types are indicated above each lane of the blot, numbers are the conditional numbers of RNA samples: (a) GB—glioblastoma, NB—human adult normal brain; (b) GB—glioblastoma, NRB—neuroblastoma, NB—human adult normal brain, AA—anaplastic astrocytoma, A—astrocytoma, OL—oligodendroglioma; (c) GB—glioblastoma, AA—anaplastic astrocytoma, AOA—anaplastic oligoastrocytoma, OL—oligodendroglioma, A—astrocytoma, MS—meningioma sarcomatous, NB—human adult normal brain, ABL—angioblastoma; (d) NB—human adult normal brain, GB—glioblastoma.

2.6. Protein Extraction from Brain Tissue and Western Blot Analysis. Total protein extracts of the brain tissues were obtained by grinding of frozen tissue in ceramic mortar and subsequent homogenization in 100 mM Tris-HCl, pH 7.5/100 mM NaCl/0.5% sodium deoxycholate with mixture of protease inhibitors (Boehringer Mannheim, Germany) on ice using a Dounce homogenizer and stored at -20°C until use. After centrifugation the protein concentration in the supernatant was determined by Bradford method [11].

Western blotting was performed as described by Burnette [12]. Briefly, 40 μg protein samples were fractionated by 12% SDS-PAGE and then electrophoretically transferred to nitrocellulose membrane (Amersham Pharmacia Biotechnology, Austria) in Criterion Blotter (Bio-Rad, USA) at 250 mA during 75 minutes. Nonspecific membrane binding

was blocked by 5% solution of dry fat-free milk in PBST for 1 hour at room temperature. The membrane was incubated for 1 hour with anti-YKL-39 polyclonal antibody, 1:300 dilution in 5% dry fat-free milk-PBST for 1 hour. The membrane was washed three times in PBST, 5 minutes each, and incubated with the secondary antirabbit antibody, horseradish peroxidase conjugated, 1:20000 dilution (Promega, USA) for 1 hour. Again, the membrane was washed three times in PBST, and antibody-bound protein was detected by adding enhanced chemiluminescence reagent-Luminol (Amersham Pharmacia Biotechnology, Austria) with hydrogen peroxide and exposing the membrane to Agfa film (Belgium). Western blotting with commercial anti-YKL-40 antibody, 1:5000 dilution, (Quidel Corporation, USA) was performed by the same way.

1	MGATTTMDQKSLWAGVVVLLLLQGGSA YKLV	YKL-39
1	- - - - M GV K ASQT GF VVL V LL QC CS AYKLV	YKL-40
31	CYFTNWSQDRQEPGKFTPENIDPFLCSHLI	YKL-39
26	CY YT S WS QY RE GD GS CF P DAL D R FL CT H T I	YKL-40
61	YSFASIEENNKVILIKDKSEVMLYQTI NSLKT	YKL-39
56	YSFA N I S ND HI DT WE WN D V T LY G ML N T L K N	YKL-40
91	KNPKLKILLSIGGYLFGSKGFHPMVDSSSTS	YKL-39
86	R N P N L K T LL S V G G WN F G S QR F S KI AS NT Q S	YKL-40
121	RLEFINSLILFLRNHNFDGLDVS WIYPDQK	YKL-39
116	R T F I K S VP P F LR T H G F DGL D L A W L Y P G R R	YKL-40
151	ENTHFTVLIHELAEAFQKDFTKSTKERLLL	YKL-39
146	DK Q H F T T L I K E MK AE F T K EA QP G K Q - L L L	YKL-40
181	TAGVSA GRQMI DNSYQVEKLAKDLDFI NLL	YKL-39
175	S A A L S A G KV T I D S S Y D I A K I S QH L D F I S I M	YKL-40
211	SFDFHGSWEKPLITGHNSPLSKGWQDRGPS	YKL-39
205	T Y D F H G A W R G - - T T G H F S P L F R G Q E D A S P D	YKL-40
241	SYYNVEYAVGYWI HKGMPSEKVV MGIP TYG	YKL-39
233	R F S N T D YAVGY ML RL G A P A S K L V M G I P T F G	YKL-40
271	HSFTLASAETTVGAPASGPGAAGPI TESSG	YKL-39
263	R S F T L A S S E T G V G A P I S G P G I P G R F T K E A G	YKL-40
301	FLAYYEICQFLKGAKITRLQDQQVPYAVKG	YKL-39
293	T L A Y Y E I C D F L R G A T V H R I L G Q Q V P Y A T K G	YKL-40
331	NQWVG YDDVKS METKVQFLKNLNLGGAMIW	YKL-39
323	NQWVG YDD QE S V K S K V Q Y L K D R Q L A G A M V W	YKL-40
361	SIDMDDFTGKSCNQGPYPLVQAVKRS LGS	YKL-39
353	A L D L D D F Q G S F C G Q D L R F P L T N A T K D A L A A	YKL-40
390	L	YKL-39
383	T	YKL-40

FIGURE 3: Proteins YKL-39 and HC-gp39 are highly homologous (50,4%) members of 18 glycosylhydrolases family. Amino-acid residues of YKL-40 differing from YKL-39 are boxed.

3. Results and Discussion

Serial Analysis of Gene Expression found *YKL-39* among the most abundant transcripts in glioblastoma, extremely aggressive form of human brain tumors. It was number one in the list of 44 genes with more than 5-fold ($P \leq .05$) increased expression level in glioblastoma. According to results of SAGE, *YKL-39* is not expressed at all in adult normal brain but increased its expression in glial tumors of II–IV grades of malignancy [3].

YKL-39 belongs to the protein family of 18 glycosyl hydrolases (chitinases and chitinase-like proteins). The members of this family have an $(\alpha\beta)_8$ barrel structure [13] and include chitotriosidase, a protein secreted from human macrophages that does have chitinase activity [14], and five proteins with no presently known enzymatic activity, HC gp-39 (*YKL-40*) [15], *YKL-39* [4], YM-1 [16], oviductal glycoprotein [17], and SI-CLP, an interacting partner of the endocytic/sorting receptor stabilin-1 [18]. *YKL-39* was found as a protein that co-purified with *YKL-40*. This 39-kDa protein has the N-terminal sequence YKL, so it was termed *YKL-39*. The 1434-nucleotide sequence of the *YKL-39* cDNA predicts a 385-residue initial translation product and a 364-residue mature *YKL-39*. *YKL-39* lacks the

active site glutamate, which is essential for the activity of chitinases, and as expected has no chitinase activity. *YKL-39* is closely related in size and sequence to *YKL-40*, a 40-kDa glycoprotein [19], more closely than to other members of this family, but in contrast to HC gp-39 (*YKL-40*), *YKL-39* is not a glycoprotein, does not bind to heparin and was not found in macrophages [20]. Same as *YKL-39*, SAGE includes *HC gp-39 (YKL-40)* to the list of 44 genes with more than 5-fold ($P \leq .05$) increased expression level in glioblastoma as compared to normal brain [3]. However, the absolute quantity of *YKL-39* transcripts is much less in glioblastomas and normal brain than those for *YKL-40*.

It was shown that high contents of *YKL-40* protein in sera of patients with so different tumors as colorectal cancer [21], recurrent breast cancer [22] and glioblastoma [23] are associated with a significantly poorer prognosis compared to patients with normal serum. Taking into account the lack of similar data for *YKL-39* and its high homology to HC gp-39, the present study was initiated to further analysis of the expression of *YKL-39* in brain neoplasms and especially in glial tumors.

Northern hybridization and Western blot analysis were used to confirm SAGE results for glioblastomas (WHO astrocytoma grade IV), anaplastic astrocytomas (WHO

astrocytoma grade III) and diffuse astrocytomas (WHO astrocytoma grade II) (Figure 2). In some cases, the Northern hybridization was performed two-three times with the same sample. The samples with low signals with human β -actin cDNA probe as a control of RNA gel loading were excluded from the calculation. Expression of *YKL-39* was determined by Northern analysis in the majority of glioblastomas (19 of 27 samples analyzed). Anaplastic astrocytomas were possible to divide on two groups: in one of them, the *YKL-39* expression was completely undetectable (12 samples), but in other group (4 samples), it was possible to detect quite high contents of *YKL-39* mRNA. *YKL-39* RNA has not been detected in eight samples of diffuse astrocytoma under investigation. Among brain tumors of nonastrocytic origin, *YKL-39* mRNA was detected in neuroblastoma and metastatic cancer, but was not detected in three samples of oligodendroglioma, one sample of each anaplastic oligoastrocytoma, angioblastoma, and sarcomatous meningioma.

Hybridization showed the presence of *YKL-39* mRNA only in 1 from 13 samples of normal brain at the level high enough for the determination by Northern analysis. It should be pointed that the “normal” brain tissue collected around the tumor samples may serve as a source of normal adult human brain RNA only with some precautions: gliomas are infiltrating tumors, and scattered tumor cells are present far away from the dense tumor area removed during surgery. Moreover, inflammation and astrocytic activation occur in this peritumoral tissue.

All 16 anaplastic astrocytomas analyzed in this work were used for the determination of expression of both genes. 1 of 4 samples with expressed *YKL-39* had no detectable *HC gp-39* mRNA; and vice versa 4 of 12 anaplastic astrocytomas, negative as related to *YKL-39* mRNA, had the expression of *HC gp-39* gene. Interesting that *YKL-39* mRNA was detected in one recurrent tumor (glioblastoma), while in the original anaplastic astrocytoma expression of both genes it was not detected. Earlier, we showed that anaplastic astrocytomas can be divided in two groups as it concerns *HC gp-39* expression [24]. Patients with high level of *HC gp-39* mRNA had shorter remission period to the secondary surgery than patients with lower level of *HC gp-39* expression. Naturally, it is necessary to investigate much more samples, however even preliminary data show that patients with undetectable expression of *YKL-39* in anaplastic astrocytomas (6 patients of 12 under investigation) did not have recurrent tumors for a quite long (more than 2-3 years) period.

To analyze the expression of *YKL-39* gene at the protein level, total RNA extracted from glioblastoma cells, was converted to cDNA by reverse transcription and oligo(dT) priming. The DNA fragment encoding *YKL-39* was amplified from the cDNA sample and the amplified DNA was then cloned in a plasmid vector pET-24a(+). *E. coli* transformed with this recombinant plasmid was used for production of the recombinant (His)₆-tagged protein. Purified protein had an expected size (about 40 kDa) although electrophoresis in denatured conditions beside the major band showed a couple of minor bands which apparently are the result of proteolytic hydrolysis. The problems occurred during (His)₆-protein purification by Ni-NTA-agarose, particularly

FIGURE 4: Detection of *YKL-39* and *YKL-40* in tumors and normal human brain. (A) Western blot analysis of total tissue lysates with rabbit polyclonal antibody to *YKL-39*. (B) Western blot analysis of total tissue lysates with rabbit polyclonal antibody to *YKL-40*. (C) Western blot analysis of total tissue lysates with antibody to β -actin. Tissue and tumor types are indicated above each lane of the blot, numbers are the conditional numbers of tissue samples: NB—human adult normal brain, Rec. protein in (A) recombinant *YKL-39*, Rec. protein in (B) recombinant *YKL-40*, AA—anaplastic astrocytoma, A—diffuse astrocytoma, M—protein weight molecular marker (PageRuler Unstained Protein Ladder SM0661, Fermentas), the position of the band of 40 kDa is indicated.

nonspecific oxidation of protein by this resin, were described also earlier [23]. The antibody to the purified recombinant protein was shown to react specifically on Western blots with purified human *YKL-39*. Preimmune sera did not react.

Western analysis of brain tumors revealed the 39 kDa product in five of eleven analyzed glioblastoma samples and in five of thirteen anaplastic astrocytomas, but in none of five samples of normal brain. High homology of nucleotide and amino acid sequences of *YKL-39* and *YKL-40* (Figure 3) suppose either some identity of their functions or at least some coordination of their activity. However, experimental results do not always confirm this hypothesis. For example, expression analysis showed high levels of both proteins in normal articular chondrocytes, with lower levels of *YKL-39* than *YKL-40*. However, whereas *HC gp-39* was significantly downregulated in late stage of osteoarthritic chondrocytes, *YKL-39* was significantly upregulated [25]. The immune response to *YKL-39* was independent of that to *YKL-40*: the level of *YKL-40* protein did not correlate with the level of *YKL-39*. As it is possible to see in Figure 4: in one case, both *YKL-39* and *YKL-40* were detected in anaplastic astrocytoma; in other cases *YKL-39* or *YKL-40* or none of them was detected. Western blots therefore revealed neither possible synergistic, nor simultaneous increasing of activity of *YKL-39* and *YKL-40*.

The increasing of *YKL-39* level in subsets of patients with glial tumors is reported and apparently represents a cellular response to changes in the extracellular matrix environment. This, coupled with the abnormal increase of the *YKL-40* gene expression, identifies a novel molecular marker for glial tumors. Clarification of the antibody and T cell responses

to autoantigens may lead to a better understanding of the pathophysiology of tumor development in patients with astrocytomas. However, high oncogenic heterogeneity of brain tumors is the considerable hindering for successful therapy of separate tumors. Continuation of investigation of new molecular markers must help in the finding of correlations between the changes of their activity and prognosis of motion of disease.

Acknowledgments

This work was supported by National Academy of Sciences of Ukraine in frames of the programs "Novel Problems of Medicine and Biology and Human Environment" as well as "Fundamental basis of genomics, proteomics and newest biotechnologies" by Ministry of Education and Science of Ukraine in frames of Program of joint research in the field of scientific and technological collaboration between Ukraine and France "DNIPRO," and also by Grant F25.5/088 from Fundamental Research State Fund of Ukraine. The authors wish to thank O. Y. Borozenko for the help in preparation of some Western blots.

References

- [1] G. Reifenberger and V. P. Collins, "Pathology and molecular genetics of astrocytic gliomas," *Journal of Molecular Medicine*, vol. 82, no. 10, pp. 656–670, 2004.
- [2] Y. Cheng, H.-K. Ng, M. Ding, S.-F. Zhang, J. C.-S. Pang, and K.-W. Lo, "Molecular analysis of microdissected de novo glioblastomas and paired astrocytic tumors," *Journal of Neuropathology and Experimental Neurology*, vol. 58, no. 2, pp. 120–128, 1999.
- [3] V. Kavsan, K. Shostak, V. Dmitrenko, Y. Zozulya, V. Rozumenko, and J. Demotes-Mainard, "Characterization of genes with increased expression in human glioblastomas," *Tsitology and Genetics*, vol. 39, no. 6, pp. 37–49, 2005.
- [4] B. Hu, K. Trinh, W. F. Figueira, and P. A. Price, "Isolation and sequence of a novel human chondrocyte protein related to mammalian members of the chitinase protein family," *The Journal of Biological Chemistry*, vol. 271, no. 32, pp. 19415–19420, 1996.
- [5] D. N. Louis, H. Ohgaki, O. D. Wiestler, et al., "The 2007 WHO classification of tumours of the central nervous system," *Acta Neuropathologica*, vol. 114, no. 2, pp. 97–109, 2007.
- [6] K. Boon and G. J. Riggins, "SAGE as a strategy to isolate cancer-related genes," *Methods in Molecular Biology*, vol. 222, pp. 463–479, 2003.
- [7] V. Kavsan, V. Dmitrenko, K. Shostak, et al., "Comparison of microarray and SAGE techniques in gene expression analysis of human glioblastoma," *Tsitology and Genetics*, vol. 41, no. 1, pp. 36–55, 2007.
- [8] V. Dmitrenko, O. I. Bojko, K. Shostak, et al., "Characterization of genes, down-regulated in human glioma, potential tumor suppressor genes," *Biopolymers and Cell*, vol. 23, no. 4, pp. 347–362, 2007.
- [9] P. Chomczynski and N. Sacchi, "Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction," *Analytical Biochemistry*, vol. 162, no. 1, pp. 156–159, 1987.
- [10] L. L. Sidorik, O. I. Gudžera, V. A. Dragovoz, M. A. Tukalo, and S. F. Beresten, "Immuno-chemical non-cross-reactivity between eukaryotic and prokaryotic seryl-tRNA synthetases," *FEBS Letters*, vol. 292, no. 1–2, pp. 76–78, 1991.
- [11] M. M. Bradford, "A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding," *Analytical Biochemistry*, vol. 72, no. 1–2, pp. 248–254, 1976.
- [12] W. N. Burnette, "'Western blotting': electrophoretic transfer of proteins from sodium dodecyl sulfate-polyacrylamide gels to unmodified nitrocellulose and radiographic detection with antibody and radioiodinated protein A," *Analytical Biochemistry*, vol. 112, no. 2, pp. 195–203, 1981.
- [13] F. Fusetti, T. Pijning, K. H. Kalk, E. Bos, and B. W. Dijkstra, "Crystal structure and carbohydrate-binding properties of the human cartilage glycoprotein-39," *The Journal of Biological Chemistry*, vol. 278, no. 39, pp. 37753–37760, 2003.
- [14] R. G. Boot, G. H. Renkema, A. Strijland, A. J. van Zonneveld, and J. M. F. G. Aerts, "Cloning of a cDNA encoding chitotriosidase, a human chitinase produced by macrophages," *The Journal of Biological Chemistry*, vol. 270, no. 44, pp. 26252–26256, 1995.
- [15] B. E. Hakala, C. White, and A. D. Recklies, "Human cartilage gp-39, a major secretory product of articular chondrocytes and synovial cells, is a mammalian member of a chitinase protein family," *The Journal of Biological Chemistry*, vol. 268, no. 34, pp. 25803–25810, 1993.
- [16] H. M. Jin, N. G. Copeland, D. J. Gilbert, N. A. Jenkins, R. B. Kirkpatrick, and M. Rosenberg, "Genetic characterization of the murine Ym1 gene and identification of a cluster of highly homologous genes," *Genomics*, vol. 54, no. 2, pp. 316–322, 1998.
- [17] E. B. Arias, H. G. Verhage, and R. C. Jaffe, "Complementary deoxyribonucleic acid cloning and molecular characterization of an estrogen-dependent human oviductal glycoprotein," *Biology of Reproduction*, vol. 51, no. 4, pp. 685–694, 1994.
- [18] J. Kzhyshkowska, S. Mamidi, A. Gratchev, et al., "Novel stabilin-1 interacting chitinase-like protein (SI-CLP) is up-regulated in alternatively activated macrophages and secreted via lysosomal pathway," *Blood*, vol. 107, no. 8, pp. 3221–3228, 2006.
- [19] J. J. Rejman and W. L. Hurley, "Isolation and characterization of a novel 39 kilodalton whey protein from bovine mammary secretions collected during the nonlactating period," *Biochemical and Biophysical Research Communications*, vol. 150, no. 1, pp. 329–334, 1988.
- [20] S. W. Krause, M. Rehli, M. Kreutz, L. Schwarzfischer, J. D. Paulauskis, and R. Andreesen, "Differential screening identifies genetic markers of monocyte to macrophage maturation," *Journal of Leukocyte Biology*, vol. 60, no. 4, pp. 540–545, 1996.
- [21] C. Cinton, J. S. Johansen, I. J. Christensen, P. A. Price, S. Sørensen, and H. J. Nielsen, "Serum YKL-40 and colorectal cancer," *British Journal of Cancer*, vol. 79, no. 9–10, pp. 1494–1499, 1999.
- [22] J. S. Johansen, C. Cinton, M. Jørgensen, C. Kamby, and P. A. Price, "Serum YKL-40: a new potential marker of prognosis and location of metastases of patients with recurrent breast cancer," *European Journal of Cancer*, vol. 31, no. 9, pp. 1437–1442, 1995.
- [23] M. K. Tanwar, M. R. Gilbert, and E. C. Holland, "Gene expression microarray analysis reveals YKL-40 to be a potential serum marker for malignant character in human glioma," *Cancer Research*, vol. 62, no. 15, pp. 4364–4368, 2002.

- [24] K. Shostak, V. Labunsky, V. Dmitrenko, et al., “*HC gp-39* gene is upregulated in glioblastomas,” *Cancer Letters*, vol. 198, no. 2, pp. 203–210, 2003.
- [25] T. Knorr, F. Obermayr, E. Bartnik, A. Zien, and T. Aigner, “YKL-39 (chitinase 3-like protein 2), but not YKL-40 (chitinase 3-like protein 1), is up regulated in osteoarthritic chondrocytes,” *Annals of the Rheumatic Diseases*, vol. 62, no. 10, pp. 995–998, 2003.