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# Glioblastoma gene network reconstruction and ontology analysis by online bioinformatics tools

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**Abstract:** Glioblastoma is the most aggressive type of brain tumors resistant to a number of antitumor drugs. The problem of therapy and drug treatment course is complicated by extremely high heterogeneity in the benign cell populations, the random arrangement of tumor cells, and polymorphism of their nuclei. The pathogenesis of gliomas needs to be studied using modern cellular technologies, genome- and transcriptome-wide technologies of high-throughput sequencing, analysis of gene expression on microarrays, and methods of modern bioinformatics to find new therapy targets. Functional annotation of genes related to the disease could be retrieved based on genetic databases and cross-validated by integrating complementary experimental data. Gene network reconstruction for a set of genes (proteins) proved to be effective approach to study mechanisms underlying disease progression. We used online bioinformatics tools for annotation of gene list for glioma, reconstruction of gene network and comparative analysis of gene ontology categories. The available tools and the databases for glioblastoma gene analysis are discussed together with the recent progress in this field.

**Keywords:** drug search; gene networks; gene ontology; glioblastoma; medical genomics.

## 1 Introduction

Glioblastomas, along with astrocytomas, are the most common primary tumors of the central nervous system and make up approximately 60–70% of all pure brain tumors, challenging search for new therapy methods. Primary glioblastoma in 60% of cases occurs in people over 50 years old, while secondary glioblastoma is more characteristic for people under 45 years old [1, 2]. Glioblastoma is characterized by rapid infiltrative growth, the presence of foci of necrosis, a change in blood vessels. The lack of clear boundaries and the ability to relapse are a particular problem for surgical removal of the tumor, and for immunotherapy [3]. Primary

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central nervous system tumors are relatively rare and account for approximately 2% of all oncological diseases. However, tumors of the brain and spinal cord are the second most common form of malignancy in children after leukemia [4]. Glioblastoma has higher burden in survival of the patients with malignant brain tumors [5]. Despite the development in early detection, and treatment, 5-year glioblastoma survival only increased from 4 to 7% in past 4 decades [5]. Gliomas are the most common heterogeneous group of malignant tumors of the brain among the adult population, which differ in morphological characteristics, clinical course, and response to radio- and chemotherapy. The incidence of gliomas is 3–5: 100,000 of the adult population. In the treatment of glioblastoma with drugs, an obstacle is its resistance to a number of antitumor drugs initially, as well as the presence of a blood-brain barrier that negates the effectiveness of most chemotherapeutic agents and targeted drugs [3, 6].

For an object such as gliomas, it is necessary to conduct new studies based on modern cellular technologies, genome-wide technologies for high-throughput sequencing, and the integration of available information from international databases and genomic projects [7, 8].

The practical task was to construct (collect) a list of genes associated with the development of glioblastoma, analyze the categories of gene ontologies for such a list, and reconstruct the gene network. For key genes of the disease, obtained by analyzing the structure of the gene network, options for searching for drugs (substances that interact with this protein) will be considered.

There are open international databases on gene expression including glioblastoma (microarrays and sequencing data of GEO NCBI, <http://www.ncbi.nlm.nih.gov/geoprofiles/>), expression in various types of tumor cells (The Cancer Gene Atlas, [cancergenome.nih.gov](http://cancergenome.nih.gov)), gene expression for brain compartments (Allen Brain Atlas), protein interactions databases such as HPRD (<http://hprd.org/>), KEGG biochemical reactions (<http://www.genome.jp/kegg/>), Interactome (<http://interactome.org/>), sequenced tumor genomes, including gliomas and glioblastomas (<https://cghub.ucsc.edu/>). The Allen Institute has developed the Ivy Glioblastoma Atlas Project database (<http://glioblastoma.alleninstitute.org/>) according to patients with glioma. We demonstrate that these publicly available online bioinformatics tools can give helpful information for annotation of gene list for glioblastoma, reconstruction of gene network and comparative analysis of the related diseases. This approach for gene network analysis of list of gene names using online bioinformatics tools presents application of bioinformatics methods to annotation of complex human diseases.

## 2 Methods

Obtaining a list of genes associated with a hereditary predisposition to glioblastoma. The Internet resource OMIM (Online Mendelian Inheritance in Man) (<https://omim.org/>, access date 10.10.2021) was used to analyze the genes of Mendelian inheritance in humans [9]. Search for the keyword ‘glioblastoma’ gave 264 genes. As a result, 264 gene names (official gene symbols) were obtained (ADAM10, ADAM17, ADAM9, ADGRB1, etc., see the Supplement).

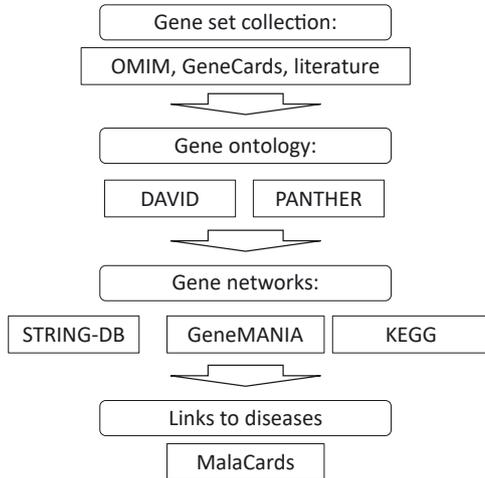
Next, we used DAVID (Database for Annotation, Visualization and Integrated Discovery) tool (<https://david.ncifcrf.gov/summary.jsp>) [10] and the PANTHER (Protein ANalysis THrough Evolutionary Relationships) (<http://pantherdb.org/>) resource [11] for gene ontology analysis. Then we applied gene network reconstruction tools described in the next sections.

The workflow of the data processing is presented in Figure 1.

Figure 1 shows main steps of data processing. Data collection starts from the OMIM database (<https://omim.org/>) and GeneCards (<https://www.genecards.org/>) resources. At next step gene ontology analysis tools PANTHER and DAVID are used as well as g:Profiler (<http://biit.cs.ut.ee/gprofiler/gost>) online tool. Then STRING-DB (<https://string-db.org>) and GeneMANIA (<https://genemania.org/>) online applications help in the network reconstruction and visualization. Finally MalaCards (<https://www.malacards.org/>) and ToppGene (<https://toppgene.cchmc.org/>) allow find disease-disease interactions and prioritize genes in the list.

## 3 Results

We have analyzed gene ontology categories for the same gene list obtained by different online bioinformatics tools. DAVID [10] and the PANTHER resource [11] were used to estimate the categories of gene ontologies.



**Figure 1:** Workflow for information data processing for glioblastoma genes.

### 3.1 Comparison of gene ontology categories

A list of 264 human genes was loaded through the DAVID interface to search for relevant categories of gene ontologies for this group of genes. 255 identifiers were used next (the Functional Annotation Chart option).

The table was limited to values of normalized  $p$ -value  $<0.00001$  (or, in power notation,  $<1.0 \times 10^{-5}$ ) (Bonferroni correction). We deleted categories (table rows) for groups of less than eight genes, and Fold enrichment (observed number of genes/expected) less than 2.

As can be seen from Table 1, the most significant GO categories for glioblastoma genes are protein binding, positive regulation of protein phosphorylation, membrane location (according to cellular compartments – GOTERM\_CC classification), and kinase activity. In general, according to the categories from the table,

**Table 1:** Categories of gene ontologies for glioblastoma genes according to DAVID

Category set	Term	# genes	Fold	Bonferroni
UP_KEYWORDS	Tumor suppressor	24	12	$1.3 \times 10^{-15}$
UP_KEYWORDS	Phosphoprotein	154	1.6	$3.0 \times 10^{-13}$
UP_KEYWORDS	Disease mutation	71	2.5	$1.2 \times 10^{-10}$
GOTERM_MF_DIRECT	Protein binding	164	1.5	$4.2 \times 10^{-10}$
GOTERM_BP_DIRECT	Negative regulation of cell proliferation	26	5	$1.8 \times 10^{-7}$
INTERPRO	Protein kinase-like domain	28	4.3	$2.1 \times 10^{-7}$
GOTERM_BP_DIRECT	Positive regulation of protein phosphorylation	16	9.6	$2.5 \times 10^{-7}$
GOTERM_MF_DIRECT	Protein kinase binding	24	5	$2.6 \times 10^{-7}$
UP_KEYWORDS	Kinase	31	3.7	$3.1 \times 10^{-7}$
UP_KEYWORDS	Proto-oncogene	18	6.7	$4.4 \times 10^{-7}$
UP_KEYWORDS	Cell cycle	27	3.7	$6.6 \times 10^{-6}$
UP_KEYWORDS	Tyrosine-protein kinase	12	9.5	$1.5 \times 10^{-5}$
INTERPRO	Protein kinase, catalytic domain	24	4	$1.8 \times 10^{-5}$
UP_SEQ_FEATURE	Binding site:ATP	25	3.9	$3.2 \times 10^{-5}$
GOTERM_MF_DIRECT	Kinase activity	17	5.5	$4.0 \times 10^{-5}$
UP_SEQ_FEATURE	Active site:Proton acceptor	27	3.5	$6.8 \times 10^{-5}$
GOTERM_BP_DIRECT	Protein phosphorylation	24	4	$7.7 \times 10^{-5}$
GOTERM_MF_DIRECT	Protein kinase activity	20	4.3	$8.7 \times 10^{-5}$
GOTERM_CC_DIRECT	Membrane	56	2	$9.6 \times 10^{-5}$

membrane proteins and kinases play an important role, apparently associated with signal transduction into the cell. UP\_KEYWORD (terms from UniProt (<https://www.uniprot.org/>) shows associations with tumors and kinase activity.

Gene ontologies were analyzed for the same list of genes using the PANTHER resource (Protein ANalysis THrough Evolutionary Relationships) [11]. Table 2 of ontologies for categories of biological processes was built using PANTHER.

In total, 238 identifiers were recognized from the same list of genes, the others were not recognized or could not be unambiguously mapped. In total, 20,595 genes were used in the PANTHER reference genome (15 October 2021). We limited the output to  $p$ -values up to  $10^{-12}$  (after Bonferroni correction) and Fold enrichment greater than two to represent the most informative results. Table 2 shows that the most significant categories for glioblastoma genes are negative regulation of biological processes, developmental processes, generation of neurons, and cell proliferation.

The categories of processes related to the development of the nervous system are also presented (this is expected, since glioblastoma is a brain disease).

Further, the categories of gene ontologies for molecular functions and cellular compartments were calculated using PANTHER (Table 3). The threshold for the significance of the categories was taken already at the  $10^{-3}$  level (after the Bonferroni correction).

The results for cellular components show that the most significant categories for glioblastoma genes are organelles, cell junction. It may be associated with signal transmission between cells and refers to neurons.

Thus, both DAVID and PANTHER for glioblastoma genes support the categories of gene ontologies of protein binding, membranes, cell adhesion, and intercellular contacts. Note that direct comparison of DAVID and PANTHER tools proved be limited due to different database version and genome annotation versions [12]. Other available resources for calculating gene ontologies – GeneOntology (<http://geneontology.org/>) and g:Profiler GOST (<http://biit.cs.ut.ee/gprofiler/>), which are not presented in this study, can also be used.

**Table 2:** Categories of gene ontologies for glioblastoma genes according to PANTHER (biological process).

GO categories (biological process complete)	# genes	Fold enrich.	$p$ value
Negative regulation of biological process	146	2.36	$2.21 \times 10^{-25}$
Negative regulation of cellular process	137	2.40	$2.20 \times 10^{-23}$
Anatomical structure development	136	2.28	$5.27 \times 10^{-21}$
System development	123	2.47	$7.48 \times 10^{-21}$
Regulation of cell population proliferation	75	3.81	$3.21 \times 10^{-20}$
Developmental process	142	2.14	$1.42 \times 10^{-19}$
Regulation of signaling	102	2.62	$1.39 \times 10^{-17}$
Regulation of cell differentiation	67	3.75	$3.90 \times 10^{-17}$
Regulation of cell death	69	3.63	$4.97 \times 10^{-17}$
Regulation of apoptotic process	64	3.76	$3.44 \times 10^{-16}$
Cellular developmental process	103	2.49	$4.11 \times 10^{-16}$
Regulation of programmed cell death	64	3.68	$9.59 \times 10^{-16}$
Regulation of signal transduction	91	2.66	$3.02 \times 10^{-15}$
Regulation of cellular component movement	53	4.33	$3.48 \times 10^{-15}$
Nervous system development	78	2.98	$5.86 \times 10^{-15}$
Regulation of epithelial cell proliferation	32	7.93	$1.10 \times 10^{-14}$
Positive regulation of phosphorus metabolic process	47	4.44	$1.96 \times 10^{-13}$
Immune system development	40	5.04	$1.21 \times 10^{-12}$
Regulation of nervous system development	33	6.31	$1.69 \times 10^{-12}$
Generation of neurons	53	3.59	$7.61 \times 10^{-12}$

**Table 3:** Categories of gene ontologies for glioblastoma genes according to PANTHER (molecular functions and cellular compartments).

	# genes	Fold enrich.	p value
<b>GO categories – molecular function complete</b>			
Protein binding	225	1.31	$7.98 \times 10^{-13}$
Enzyme binding	68	2.76	$2.84 \times 10^{-8}$
Kinase binding	36	3.94	$1.54 \times 10^{-8}$
Phosphotransferase activity alcohol group as acceptor	31	3.76	$1.57 \times 10^{-6}$
Protein tyrosine kinase activity	14	8.20	$1.61 \times 10^{-5}$
Transferase activity transferring phosphorus-containing groups	34	3.09	$2.78 \times 10^{-5}$
Beta-catenin binding	9	8.66	$5.97 \times 10^{-3}$
Growth factor receptor binding	11	6.49	$6.20 \times 10^{-3}$
<b>GO categories – cellular component complete</b>			
Organelle lumen	115	1.74	$6.06 \times 10^{-8}$
Cytosol	111	1.72	$3.16 \times 10^{-7}$
Cell junction	59	2.35	$8.77 \times 10^{-7}$
Cell projection	63	2.23	$1.75 \times 10^{-6}$

### 3.2 Reconstruction of gene networks for glioblastoma genes

To reconstruct the gene network of interactions between glioblastoma genes, the resources of GeneMANIA (<https://genemania.org/>) and STRING-DB (<https://string-db.org/>) were used. The following Figure 2 shows the glioblastoma gene network reconstructed with GeneMANIA and STRING-DB.

In the centre of the network there are the genes (proteins) of glioblastoma, which have a large number of connections with other elements – PTRN, TP53, KRAS.

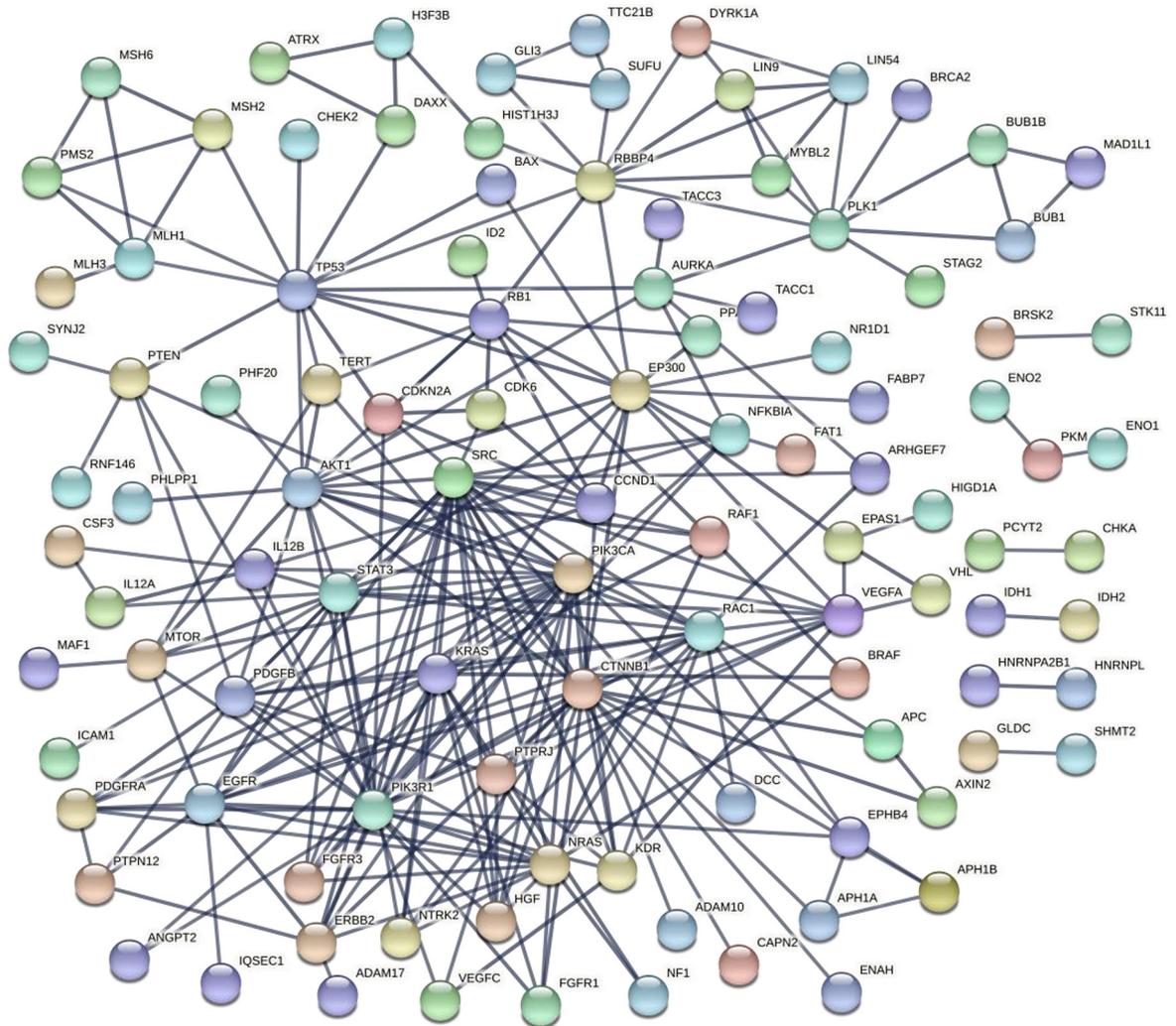
Consider the results of network reconstruction using STRING-DB (<https://string-db.org/>) for the same list of glioblastoma genes (Figure 2). It can be seen that the network is quite sparse, some objects (proteins) do not contact others. A central, strongly linked cluster of genes is highlighted.

In total, 243 genes from the list were recognized by STRING-DB. Statistics on STING-DB show that the network has a reasonably large number of connections (with a significance  $<1.0 \times 10^{-16}$ ), the average degree of connectivity of the network node (protein) is 13.6, the clustering coefficient is 0.5. The drawing from the STING-DB was iteratively rebuilt with the removal of unrelated genes and the replacement of several colored connections between the nodes of the network – with one line (confidence), taking into account only experimentally proven interactions. Than we increase parameter ‘confidence level’ to keep only verified links in the network.

The figure shows several hubs of the network – the largest includes genes RBBP4, AKT1, MTOR, KRAS, STAT3, PTEN, several smaller, but also connected in a common network of clusters, the largest number of connections in the TP53 gene (a well-known oncogene) can be seen.

In general, the analysis of the structure of the gene network for glioblastoma genes shows the existence of a dense, connected, sufficiently large cluster of genes (network nodes) including known oncogenes, such as TP53.

Thus, standard online bioinformatics tools allow to model main parameters of gene network related to given disease. This approach is quite useful in distant education for medical students due to open access tools, fast visualization, interactive user interface not demanding programming. Integration of the results obtained by online bioinformatics tools and genetics data leads to novel knowledge in the study of complex oncological diseases.



**Figure 2:** Gene network for glioblastoma genes reconstructed by STRING-DB.

## 4 Related works

Let's consider functional annotation of the glioblastoma genes using different data sources and their combinations.

According to GeneCards ([genecards.org](https://www.genecards.org)), the following 20 genes are the most significant.

Table 4 shows large fraction of non-protein coding genes in the list. The score in the table column show relevance of the gene to the search query to the literature database (<https://www.genecards.org/Guide/Search#relevance>). Larger the GeneCards score, higher relevance to the disease is. GeneCards Inferred Functionality Scores (GIFtS) parameter shows gene functionality annotated in the databases (<https://www.genecards.org/Guide/GeneCard#GIFtS>) [13]. Note that the hub genes in the network have high functionality, while RNA less annotated and included in standard gene network (in STRING-DB).

Prioritization of genes was performed using the resource ToppGene: Candidate gene prioritization (<https://toppgene.cchmc.org>) [14].

The genes presented were found to be associated with the following diseases (Table 5).

**Table 4:** Gene list for glioblastoma by GeneCards.

	Symbol	Description	Category	GiftS	Relevance score
1	TP53	Tumor protein P53	Protein coding	52	52.29
2	EGFR	Epidermal growth factor receptor	Protein coding	52	40.05
3	IDH1	Isocitrate dehydrogenase (NADP(+)) 1	Protein coding	51	38.95
4	MSH2	MutS homolog 2	Protein coding	48	33.76
5	MGMT	O-6-Methylguanine-DNA methyltransferase	Protein coding	48	31.36
6	ERBB2	Erb-B2 receptor tyrosine kinase 2	Protein coding	52	29.57
7	MIR21	MicroRNA 21	RNA gene	24	28.37
8	BRCA2	BRCA2 DNA repair associated	Protein coding	48	27.59
9	PTEN	Phosphatase and tensin homolog	Protein coding	51	26.68
10	PIK3CA	Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha	Protein coding	52	25.74
11	MIR221	MicroRNA 221	RNA gene	21	24.00
12	MIR222	MicroRNA 222	RNA gene	21	23.89
13	FGFR1	Fibroblast growth factor receptor 1	Protein coding	53	22.81
14	MIR34A	MicroRNA 34a	RNA gene	22	22.63
15	MIR296	MicroRNA 296	RNA gene	17	22.51
16	H3-3A	H3.3 histone A	Protein coding	34	21.76
17	MIR137	MicroRNA 137	RNA gene	19	44.398
18	IDH2	Isocitrate dehydrogenase (NADP(+)) 2	Protein coding	51	20.63
19	PPARG	Peroxisome proliferator activated receptor gamma	Protein coding	51	20.37
20	MIR326	MicroRNA 326	RNA gene	21	20.14

**Table 5:** Diseases found by prioritization of shared genes to glioblastoma.

No.	Description	Category	Score
1	Glioma	$1.882 \times 10^{-25}$	$9.333 \times 10^{-22}$
2	Pilocytic astrocytoma	$3.147 \times 10^{-25}$	$1.561 \times 10^{-21}$
3–4	Adult pilocytic astrocytoma/childhood pilocytic astrocytoma	$1.829 \times 10^{-22}$	$9.069 \times 10^{-19}$
5	Malignant glioma	$3.741 \times 10^{-21}$	$1.855 \times 10^{-17}$
6	Mixed gliomas	$3.741 \times 10^{-21}$	$1.855 \times 10^{-17}$
7	Neurofibromatosis 1	$6.750 \times 10^{-21}$	$3.348 \times 10^{-17}$
8	Malignant neoplasm of soft tissue	$2.560 \times 10^{-20}$	$1.270 \times 10^{-16}$
9	Ganglioglioma	$4.231 \times 10^{-20}$	$2.098 \times 10^{-16}$
10–11	Childhood oligodendroglioma/adult oligodendroglioma	$4.616 \times 10^{-20}$	$2.290 \times 10^{-16}$
12	Sarcoma	$2.272 \times 10^{-19}$	$1.127 \times 10^{-15}$

One can see in the table relevant diseases categories related to brain, glioma types and soft tissues cancers.

The approaches of gene ontology analysis and gene network reconstruction using similar bioinformatics tools to search for glioma hub genes are published in series of papers. In recent work by [15] hub genes in the tumor microenvironment were identified using TCGA data. The hub genes for glioblastoma were identified by the Cytoscape tool (<https://cytoscape.org/>), and pathway enrichment analysis of the genes was performed using Database for Annotation, Visualization and Integrated Discovery (DAVID). Functional enrichment analysis identified set of upregulated and downregulated cross genes, which were mainly linked to immune response, inflammatory response, cell membrane, and receptor activity. Li and co-authors [16] used weighted gene co-expression network analysis to define hub genes.

Gene Set Enrichment Analysis (GSEA) identified further hub genes-related pathways. The Cancer Genome Atlas (TCGA) database was used to identify differentially expressed genes [17]. Then Gene Ontology and Kyoto

Encyclopedia of Genes and Genomes (KEGG) analyses were used to determine the related functions and pathways of these genes.

Such analysis can lead to the identification of potential target genes and related drugs. Yang and Yang [18] recently analyzed glioma genes by GSEA, and the significantly enriched KEGG pathways involved in synapse signaling and oxytocin signaling pathways. The core molecules of GBM and the DrugBank database were assessed to identify 10 drugs included tetrachlorodecaoxide related to cancer and neuropsychiatric diseases [18].

## 5 Conclusions

After years of intensive research glioblastoma remains a dangerous disease with a low survival rate. The existing methods of therapy (chemotherapy, radio- and immunotherapy) can prolong the patient's life without remission and glioblastoma data integration and analysis can lead to promising therapeutic targets [19].

The study of the structure of the gene network shows a high connectivity of genes and their products. Glioma progression is strongly connected with different types of epigenetic phenomena, such as histone modifications, DNA methylation, chromatin remodeling, and aberrant microRNA [18]. Amid the epigenetic therapies, histone deacetylase inhibitors (HDACIs) and DNA methyltransferase inhibitors have been used for treating tumors [20].

Long non-coding RNAs, circular RNAs, and transcribed pseudogenes act as ceRNA (competing endogenous RNA) to regulate the expression of related genes by sponging the shared microRNAs [21]. Circular (circ)RNAs serve important roles in the development and progression of glioma and may have potential as therapeutic targets too [22].

A set of glioma-related genes obtained from the Comparative Toxicogenomics Database were predicted to be regulated by 15 miRNAs via the miRwalk 2.0 database [22]. Zhu and colleagues [23] provided a framework of workflow for potential therapeutic drug discovery and predicted 10 potential drugs for glioblastoma therapy. Effectiveness of drugs has to be tested on the experimental models [24]. Chemotherapy for glioblastoma remains ineffective due to insufficient penetration of therapeutic agents into the tumor [25]. Recent works show limits on the isotope distribution in tissues in a glioma xenograft animal model [26].

An analysis of the literature (PubMed) showed continued growth of publications on the topic – there are about 54 thousand publications up to date. Integration of large biological databases and re-analysis, combination of different tools allows keeping current structure of the disease description and annotation, as we have discussed at the international conferences series on computational genomics [27, 28]. Network analysis of interacting genes shows existing gene clusters in the network, and potential new targets for therapy [29]. Overall, such application of available bioinformatics tools for gene list analysis could serve as a standard for education [30, 31], allows to reveal novel features for cancer diseases. The network analysis complements analysis of microarray gene expression and transcriptomics data for glioblastoma research [7, 32]. The network modelling using the described tools proved to be fruitful in other complex disease analysis, such as Parkinson disease, mental disorders, and diabetes [33, 34].

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**Author contribution:** NVG planned the study, NGO and AID did the computation and statistical analysis, NYO described disease network and drug target search, YLO planned the work and wrote the manuscript. All the authors have accepted responsibility for the entire content of this manuscript and approved its submission.

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## References

1. Ohgaki H, Kleihues P. Genetic pathways to primary and secondary glioblastoma. *Am J Pathol* 2007;170:1445–53.
2. Bowman RL, Wang Q, Carro A, Verhaak RG, Squatrito M. GlioVis data portal for visualization and analysis of brain tumor expression datasets. *Neuro Oncol* 2017;19:139–41.
3. McGranahan T, Li G, Nagpal S. History and current state of immunotherapy in glioma and brain metastasis. *Ther Adv Med Oncol* 2017;9:347–68.
4. Franceschi S, Civita P, Pasqualetti F, Lessi F, Modena M, Barachini S, et al. Multiregional sequencing of IDH-WT glioblastoma reveals high genetic heterogeneity and a dynamic evolutionary history. *Cancers* 2021;13:2044.
5. Miller KD, Ostrom QT, Kruchko C, Patil N, Tihan T, Cioffi G, et al. Brain and other central nervous system tumor statistics. *CA Cancer J Clin* 2021;71:381–406.
6. Stavrovskaya AA, Shushanov SS, Rybalkina EY. Problems of glioblastoma multiforme drug resistance. *Biochemistry* 2016;81:91–100.
7. Babenko VN, Gubanova NV, Bragin AO, Chadaeva IV, Vasiliev GV, Medvedeva IV, et al. Computer analysis of glioma transcriptome profiling: alternative splicing events. *J Integr Bioinform* 2017;14:20170022.
8. Kovalev SS, Lieberfarb EY, Gubanova NV, Bragin AO, Galieva AG, Tsukanov AV, et al. Computer analysis of gene alternative splicing in glioma cell cultures by RNA-seq data. *Vestnik NSU. Series: Information Technologies* 2018;16:22–36.
9. Amberger JS, Bocchini CA, Scott AF, Hamosh A. OMIM.org: leveraging knowledge across phenotype-gene relationships. *Nucleic Acids Res* 2019;47:D1038–43.
10. Dennis G Jr, Sherman BT, Hosack DA, Yang J, Gao W, Lane HC, et al. DAVID: database for annotation, visualization, and integrated discovery. *Genome Biol* 2003;4:3.
11. Mi H, Muruganujan A, Casagrande JT, Thomas PD. Large-scale gene function analysis with the PANTHER classification system. *Nat Protoc* 2013;8:1551–66.
12. Talbott H, Hou X, Qiu F, Zhang P, Guda C, Yu F, et al. Transcriptomic and bioinformatics analysis of the early time-course of the response to prostaglandin F2 alpha in the bovine corpus luteum. *Data Brief* 2017;14:695–706.
13. Harel A, Inger A, Stelzer G, Strichman-Almashanu L, Dalah I, Safran M, et al. GIFTS: annotation landscape analysis with GeneCards. *BMC Bioinf* 2009;10:348.
14. Chen J, Bardes EE, Aronow BJ, Jegga AG. ToppGene suite for gene list enrichment analysis and candidate gene prioritization. *Nucleic Acids Res* 2009;37:W305–11.
15. Jiang Z, Shi Y, Zhao W, Zhang Y, Xie Y, Zhang B, et al. Development of an immune-related prognostic index associated with glioblastoma. *Front Neurol* 2021;12:610797.
16. Li C, Pu B, Gu L, Zhang M, Shen H, Yuan Y, et al. Identification of key modules and hub genes in glioblastoma multiforme based on co-expression network analysis. *FEBS Open Bio* 2021;11:833–50.
17. Xu B. Prediction and analysis of hub genes between glioblastoma and low-grade glioma using bioinformatics analysis. *Medicine* 2021;100:e23513.
18. Yang J, Yang Q. Identification of core genes and screening of potential targets in glioblastoma multiforme by integrated bioinformatic analysis. *Front Oncol* 2021;10:615976.
19. Corso CD, Bindra RS. Success and failures of combined modalities in glioblastoma multiforme: old problems and new directions. *Semin Radiat Oncol* 2016;26:281–98.
20. Uddin MS, Mamun AA, Alghamdi BS, Tewari D, Jeandet P, Sarwar MS, et al. Epigenetics of glioblastoma multiforme: from molecular mechanisms to therapeutic approaches. *Semin Cancer Biol* 2020;S1044-579X:30275–3.
21. Cen L, Liu R, Liu W, Li Q, Cui H. Competing endogenous RNA networks in glioma. *Front Genet* 2021;12:675498.
22. Wei B, Wang L, Zhao J. Circular RNA hsa\_circ\_0005114-miR-142-3p/miR-590-5p-adenomatous polyposis coli protein axis as a potential target for treatment of glioma. *Oncol Lett* 2021;21:58.
23. Zhu B, Mao X, Man Y. Potential drug prediction of glioblastoma based on drug perturbation-induced gene expression signatures. *BioMed Res Int* 2021;2021:6659701.
24. Raj D, Agrawal P, Gaitsch H, Wicks E, Tyler B. Pharmacological strategies for improving the prognosis of glioblastoma. *Expert Opin Pharmacother* 2021;22:2019–31.
25. Ahmed T, Liu FF, He C, Abbasi AZ, Cai P, Rauth AM, et al. Optimizing the design of blood-brain barrier-penetrating polymer-lipid-hybrid nanoparticles for delivering anticancer drugs to glioblastoma. *Pharm Res* 2021. <https://doi.org/10.1007/s11095-021-03122-9>.
26. Gubanova NV, Tsygankova AR, Zavjalov EL, Romashchenko AV, Orlov YL. Biodistribution of 10B in glioma orthotopic xenograft mouse model after injection of L-para-boronophenylalanine and sodium borocaptate. *Biomedicines* 2021;9:722.
27. Orlov YL, Baranova AV, Hofestädt R, Kolchanov NA. Computational genomics at BGRS\SB-2016: introductory note. *BMC Genom* 2016;17:996.
28. Orlov YL, Baranova AV. Editorial: bioinformatics of genome regulation and systems biology. *Front Genet* 2020;11:625.
29. Orlov YL, Anashkina AA, Klimontov VV, Baranova AV. Medical genetics, genomics and bioinformatics aid in understanding molecular mechanisms of human diseases. *Int J Mol Sci* 2021;22:9962.

30. Babenko VN, Bragin AO, Spitsina AM, Chadaeva IV, Galieva ER, Orlova GV, et al. Analysis of differential gene expression by RNA-seq data in brain areas of laboratory animals. *J Integr Bioinform* 2016;13:292.
31. Chen M, Harrison A, Shanahan H, Orlov Y. Biological big bytes: integrative analysis of large biological datasets. *J Integr Bioinform* 2017;14:20170052.
32. Gnanavel M, Murugesan A, Konda Mani S, Yli-Harja O, Kandhavelu M. Identifying the miRNA signature association with aging-related senescence in glioblastoma. *Int J Mol Sci* 2021;22:517.
33. Orlov YL, Galieva AG, Orlova NG, Ivanova EN, Mozyleva YA, Anashkina AA. Rekonstruktsiia gennoĭ seti bolezni Parkinsona dlia poiska genov-misheneĭ [reconstruction of gene network associated with Parkinson disease for gene targets search]. *Biomed Khim* 2021;67:222–30.
34. Saik OV, Klimontov VV. Bioinformatic reconstruction and analysis of gene networks related to glucose variability in diabetes and its complications. *Int J Mol Sci* 2020;21:8691.

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