Analysis of the gene-protein interaction network in glioma

C. Zhou[1]*, W.J. Teng[2]*, J. Zhuang[1], H.L. Liu[3], S.F. Tang[1], X.J. Cao[4], B.N. Qin[4], C.C. Wang[4] and C.G. Sun[1]

[1]Cancer Center, WeiFang Traditional Chinese Hospital, Weifang, Shandong, China
[2]Shangai Municipal Hospital of Traditional Chinese Medicine,
Affiliated to Shangai Univerity of Traditional Chinese Medicine, Shanghai, China
[3]Guang'anmen Hospital, China Academy of TCM, Beijing, China
[4]Shandong University of Chinese Traditional Medicine, Jinan, Shandong, China

*These authors contributed equally to this study.
Corresponding author: C.G. Sun
E-mail: zhongliuyike@163.com

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ABSTRACT. Glioma is the most aggressive type of brain tumor. Great progress has been achieved in glioma treatment, but the protein-protein interaction networks underlining glioma are poorly understood. We identified the protein-protein interaction network for glioma based on gene expression and predicted biological pathways underlying the molecular complexes in the network. Genes involved in glioma were selected from the Online Mendelian Inheritance in Man (OMIM) database. A literature search was performed using the Agilent Literature Search plugin, and Cytoscape was used to establish a protein-protein interaction network. The molecular complexes in the network were detected using the Clusterviz plugin, and pathway enrichment of molecular complexes was performed using DAVID online. There were 378 glioma genes in the OMIM database. The protein-protein interaction network in glioma contained 1814 nodes, 6471 edges, and 8 molecular complexes. There were 17 pathways (false discovery rate <1), which were related to cytokine-cytokine receptor interaction, Toll-like receptor signaling pathway, chemokine signaling pathway, oocyte meiosis, progesterone-mediated oocyte maturation, transmembrane transport of small molecules, metabolism of amino acids, and notch signaling pathway, among others. Our results provide a bioinformatic

foundation for further studies of the mechanisms of glioma.

Key words: Molecular complexes; Protein-protein interaction networks; Glioma; Pathways

INTRODUCTION

Gliomas, which are the most aggressive type of brain tumor, show high morbidity, a high recurrence rate, and high mortality. Glioma accounts for approximately 30% of brain and central nervous system tumors and 80% of malignant brain tumors (Goodenberger and Jenkins, 2012; Shao et al., 2014). Survival of gliomas depends on the tumor type and malignancy grade (Constantin et al., 2012). According to World Health Organization standards, gliomas are classified into 4 malignant grades. Grade I-II gliomas can be treated with surgery and chemoradiotherapy, and are generally associated with a survival time of 5-10 years. The most lethal is grade IV glioblastoma, with a median survival of only 15 months (Wen and Kesari, 2008) because of the inefficacy of surgery and chemoradiotherapy. In addition, over 50% of low-grade gliomas undergo malignant transformation into high-grade gliomas within 5-10 years during recurrence (Dell'Albani, 2008). The prognosis of glioma, particularly high-grade (III-IV) glioma, is typically poor. Glioblastoma multiforme is the most predominant and most malignant form of glioma. Despite the high incidence of glioma, the etiology of this disease remains largely unknown.

Recently, the development of high-throughput experimental strategies has facilitated the study of characteristics underlying cancer progression. Several studies have investigated the gene expression signature in glioma patients (Ideker and Sharan, 2008; Zhao et al., 2008). Previous studies have mainly used regression or variance analysis to identify deregulated genes that may contribute to the glioma pathomechanism. However, these methods cannot address other array-specific factors, such as various background biological and environmental factors. Identifying the molecular characteristics of glioma patients may increase the understanding of the mechanism underlying glioma.

Because of the large number of targets involved in gliomas, the gene-protein network cannot be constructed using standard experiments. Numerous previous studies have examined gliomas, indicating that the gene-protein network can be constructed using a literature-mining method (Yang et al., 2009; Giacomelli and Covani, 2010). Construction through literature mining involves bioinformatics and computer science, among other fields, to sort and analyze existing data based on gene-protein interaction relationships to construct a regulation network of biological molecules in a cell. This method is important for identifying regulators and network-stable, therefore it has great application space (Strogatz, 2001; Pospisil et al., 2006).

To further examine glioma on the gene-protein network level, we used the human Mendel database to identify confirmed genes associated with gliomas, and then used Cytoscape application software to establish gliomas based on biological function gene-protein interaction networks. Subsequently, we determined topological properties and conducted modularity analysis of the network, and enriched the functional analysis and functional modules using DAVID software. We identified and analyzed key genes and signaling pathways in the network to predict the pathogenic site of the disease and the molecular mechanisms of gliomas.

MATERIAL AND METHODS

Data acquisition

On April 5, 2014 after searched "glioma" on the OMIM home page (http://www.ncbi.nlm.

nih.gov/omim), gene information associated with gliomas was screened to remove duplicate genes (Amberger et al., 2009).

Construction of gene-protein interaction networks

Glioma-associated genes were searched in the Cytoscape 2.8.2 plug-in Agilent Literature Search 2.7.7 (USA Agilent Technologies, Santa Clara, CA, USA) and in Pubmed (Vailaya et al., 2005). False-positive interaction information was removed from the results. Next, gene/protein interaction relationships were read in Cytoscape 2.8.2 and visualized (Shannon et al., 2003).

Network analysis

The MCOMD algorithm in Cytoscape 2.8.2 web analytics plug-in Clusterviz of 1.2 was used for correlation analysis to construct biological networks (Saito et al., 2012). By analyzing the network structure, proteins were grouped to form molecular compounds in the entire network and were viewed in Cytoscape based on the correlation integral value. The areas with integral values higher than 3 were regarded as molecular compounds. The gene/protein names contained in the molecular compounds were submitted to The Database for Annotation, Visualization, and Integrated Discovery (Huang et al., 2009). Using the Kyoko Encyclopedia of Genes and Genomes (KEGG) Database, biological pathways involved in glioma heredity were identified.

Main outcome measures

Protein networks were constructed based on glioma-related genes, nodes (proteins) and edges (interaction between), molecular complexes in the network and its associated interaction points, and nodes (protein) and the edges (interaction between) to analyze the biological pathways involved in the molecular complexes.

RESULTS

Glioma-related genes in OMIM

Through OMIM database retrieval, we identified 378 genes related to glioma, as shown in Table 1.

Protein interaction networks

The 378 glioma-related genes identified were constructed into a network diagram with 1814 nodes (proteins) and 6471 edges. As shown in Figure 1, the triangles represent OMIM genetic disease-related proteins, while the diamonds represent proteins obtained from text mining.

Network topology attribute analysis

Network topology attribute analysis revealed that the connectivity of nodes in the network (the number of nodes in the network) had a descending distribution; as the edges connected to the node increased, the number of nodes decreased. Thus, the gene-protein interaction networks are scale-free networks (Burkard et al., 2010). We found that the degree of nodes in the network greater than or equal to 50 corresponded to a sharp reduction in the number of nodes (Figure 2).

Table 1. Glioma related genes in OMIM.

ID	GENES	ID	GENES	ID	GENES	ID	GENES	ID	GENES
1	ABCB1	77	CTLA4	153	HOXD9	229	MRC2	305	REV3L
2	ABCC3	78	CTNNB1	154	IDH1	230	MSI1	306	RFX1
3	ABCG2	79	CTNNBIP1	155	IDH2	231	MST1R	307	RICTOR
4	ACSL5	80	CX3CR1	156	IDH3B	232	MTHFR	308	RTN4
5	ADAM17	81	CXCR4	157	IGF1	233	MYB	309	S100A13
6	ADAM22	82	CYP1B1	158	IGF1R	234	MYBL1	310	SCG5
7	ADAM3A	83	CYR61	159	IKBKB	235	MYC	311	SEMA3G
8	ADAM8	84	DKK1	160	IL10	236	NANOG	312	SERPINE1
9	AHR	85	DLK1	161	IL16	237	NCOR1	313	SETD2
10	AJAP1	86	DLL4	162	IL24	238	NCOR2	314	SH3GL1
11	AKT1	87	DMBT1	163	INA	239	NDRG2	315	SH3GL3
12	AKT2	88	DNAJA3	164	ING1	240	NDRG4	316	SIRT2
13	ALCAM	89	DPP4	165	ING4	241	NDUFA13	317	SLC16A4
14	ALK	90	DVL2	166	ITGAV	242	NEDD4L	318	SLC22A17
15	ALOX12	91	EBAG9	167	JAK2	243	NEO1	319	SLC38A3
16	ALOX15	92	EFEMP1	168	KCNN4	244	NES	320	SLC3A2
17	ALOX5	93	EFNA1	169	KDM1A	245	NEWENTRY	321	SLC5A8
18	ANGPT2	94	EGF	170	KEAP1	246	NF1	322	SLC7A11
19	ANGPTL4	95	EGFR	171	KIAA1549	247	NFKB1	323	SLC7A5
20	APEX1	96	EMC10	172	KIF14	248	NGF	324	SLC9A3R1
21	APLN	97	EMP3	173	KIT	249	NGFR	325	SLIT2
22	APLNR	98	ENTPD1	174	KLF6	250	NKIRAS1	326	SMAD1
23	AQP1	99	EPHA2	175	KLF8	251	NME1	327	SNAI2
24	AQP4	100	ERCC1	176	KPNA2	252	NOTCH1	328	SOCS3
25	ASPM	101	ERCC2	177	L1CAM	253	NOTCH2	329	SOX10
26	ATG5	102	ERCC5	178	LAMC2	254	NR2E1	330	SOX2
27	ATM	103	ERCC6	179	LCN2	255	NRG1	331	SOX6
28	ATRX	104	ESM1	180	LETMD1	256	NRP2	332	SOX9
29	AURKA	105	F2	181	LGALS1	257	NT5E	333	SP1
30	AURKB	106	FAS	182	LGALS3	258	NUMBL	334	SP3
31	BCAN	107	FAT1	183	LGI1	259	OLIG2	335	SPDYA
32	BCL2	108	FBXW7	184	LIG4	260	OSM	336	SPP1
33	BDNF	109	FGF2	185	LMX1A	261	PAX6	337	SPRY2
34	BECN1	110	FOCAD	186	LNX1	262	PCDHGA11	338	STAT3
35	BIRC5	111	FOSL1	187	LOC652614	263	PCSK6	339	Symbol
36	BMI1	112	FRAT1	188	LRRC4	264	PDCD4	340	TAX1BP3
37	BMP2	113	FUBP1	189	LRRN2	265	PDCD5	341	TERF1
38	BMP4	114	GDF15	190	MAGED1	266	PDGFA	342	TERT
39	BMPR1B	115	GEMIN2	191	MAPK14	267	PDGFB	343	TET1
40	BNIP3	116	GFAP	192	MAPK3	268	PDGFRA	344	TET2
41	BNIP3L	117	GFI1	193	MARK4	269	PEBP1	345	TGFB1
42	BRAF	118	GOLPH3	194	MBD4	270	PER1	346	TGFB2
43	BSG	119	GPC1	195	MCTS1	271	PER2	347	THBS1
44	CADM1	120	GPNMB	196	MDK	272	PIN1	348	THY1
45	CASP3	121	GPR26	197	MDM2	273	PIWIL1	349	TIMD4
46	CCK	122	GPRASP1	198	MDM4	274	PKM	350	TIMP3
47	CCL20	123	GRIA1	199	MET	275	PLAUR	351	TK1
48	CCL3	124	GRIA2	200	MGMT	276	POLK	352	TKTL1
49	CCL7	125	GSK3B	201	MIF	277	POU5F1	353	TNC
50	CCND1	126	GSTM1	202	MIIP	278	PPM1D	354	TNFRSF10B
51	CCR6	127	GSTP1	203	MIR106B	279	PPME1	355	TNFRSF11A
52	CD24	128	GSTT1	204	MIR137	280	PRAF2	356	TNFSF10
53	CD274	129	GUCY1A3	205	MIR182	281	PRKAA2	357	TOP2A
54	CD40	130	H3F3A	206	MIR183	282	PROM1	358	TP53
55	CD44	131	HDAC1	207	MIR196A1	283	PROX1	359	TRAF1
56	CD74	132	HDAC2	208	MIR196B	284	PTBP2	360	TRAF2
57	CDC25A	133	HDAC3	209	MIR203A	285	PTEN	361	TRIM11
58	CDH1	134	HDGF	210	MIR20A	286	PTGER2	362	TRIM3
59	CDH11	135	HEXA	211	MIR21	287	PTGES	363	TWIST1
60	CDH2	136	HEXB	212	MIR218-1	288	PTGES2	364	VCAN
61	CDK1	137	HGF	213	MIR221	289	PTGES3	365	VEGFA
62	CDKN1B	138	HIF1A	214	MIR222	290	PTGS1	366	WDR11

Continued on next page

Table 1. Glioma related genes in OMIM.

ID	GENES	ID	GENES	ID	GENES	ID	GENES	ID	GENES
63	CDKN2A	139	HJURP	215	MIR27B	291	PTGS2	367	WNT1
64	CHEK2	140	HK2	216	MIR30A	292	PTK2	368	WNT2
65	CHI3L1	141	HLA-B	217	MIR335	293	PTP4A3	369	WNT5A
66	CHN2	142	HLA-C	218	MIR372	294	PVR	370	WRN
67	CIC	143	HLA-DQB1	219	MIR375	295	RAC2	371	WT1
68	CLCN3	144	HLA-DRB1	220	MIR383	296	RASL10A	372	WWTR1
69	CLIC1	145	HLA-DRB3	221	MIR410	297	RASSF10	373	XBP1
70	CNR1	146	HMG20B	222	MIR452	298	RB1	374	XRCC1
71	CNR2	147	HMGA1	223	MIR483	299	RBL2	375	XRCC3
72	CNTFR	148	HMGN5	224	MKI67	300	RBP1	376	XRCC4
73	COL18A1	149	HNRNPA1	225	MMP14	301	RBPJ	377	YY1
74	CRABP2	150	HNRNPA2B1	226	MMP2	302	RECQL	378	ZAR1
75	CSF2	151	HNRNPH1	227	MMP3	303	REG4		
76	CTGF	152	HOXA9	228	MMP9	304	RELA		

Therefore, we regarded the nodes which connectivity is greater than or equal to 50 as key nodes (hub). Key nodes included akt1, tnfsf13, tp53, ephb2, pik3ca, mapk3, mapk14, il6, cdkn1a, vegfa, mapk8, stat3, egfr, myc, bcl2, cdkn2a, apc, ptgs2, pten, hcc, ccl2, and ervk2.

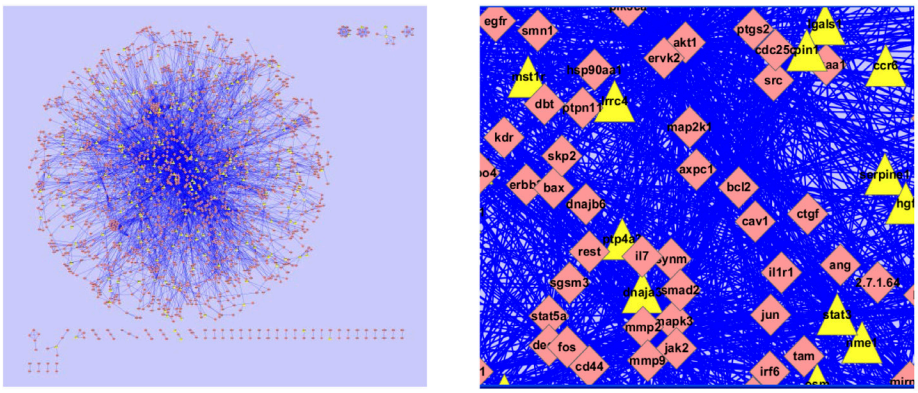

Figure 1. Network map of glioma protein interaction (overall + partial).

Detection of molecular complexes

Through MCOMD algorithm analysis, we identified 8 molecular complexes whose correlation integral values were higher than 3 (Figure 3).

Molecular complex pathway enrichment

The 8 names of protein molecule complexes were searched online to identify the relevant pathways (Table 2). Using hypergeometric distribution test software (DAVID) (Bader and Hogue, 2003) (parameters: count = 2, EASE = 0.1, "species and background" choosing "*Homo sapiens*"), we conducted function analysis of modules contained in the 2 networks. According to the pathway annotations, we identified biological signaling pathways (Ashburner et al., 2000) corresponding to the modules and sorted false-discovery rate values of biological processes, considering a false-discovery rate ≤ 1 as a statistically significant difference in the biological process (Burkard et al., 2010).

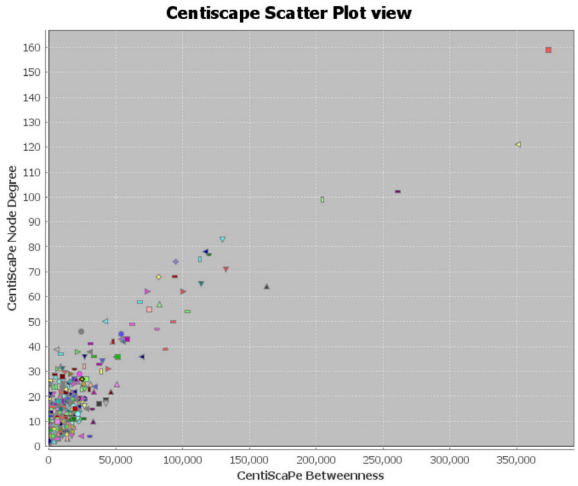

Figure 2. Connectivity degree of each node and betweenness (betweenness) comparison (horizontal axis represents betweenness, and the ordinate represents the connectivity degree. The graphic in the table represents each node in the network). The connectivity (number of nodes in the network) of nodes in the network obeys descending distribution, while the connectivity is greater than or equal to 50, and the number of nodes corresponds to a sharp decrease.

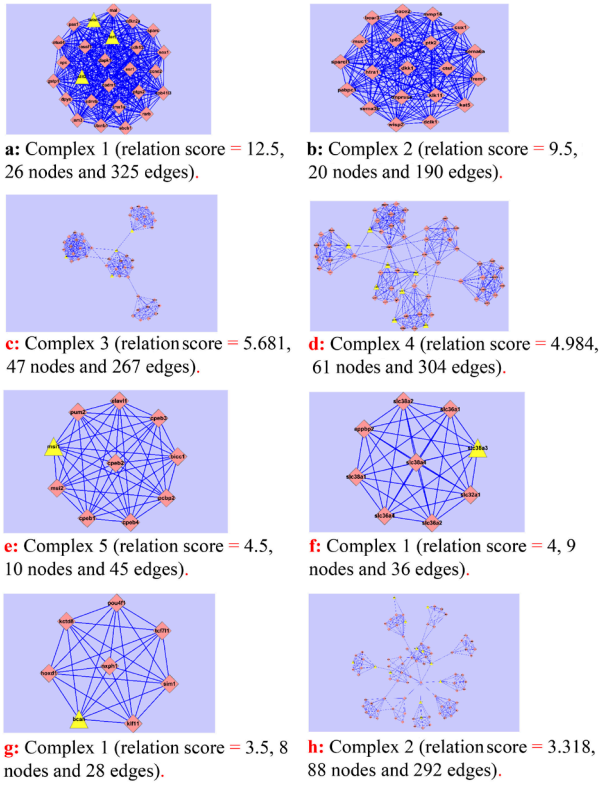

Figure 3. Molecular complexes obtained by MCOMD algorithm analysis.

Table 2. Enrichment of pathways related to molecular complexes.

Category	Term	P value	Genes	FDR
Complex 1				
KEGG_PATHWAY	hsa05200:Pathways in cancer	1,79E-5	CDKN2A, PTGS2, RASSF1, RARB, GSTP1, DAPK1, CTNNB1, APC	0,016
Complex 3				
KEGG_PATHWAY	hsa04060:Cytokine-cytokine receptor interaction	5,42E-8	CCL1,CXCL1,CSF3,IL17A,CCL3,IL6,IL23A,CCL2,TNFRSF10D,CCL5,CCL4, CXCL10	5,35E-5
BBID	33.GProt-coupled_Rec_T_Cell_med_Inflamm	5,20E-6	CCL1, CCL3, CCL2, CCL5, CXCL10	0,005
BBID	34.Chemokines_in_EAE	5,20E-6	CCL1, CCL3, CCL2, CCL5, CXCL10	0,005
KEGG_PATHWAY	hsa05213:Endometrial cancer	1,66E-4	TP53,MLH1,CDH1,PTEN, AXIN1	0,164
KEGG_PATHWAY	hsa04620:Toll-like receptor signaling pathway	1,91E-4	FOS,CCL3,IL6,CCL5,CCL4, CXCL10	0,189
KEGG_PATHWAY	hsa05200:Pathways in cancer	2,73E-4	FOS,CDKN1A,IL6, TP53, MLH1,CDH1,CCNA1, PTEN, AXIN1	0,269
KEGG_PATHWAY	hsa04062:Chemokine signaling pathway	4,37E-4	CCL1,CXCL1,CCL3,CCL2,CCL5, CCL4, CXCL10	0,431
PANTHER_PATHWAY	P04398:p53 pathway feedback loops 2	9,13E-4	CDKN1A, TP53, CCNA1, PTEN, TP73	0,693
BBID	109.Chemokine_families	9,73E-4	CCL1,CCL3,CCL2,CCL5, CCL4, CXCL10	0,871
Complex 4				
KEGG_PATHWAY	hsa04114:Oocyte meiosis	7,34E-5	CCNB1,CCNB2, MAPK3, BUB1, CDC20, AURKA, CDC25C	0,077
PANTHER_PATHWAY	P00054:Toll receptor signaling pathway	2,20E-4	IRAK3, MYD88, MAPK3, TLR2, MAPK8, TLR4	0,199
KEGG_PATHWAY	hsa04914:Progesterone-mediated oocyte maturation	2,33E-4	CCNB1,CCNB2, MAPK3, BUB1, MAPK8, CDC25C	0,245
Complex 6				
REACTOME_PATHWAY	REACT_15518:Transmembrane transport of small molecules	2,26E-10	SLC36A1,SLC38A4,SLC38A3,SLC36A2, SLC38A2, SLC38A1	7,11E-8
REACTOME_PATHWAY	REACT_13:Metabolism of amino acids	2,39E-7	SLC36A1,SLC38A4,SLC38A3,SLC36A2, SLC38A2, SLC38A1	7,54E-5
Complex 8				
PANTHER_PATHWAY	P00045:Notch signaling pathway	5,16E-5	NOTCH3,HES1,NOTCH2,NOTCH1,HES5,HEY2, NOTCH4	0,051
REACTOME_PATHWAY	REACT_299:Signaling by Notch	8,19E-4	NOTCH3,NOTCH2,NOTCH1, NOTCH4	0,689

DISCUSSION

Based on the 378 genes identified by OMIM, we constructed a glioma protein interaction network containing 1814 nodes (proteins) with 1830 edges (interaction). We next examined whether the network could describe the molecular regulation of glioma development. According to the existing literature, the anti-apoptotic protein B-cell lymphoma 2 (BCL2) has been implicated in the pathogenesis of glioma. BCL2A1 is a potential biomarker that influences preoperative seizure occurrence and postoperative seizure control in patients with low-grade gliomas (You et al., 2013; Li et al., 2014). TP53 is a pivotal gene frequently mutated in diffuse gliomas and particularly in astrocytic tumors (Takami et al., 2014); CCL2 was among the first identified in gliomas, and it is overexpressed in colon carcinomas. Its silencing inhibits colon cancer cell proliferation or increases the sensitivity to apoptotic stimuli of glioma cells, suggesting an oncogenic role (Carrillo-de Sauvage et al., 2012). Increasing evidence suggests that interplay between the Wnt/β-catenin and phosphoinositide 3-kinase /AKT signaling cascades are involved in tumor development and progression. Chen found that the expression levels of AKT1 in glioma cells were significantly correlated with the transcriptional activity of β-catenin (Pan et al., 2012). Mori found that the adenomatous polypopsis coli mutations in brain tumors were associated with the pathogenesis of one feature of Turcot syndrome (Siesjö et al., 1996); Zadeh et al., (2007) found that CDKN2A-deleted patients were younger than CDKN2A non-deleted patients in malignant gliomas in Iranian patients. Faulkner et al. found that neither epidermal growth factor receptor vIII (EGFRvIII) or EGFR were predictive of overall survival in their cohort; 49% of glioblastoma cases showed EGFR alterations, including 31% with EGFRvIII, and thus EGFR and EGFRvIII can be used as therapeutic biomarkers of glioblastoma (Cherry and Stella, 2014). The microenvironment of glioblastoma contains high levels of inflammatory cytokine interleukin 6, which contributes to tumor progression and invasion (Gurgis et al., 2014). Among the factors and pathways implicated in glioma development and growth, the kinases phosphoinositide 3-kinase and mitogen-activated protein kinase are among the most studied (Daniel et al., 2014). Annibali et al., (2014) found that Myc inhibition reduces proliferation, increases apoptosis, and, remarkably, elicits the formation of multinucleated cells that then arrest or die by mitotic catastrophe, revealing a new role for Myc in the proficient division of glioma cells. Some results confirmed that PIK3CA mutations occurred in a significant number of human glioblastomas, making it a promising target for therapy, particularly for primary glioblastomas (Weber et al., 2011; Derakhshandeh-Peykar et al., 2012). A meta-analysis by Xiao et al. (2012) provided direct and strong evidences that mutations in the PTEN gene were correlated with the poor prognosis of glioma patients (Han et al., 2014). The PTGS2, EGFR, and various types of EGFR ligands are highly expressed in human gliomas and other cancers and are involved in tumor progression (Dancey, 2004). In gliomas, STAT3 can play tumor-suppressive or oncogenic roles depending on the tumor genetic background of the patient, but the target genes are largely unknown (Kruczyk et al., 2014).

These are relational pathogenesis of glioma. We constructed a network that comprises these genes or proteins The network appeared to be reliable and can be used to describe the interactions between molecules related to glioma.

Because the network is very large, we used the MCOMD algorithm to evaluate the network's regional integration using the correlation integral. The correlation integral describes proteins associated with the degree within the region. Proteins in the same molecular complexes generally have the same biological function, and thus unknown gene functions or new molecular functional

groups can be identified. Eight molecular complexes showed correlation integrals of greater than 3. DAVID is not only extensive in gene annotation in different species, but also enriched with biological information for single genes. The protein molecule biological pathways of complexes 2, 5, and 7 are not existent, which may have two explanations. First, although the relevance of these molecular complex correlation integrals was higher, a protein with similar biological functions could not be confirmed. Second, existing studies have not revealed the biological pathways involved. Molecular complexes 1, 3, 4, 6, and8 were found to be involved many biological pathways. Table 2 shows its complexity, for which there was 1 biological pathway whose false-discovery rate < 1 in molecular complex 1, 9 pathways in molecular complex 3, 3 pathways in molecular complex 4, 2 pathways in molecular complex 6, and 2 in molecular complex 8.

Molecular complex 3 was predicted to be related to the cytokine-cytokine receptor interaction, Toll-like receptor signaling pathway, chemokine signaling pathway, p53 pathway feedback loops 2, and endometrial cancer. A previous study indicated that chemokine and chemokine receptor expression by tumor cells contributed to tumor growth and angiogenesis and thus these factors may be tumor markers and have crucial impacts on therapeutic interventions (Razmkhah et al., 2014).

There is increasing evidence that cytokines play roles in these processes. Cytokines directly influence the progression of malignant glioma, promoting or suppressing tumor progression (Zhou et al., 2014). Thus, the cytokine-cytokine receptor interaction pathway (CCL1, CXCL1, CCL3, CCL2, CCL5, CCL4, and CXCL10) and chemokine signaling pathway (CCL1, CXCL1, CSF3, IL17A, CCL3, IL6, IL23A, CCL2, TNFRSF10D, CCL5, CCL4, and CXCL10) require further analysis.

In addition to the other molecular complexes, progesterone-mediated oocyte maturation is related to the pathogenesis of glioma (Hassanzadeh and Arbabi, 2012); high notch pathway activation predicts a response to γ secretase inhibitors in the proneural subtype of glioma tumor-initiating cells (Saito et al., 2012). The genes involved in this signaling pathway may provide a basis for the molecular therapy to treat glioma. Glioma is not simply controlled by a particular gene or signaling pathway, but by a complex network system coordinately regulated and consisting of a variety of signaling pathways and multiple genes. In the signaling network, it is likely that there are some “key regulatory points”.

Our study extended the original method used for glioma analysis from a single factor to a systematic, overall point perspective by constructing a network. Our results may provide new drug development guidance for treating glioma on the gene-protein network level. We used Cytoscape 2.82 for data mining and module analysis based on the OMIM database, and a small number of genes were identified because a single source of data was used, and because of the software features. Our constructed gene-protein interaction network did not reflect the regulatory relationship between the genes and proteins, and thus, further analysis is required.

Conflicts of Interest

The authors declare no conflict of interest.

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