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Identification of key genes in glioblastoma-associated stromal cells using bioinformatics analysis

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Abstract. The aim of the present study was to identify key genes and pathways in glioblastoma-associated stromal cells (GASCs) using bioinformatics. The expression profile of microarray GSE24100 was obtained from the Gene Expression Omnibus database, which included the expression profile of 4 GASC samples and 3 control stromal cell samples. Differentially expressed genes (DEGs) were identified using limma software in R language, and Gene Ontology and Kyoto Encyclopedia of Genes and Genomes pathway analysis of DEGs were performed using the Database for Annotation, Visualization and Integrated Discovery software. In addition, a protein-protein interaction (PPI) network was constructed. Subsequently, a sub-network was constructed to obtain additional information on genes identified in the PPI network using CFinder software. In total, 502 DEGs were identified in GASCs, including 331 upregulated genes and 171 downregulated genes. Cyclin-dependent kinase 1 (*CDK1*), cyclin A2, mitotic checkpoint serine/threonine kinase (*BUB1*), cell division cycle 20 (*CDC20*), polo-like kinase 1 (*PLK1*), and transcription factor breast cancer 1, early onset (*BRCA1*) were identified from the PPI network, and sub-networks revealed these genes as hub genes that were involved in significant pathways, including mitotic, cell cycle and p53 signaling pathways. In conclusion, *CDK1*, *BUB1*, *CDC20*, *PLK1* and *BRCA1* may be key genes that are involved in significant pathways associated with glioblastoma. This information may lead to the identification of the mechanism of glioblastoma tumorigenesis.

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Introduction

Glioblastoma is the most common and fatal malignant primary brain tumor in adults, with an incidence rate of 2.8 cases per 100,000 individuals per year and a perioperative mortality rate of 2.2% (1). It is estimated that 44,500 new cases of primary brain tumors were diagnosed in the USA in 2005, of which glioblastoma accounted for ~20% (2). The traditional treatment method is surgical resection combined with fractionated radiotherapy and adjuvant chemotherapy with temozolomide (3). However, despite advances in surgical techniques, postoperative supportive care, radiation and adjuvant systemic chemotherapy, the 5-year survival rate of glioblastoma remains at <10% (4). The disease generally recurs at the resection margin, and the median survival time is ~14 months; extremely few patients have a long-term survival, which highlights the importance of understanding the peripheral brain tumor region (5).

Glioblastoma cells are capable of infiltrating deep into the surrounding tissue, which allows these cells to migrate for long distances. This is typical behavior of neural stem cells, from which glioblastoma cells originate (6). Previous studies have demonstrated that malignant tumors may be affected by stromal cells, and that cancer cells may be controlled by the microenvironment; it has been reported that the non-neoplastic, stromal compartment of the majority of solid cancers is involved in tumor invasion, proliferation and metastasis (7-9).

In glioblastoma, a novel population of stromal cells that surround the tumor, termed glioblastoma-associated stromal cells (GASCs), has been isolated and analyzed. These cells have a different molecular expression profile compared with that of control stromal cells derived from non-glioblastoma peripheral brain tissues (7). GASCs have been revealed to have a phenotype and functional properties similar to that of cancer-associated fibroblasts located in the stroma of carcinomas, which are known to be important in the growth and progression of tumors (10). However, the genetic information concerning this novel cell population is relatively scarce.

The aim of the present study was to analyze the transcriptome and differentially expressed genes (DEGs) in GASCs. Bioinformatics analysis was performed using the microarray

GSE24100, which is based on samples of GASCs and control stromal cells. In addition, functional and pathway enrichment analysis was performed and a protein-protein interaction (PPI) network was constructed. A sub-network was also constructed for additional analysis.

Materials and methods

Microarray data. Microarray data was obtained from the study by Clavreul *et al* (7), which is referenced in the Gene Expression Omnibus (GEO) database (www.ncbi.nlm.nih.gov/geo/) under accession number GSE24100. The microarray GSE24100 was detailed using Whole Human Genome Microarray 4x44K (catalog no., G4112F; design ID, 014850; Agilent Technologies, Santa Clara, CA, USA), and the data contains a total of 7 samples, consisting of 3 control stromal cell samples and 4 GASC samples.

Data preprocessing and DEG analysis. Using the limma model (11) on R/Bioconductor software version 2.15.1 (www.bioconductor.org/) and the microarray probe annotation profile from Brain Array Lab (brainarray.mbni.med.umich.edu/Brainarray/), the probe-level data was converted into expression measures, during which background correction, quantile normalization and probe summarization were performed. A *t*-test (12) was used to identify the significantly expressed DEGs in GASC samples, with a combination of $P<0.05$ and the $|\log_2 FC \text{ (fold change)}| >1$ used as the threshold. A heat map was generated using Z-score normalization of $\log_2$ expression values to illustrate the relative expression levels of DEGs in GASCs.

Gene ontology (GO) and pathway enrichment analysis of DEGs. GO is a commonly used approach for functional studies, and three independent ontologies (biological process, molecular function and cellular component) are accessible on the world-wide web (www.geneontology.org) (13). Kyoto Encyclopedia of Genes and Genomes (KEGG; www.genome.jp/kegg/) is a knowledge base for the systematic analysis of gene functions, which links genomic information with higher order functional information (14). In the present study, GO biological processes and KEGG pathway analysis were performed using the Database for Annotation, Visualization and Integrated Discovery; (http://david.abcc.ncifcrf.gov/home.jsp) (15) where $P<0.05$.

Functional annotation of DEGs. Functional annotation of DEGs was performed for the detection of transcription factors and tumor-associated genes. Two databases, Tumor Suppressor Gene Database version 2.0 (16) (bioinfo.mc.vanderbilt.edu/TSGene/)and Tumor Associated Gene database (last modified, 10/03/2014) (17) (www.binfo.ncku.edu.tw/TAG/GeneDoc.php) were used to screen tumor suppressor genes and oncogenes.

PPI network construction. The Search Tool for the Retrieval of Interacting Genes/Proteins (STRING; string-db.org/) database is a pre-computed global resource for the investigation and analysis of associations between proteins. The database reveals protein interactions, including experimental and predicted protein interaction information (18). In the present study, STRING was used to analyze the interactions between DEGs with the PPI required confidence (combined score, 0.9) and a PPI network was constructed. In addition, the degree of the nodes in the PPI network were calculated, and the nodes with a higher degree were deemed to be hub proteins compared with the other nodes in the PPI network.

Selection and pathway enrichment analysis of sub-network. To obtain additional information on the genes identified in the PPI network, a sub-network was constructed using CFinder (www.cfinder.org/) and Clique Percolation Method (k=3) (19). Four networks were obtained, but only one was associated with additional nodes and was additionally analyzed. GO and KEGG enrichment analysis were performed on the sub-network for the majority of nodes, and the interactions were selected using CFinder version 2.0.5 for the identification of significant pathways.

Results

DEG selection. In total, 512 transcripts were observed to be expressed differentially, including 337 upregulated transcripts and 175 downregulated transcripts, corresponding to 331 upregulated genes and 171 downregulated genes. The heat map of DEGs in GASCs and control stromal cells is shown in Fig. 1.

GO categories and KEGG pathway enrichment analysis of DEGs. Pathways obtained by KEGG enrichment are presented in Table I. According to the results, upregulated genes were primarily enriched in pathways associated with the cell cycle, DNA replication, oocyte meiosis and p53 signaling (Table IA). Downregulated genes were primarily enriched in pathways associated with adipocytokine signaling, aldosterone-regulated sodium reabsorption and nucleotide oligomerization domain-like receptor signaling (Table IB).

Several GO categories were enriched among DEGs and are shown in Table I. The upregulated genes were primarily enriched in categories associated with mitotic sister chromatid segregation, cell cycle checkpoint and DNA metabolic processes, which are all associated with cell mitosis and DNA replication (Table IC). Among downregulated genes, categories with increased transcripts included regulation of blood pressure and cellular response to mechanical stimulus (Table ID).

Functional annotation of DEGs. According to the annotation results (Table II), 11 transcriptional factors were upregulated, including breast cancer 1, early onset (*BRCA1*) and BRCA1 interacting protein C-terminal helicase 1, and 6 transcriptional factors were downregulated, including ary-hydrocarbon receptor nuclear translocator 2 and DNA damage inducible transcript 3 (*DDIT3*).

Additionally, among the upregulated genes, 9 oncogenes were identified [including cyclin A2 (*CCNA2*) and cyclin D2 (*CCND2*)] in addition to 23 tumor suppressor genes (including kinase anchoring protein 12 and BRCA1-associated RING domain 1). The downregulated genes included 3 oncogenes (such as Rho guanine nucleotide exchange factor 5 and *DDIT3)* and 10 tumor suppressor genes (such as cadherin 4,

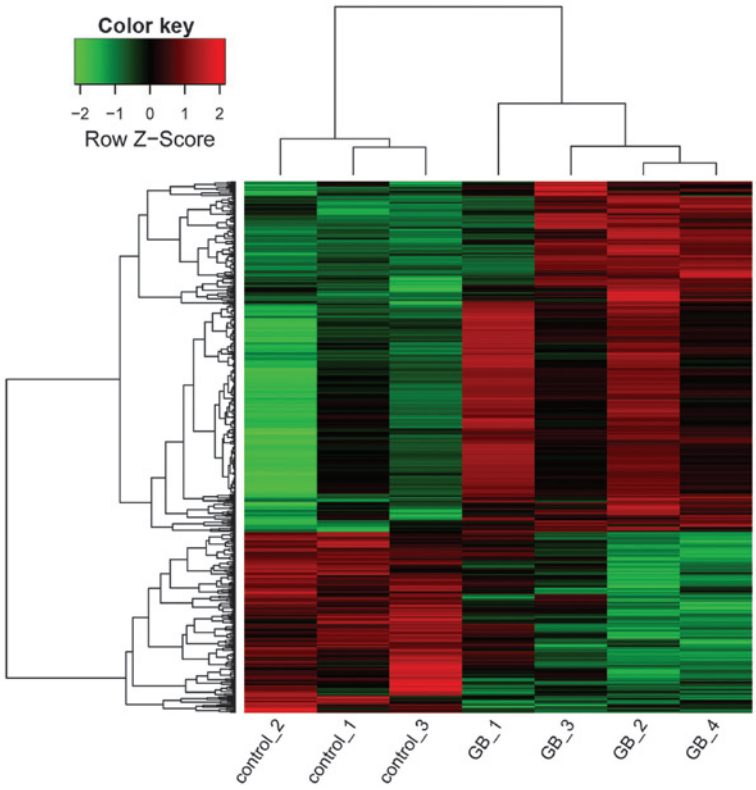

Figure 1. Heat map of gene expression in GB-associated stromal and control stromal cells. The rows indicate the expression levels of genes, and columns indicate different samples. Red, upregulated genes; green, downregulated genes. GB, glioblastoma.

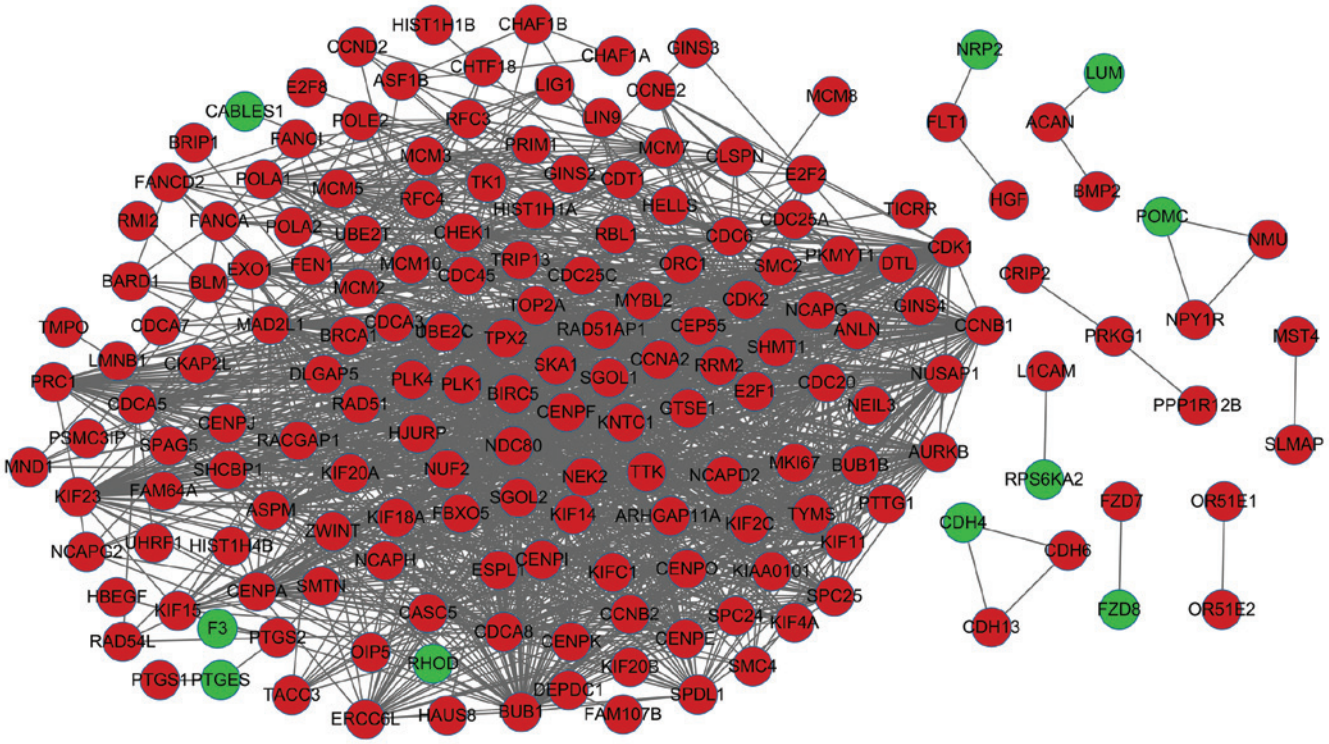

Figure 2. Protein-protein interaction network of differentially expressed genes in glioblastoma-associated stromal cells. Red nodes, upregulated genes; green nodes, downregulated genes.

type 1, R-cadherin and ATPase, aminophospholipid transporter, class I, type 8A, member 2). The details are presented in Table II.

PPI network construction. The PPI network constructed for the DEGs is shown in Fig. 2, in which 181 nodes and 1,740 interactions were identified. In this network, 8 nodes with higher

Table I. Enriched GO categories and KEGG pathway enrichment analysis of DEGs in glioblastoma-associated stromal cells.

A, KEGG analysis of upregulated DEGs

Category	Term	Biological process	Count	P-value
KEGG	4110	Cell cycle	124	0
KEGG	3030	DNA replication	36	3.47×10^{-13}
KEGG	4114	Oocyte meiosis	112	5.49×10^{-10}
KEGG	4914	Progesterone-mediated oocyte maturation	86	2.10×10^{-7}
KEGG	4115	p53 signaling pathway	68	2.13×10^{-6}
KEGG	3430	Mismatch repair	23	5.90×10^{-4}
KEGG	240	Pyrimidine metabolism	99	1.57×10^{-3}
KEGG	3410	Base excision repair	33	2.39×10^{-3}
KEGG	3420	Nucleotide excision repair	44	6.85×10^{-3}
KEGG	3440	Homologous recombination	28	1.22×10^{-2}

B, KEGG analysis of downregulated DEGs

Category	Term	Biological process	Count	P-value
KEGG	4920	Adipocytokine signaling pathway	68	3.85×10^{-3}
KEGG	4960	Aldosterone-regulated sodium reabsorption	42	7.26×10^{-3}
KEGG	4621	NOD-like receptor signaling pathway	58	1.75×10^{-2}
KEGG	4964	Proximal tubule bicarbonate reclamation	23	1.99×10^{-2}
KEGG	640	Propanoate metabolism	32	3.69×10^{-2}
KEGG	4060	Cytokine-cytokine receptor interaction	265	3.93×10^{-2}

C, GO analysis of upregulated DEGs

Category	Term	Biological process	Count	P-value
BP	GO:0000070	Mitotic sister chromatid segregation	53	0
BP	GO:0000075	Cell cycle checkpoint	226	0
BP	GO:0000226	Microtubule cytoskeleton organization	297	0
BP	GO:0000278	Mitotic cell cycle	816	0
BP	GO:0000280	Nuclear division	346	0
BP	GO:0000819	Sister chromatid segregation	56	0
BP	GO:0006259	DNA metabolic process	896	0
BP	GO:0006260	DNA replication	277	0
BP	GO:0006261	DNA-dependent DNA replication	100	0
BP	GO:0006270	DNA replication initiation	29	0

D, GO analysis of downregulated DEGs

Category	Term	Biological process	Count	P-value
BP	GO:0008217	Reg. of blood pressure	147	9.85×10^{-6}
BP	GO:0045776	Negative regulation of blood pressure	35	3.08×10^{-4}
BP	GO:0071260	Cellular response to mechanical stimulus	57	1.98×10^{-3}
BP	GO:0035094	Response to nicotine	31	2.98×10^{-3}
BP	GO:0016486	Peptide hormone processing	32	3.27×10^{-3}
BP	GO:0002864	Reg. of acute inflammatory response to antigenic stimulus	10	3.73×10^{-3}
BP	GO:0031272	Reg. of pseudopodium assembly	10	3.73×10^{-3}
BP	GO:0016485	Protein processing	160	3.95×10^{-3}
BP	GO:0051239	Reg. of multicellular organismal processes	1963	4.20×10^{-3}
BP	GO:0006952	Defense response	1372	4.25×10^{-3}

GO, gene ontology; DEG, differentially expressed gene; BP, biological process; KEGG, Kyoto Encyclopedia of Genes and Genomes; Reg., regulation.

Table II. Functional annotation of differentially expressed genes in glioblastoma-associated stromal cells.

Category	n	Gene
Upregulated		
TF	11	BRCA1, BRIP1, CDK2, HEYL, HMGB2, IRX5, MEF2C, MEIS2, MYBL2, RBL1, TBX2
TAG oncogene	9	CCNA2, CCND2, CEP55, DUSP26, FGF5, HGF, MYBL2, NET1, PTTG1
TAG tumor suppressor	23	AKAP12, BARD1, BLM, BMP2, BRCA1, BUB1B, CDH13, CHEK1, DAB2IP, E2F1, FANCD2, ID4, ITGB3, LIMD1, LIN9, MFSD2A, PCDH10, PTPN3, RBL1, STARD13, TFPI2, TMEFF2, ZFHX3
Downregulated		
TF	6	ARNT2, DDIT3, HES2, MITF, NFIA, NR3C2
TAG oncogene	3	ARHGEF5, DDIT3, MRAS
TAG tumor suppressor	10	ATP8A2, BHLHE41, CABLES1, CDH4, DAB2, HRASLS2, LGI1, PLA2G16, RARRES3, RPS6KA2

TF, transcription factor; TAG, tumor-associated genes. BRCA1, breast cancer 1, early onset; BRIP1, BRCA1 interacting protein C-terminal helicase 1; CDK2, cyclin-dependent kinase 2; HEYL, hes-related family bHLH transcription factor with YRPW motif-like ; HMGB2, high mobility group box 2; IRX5, iroquois homeobox 5; MEF2C, myocyte enhancer factor 2C; MEIS2, Meis homeobox 2; MYBL2, v-myb avian myeloblastosis viral oncogene homolog-like 2; RBL1, retinoblastoma-like 1; TBX2, T-box 2; CCNA2, cyclin A2; CCND2, cyclin D2; CEP55, centrosomal protein 55kDa; DUSP26, dual specificity phosphatase 26 (putative); FGF5, fibroblast growth factor 5; HGF, hepatocyte growth factor; NET1, neuroepithelial cell transforming 1; PTTG1, pituitary tumor-transforming 1; AKAP12, a kinase anchor protein; BARD1, BRCA1 associated RING domain 1; BLM, Bloom syndrome RecQ like helicase; BMP2, bone morphogenetic protein 2; BUB1B, BUB1 mitotic checkpoint serine/threonine kinase B; CDH13, cadherin 13; CHEK1, checkpoint kinase 1; DAB2IP, DAB2 interacting protein; E2F1, E2F transcription factor 1; FANCD2, Fanconi anemia complementation group D2; ID4, inhibitor of DNA binding 4, dominant negative helix-loop-helix protein; ITGB3, integrin subunit beta 3; LIMD1, LIM domains containing 1; LIN9, lin-9 DREAM MuvB core complex component; MFSD2A, major facilitator superfamily domain containing 2A; PCDH10, protocadherin 10; PTPN3, protein tyrosine phosphatase, non-receptor type 3; STARD13, StAR related lipid transfer domain containing 13; TFPI2, tissue factor pathway inhibitor 2; TMEFF2, transmembrane protein with EGF like and two follistatin like domains 2; ZFHX3, zinc finger homeobox 3; ARNT2, ary-hydrocarbon receptor nuclear translocator 2; DDIT3, DNA damage inducible transcript 3; HES2, hairy and enhancer of split 2; MITF, microphthalmia-associated transcription factor; NFIA, nuclear factor I/A; NR3C2, nuclear receptor subfamily 3 group C member 2; ARHGEF5, Rho guanine nucleotide exchange factor 5; MRAS, muscle RAS oncogene homolog; ATP8A2, ATPase, aminophospholipid transporter, class I, type 8A, member 2; BHLHE41, basic helix-loop-helix family member e41; CABLES1, Cdk5 and Abl enzyme substrate 1; CDH4, cadherin 4, type 1, R-cadherin; DAB2, Dab, mitogen-responsive phosphoprotein, homolog 2 (Drosophila); HRASLS2, HRAS like suppressor 2; LGI1, leucine-rich, glioma inactivated 1; PLA2G16, phospholipase A2 group XVI; RARRES3, retinoic acid receptor responder (tazarotene induced) 3; RPS6KA2, ribosomal protein S6 kinase, 90kDa, polypeptide 2.

degrees were identified, including cyclin-dependent kinase 1 (*CDK1*), *CCNA2*, mitotic checkpoint serine/threonine kinase (*BUB1*), cell division cycle 20 (*CDC20*), kinetochore complex component 80 (*NDC80*), non-SMC condensing I complex, subunit G (*NCAPG*), cell division cycle associated-8 and polo-like kinase 1 (*PLK1*).

Analysis of sub-network. The sub-network obtained using CFinder is shown in Fig. 3, in which 135 nodes and 1,694 interactions were identified, and all nodes were upregulated genes. KEGG enrichment in the sub-network is presented in Table IIIA; cell cycle, p53 signaling pathway, oocyte meiosis and progesterone-mediated oocyte maturation were the predominant pathways enriched by these DEGs. GO enrichment analysis was also performed and is presented in Table IIIB; mitotic cell cycle, DNA metabolic process and nuclear division were the predominant categories.

Discussion

Glioblastoma is the most aggressive cerebral tumor in humans, and has a high annual mortality rate (20). GASCs represent a novel stromal cell population that express mesenchymal markers and exert tumor-promoting effects (7). In the present study, 3 samples of GASCs and 4 of control stromal cells were used to identify DEGs, and the functional categories associated with those DEGs, that are altered between GASCs and control stromal cells in glioblastoma. In total, 502 DEGs were identified, including 331 upregulated genes and 171 downregulated genes, including *CDK1*, *BUB1*, *CDC20*, *CCNA2*, *NDC80*, *NCAPG* and *PLK1*. These are hub genes and serve major roles in pathways of the cell cycle, p53 signaling, oocyte meiosis and progesterone-mediated oocyte maturation as determined from the results of KEGG pathway enrichment analysis. In addition, the upregulated gene BRCA1 was identified to be a transcription factor. The predominant pathway in which the majority of hub genes were enriched was the cell cycle, which is expected as glioblastoma cell invasion requires that cells have enhanced motility and the ability to degrade local tissue barriers (21).

CDK1 protein belongs to the CDK family, which controls the cell cycle by catalyzing the transfer of phosphate from ATP to specific protein substrates. CDKs have been established as master regulators of cell proliferation (22). As expected, in the present study, *CDK1* was upregulated in GASCs and

Table III. GO terms and KEGG pathways of DEGs in the sub-network.

A, Enriched pathways of DEGs in sub-network

Category	Term	Biological Process	Count	P-value
KEGG	3030	DNA replication	12	0
KEGG	4110	Cell cycle	29	0
KEGG	4114	Oocyte meiosis	15	$1.55x10^{-14}$
KEGG	4914	Progesterone-mediated oocyte maturation	11	$1.29x10^{-10}$
KEGG	4115	p53 signaling pathway	9	$5.41x10^{-9}$
KEGG	240	Pyrimidine metabolism	7	$2.25x10^{-5}$
KEGG	3430	Mismatch repair	4	$4.28x10^{-5}$
KEGG	3420	Nucleotide excision repair	4	$5.72x10^{-4}$
KEGG	3440	Homologous recombination	3	$1.84x10^{-3}$
KEGG	3410	Base excision repair	3	$2.97x10^{-3}$

B, Enriched GO terms of DEGs in sub-network

Category	Term	Biological Process	Count	P-value
BP	GO:0000070	Mitotic sister chromatid segregation	20	0
BP	GO:0000075	Cell cycle checkpoint	27	0
BP	GO:0000082	G1/S transition of mitotic cell cycle	24	0
BP	GO:0000226	Microtubule cytoskeleton organization	36	0
BP	GO:0000278	Mitotic cell cycle	101	0
BP	GO:0000280	Nuclear division	62	0
BP	GO:0000819	Sister chromatid segregation	21	0
BP	GO:0006259	DNA metabolic process	72	0
BP	GO:0006260	DNA replication	39	0
BP	GO:0006261	DNA-dependent DNA replication	22	0

GO, gene ontology; DEG, differentially expressed gene; BP, biological process; KEGG, Kyoto Encyclopedia of Genes and Genomes.

was primarily enriched in pathways involved in the cell cycle, mitotic cell cycle and DNA replication, all of which are closely associated with the mechanisms of tumor growth (23,24). In the cell cycle, CDK1 controls a widespread regulatory system, which involves phosphorylation of other regulatory molecules and phosphorylation of the molecular machinery that drives the cell-cycle (25). Furthermore, in the current study, CDK1 was observed to be enriched in the p53 signaling pathway, which is induced by a number of stress signals, including DNA damage, oxidative stress and activated oncogenes. The p53 signaling network is an integral tumor suppressor pathway in glioblastoma pathogenesis that affects cellular processes, including cell cycle control and cell death execution (26). In this pathway, the tumor suppressor p53 protein acts as a transcriptional activator of p53-regulated genes (27) and is primarily involved in control of numerous genes governing cell survival, cell proliferation, angiogenesis and metabolism (28). Stegh *et al* (26) reported that the p53 signaling pathway is inhibited in glioblastoma, which causes aberrant cell cycling and tumorigenesis. In the present study, several DEGs were enriched in the p53 signaling pathway, including *CDK1*, *CDK2*, *CCNB1* and *CCND2*, which may be associated with the inhibition of p53 signaling (29). Therefore, according to the current study, upregulated *CDK1* may increase the growth of glioblastoma by promoting cell cycle pathways and inhibiting the p53 signaling pathway.

BUB1 was identified to be upregulated in the present study, and was primarily enriched in biological processes associated with the mitotic cell cycle, including cell cycle chromatid segregation, G1/S transition of mitotic cells and DNA replication. The *BUB* family of genes encode proteins that are involved in a large multi-protein kinetochore complex, and are hypothesized to be key components of the checkpoint regulatory pathway (30). *BUB1* encodes a serine/threonine-protein kinase that is critical in mitosis, and functions partly through the phosphorylation of members of the mitotic checkpoint complex and activation of the spindle checkpoint (31). *BUB1* accumulates at unattached kinetochores where it mediates the recruitment of mitotic arrest deficient (Mad) dimers (32). Combined with Mad, *BUB1* prevents the premature separation of sister chromatids until all the chromosomes are correctly attached to kinetochores, which leads to correct chromosome segregation (33). Therefore, *BUB1* may promote the growth of cancer cells in glioblastoma primarily by regulating the mitotic cell cycle. In addition, it appears that the mutation of mitotic spindle checkpoint genes is associated with the

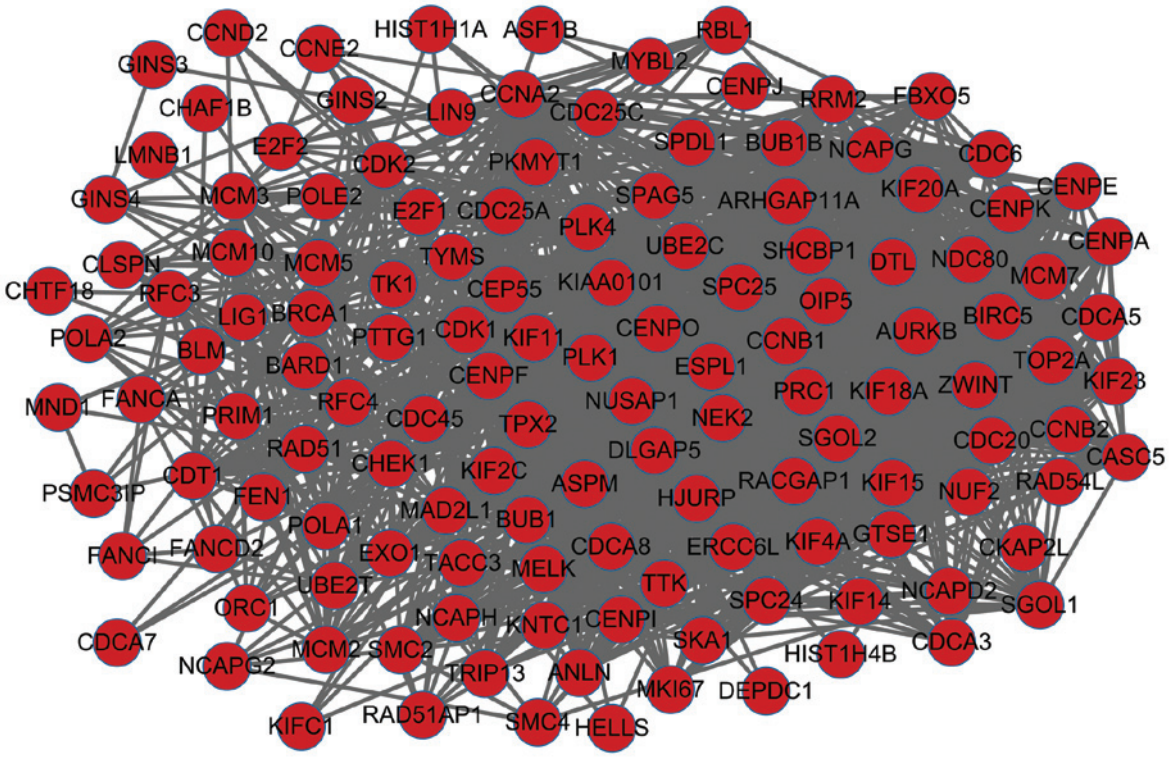

Figure 3. Sub-network constructed from the protein-protein interaction network. Red nodes, upregulated genes.

evolution of certain human cancers, particularly those with aneuploidy (34). Glioblastoma exhibits a high degree of aneuploidy (35) and the upregulation of *BUB1* in GASCs may increase the tumorigenesis of glioblastoma.

CDC20 appears to act as a regulatory protein interacting with several other proteins at multiple points in the cell cycle (36). In the present study, the *CDC20* gene was upregulated and enriched in cell cycle and oocyte meiosis pathways. *CDC20* is an activator protein that regulates the anaphase-promoting complex ubiquitin ligase, which is considered to be crucial in governing certain cellular processes (37), including the interaction with specific ubiquitin substrates for their subsequent degradation by the 26S proteasome at various points during cell cycle progression; this results in the forwards progression of the cell cycle in a unidirectional manner (38). Previous studies have demonstrated that *CDC20* is highly expressed in various types of human tumors, including breast (39) and cervical cancer (40), where it functions as an oncoprotein. Marucci *et al* (41) reported that, in glioblastoma, *CDC20* expression is upregulated, which is consistent with the present results. This implies that *CDC20* may promote glioblastoma occurrence by regulating cellular processes. In addition, Bie *et al* (42) observed that the expression levels of mitotic spindle assembly checkpoint gene *CDC20* is correlated with the grade of glioblastoma. The expression of *CDC20* is regulated by *BRCA1*, a susceptibility gene that greatly increases the risk of breast and other types of cancer (43), and is expressed differently depending on the age of the patient (44). In the present study, *BRCA1* and its target gene, *CDC20,* were upregulated. This leads to the hypothesis that *BRCA1* acts on glioblastoma, and is expressed at various levels in patients of various ages, regulating the expression of target genes that are associated with tumor grade or age of the patient, including *CDC20*. Therefore, *BRCA1* and its target genes are of significant value in clinical research, and *BRCA1* may be used as an anti-cancer drug target.

According to the present study, *PLK1* was upregulated and enriched in pathways associated with the cell cycle, oocyte meiosis and progesterone-mediated oocyte maturation. PLK1 is a serine/threonine kinase and is critical in centrosome maturation (45), mitotic entry (46), bipolar spindle formation (47,48), metaphase-to-anaphase transition (49) and cytokinesis (50) in the mitotic phase of the cell cycle. Foong *et al* (51) demonstrated that increased expression of PLK1 is an independent, negative prognostic factor in glioma and is associated with proliferative and mesenchymal molecular subclasses, which characterize highly recurrent and aggressive tumors (52). PLK1 has become a primary target in brain tumor treatment, and its inhibition has been shown to result in 80-90% growth suppression in a panel of pediatric cancer cells, including glioblastoma, following 72 h of treatment (52). Therefore, in GASCs, *PLK1* upregulation may promote the cell cycle, leading to the growth of glioblastoma.

CCNA2 belongs to a highly conserved cyclin family and is expressed in almost all tissues of the human body (53). The encoded protein is crucial in the control of the cell cycle at G1/S and G2/M transition points, and this is essential in embryonic cells and the hematopoietic lineage (54). Overexpression of *CCNA2* is involved in tumor transformation and progression in numerous types of cancer (55). Another member of the cyclin family, CCND2, is critical in cell cycle progression and tumorigenicity of glioblastoma stem cells (56). As expected, the present data revealed that *CCNA2* was upregulated, which is in accordance with the function of CCNA2 in

cancer. According to the pathway enrichment results, *CCNA2* was enriched in cell cycle and progesterone-mediated oocyte maturation pathways, in which *CDK1*, *BUB1* and *PLK1* were also involved. The present results indicate that *CCNA2* promotes the growth of glioblastoma by participating in the cell cycle. However, few studies have reported the association between oocyte maturation and glioblastoma, revealing that this may be a novel insight in glioblastoma.

In conclusion, the present study identified several significant genes in glioblastoma, including *CDK1*, *BUB1*, *CDC20*, *CCNA2*, *PLK1* and *BRCA1*, which are all upregulated and may play various roles in the biological function of GASCs. These significant DEGs may promote the tumorigenesis of glioblastoma as they are involved in major biological pathways, including cell cycle, mitosis, p53 signaling and DNA replication. However, since the sample size used in this study is small and no experiments have been performed to confirm the conclusions, additional analyses of experimental studies are required to investigate the genes associated with glioblastoma.

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