Bioinformatics analysis of potential glioblastoma circular RNA sponge network

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Background: Circular RNA is emerging functional molecule for glioblastoma. However, the function and regulatory of circular RNA (circRNA) remains unclear. In this study, the circRNA sequencing and array data of glioblastoma were analyzed by multiple bioinformatics methods to establish a potential molecular sponge mechanism regulation network.

Methods: Gene Expression Omnibus datasets were used to extract circRNAs. CircInteractome was used to predict microRNAs binding to circRNAs. Chinese Glioma Gene Atlas database was used to screen the microRNAs with expression and survival trends. MiRabel database was used to predict potential gene targets of microRNAs. The Cancer Genome Atlas database was used to screen the gene targets of sponge network. Gene Ontology and Kyoto Encyclopedia of Genes and Genomes analysis were to explain the gene targets functions. R software, Cytoscape software and Bioinformatics website were used to establish the network and visualize the results.

Results: Hsa_circ_0000219, hsa_circ_0001073 and hsa_circ_0070700 were selected from more than 2000 differentially expressed circRNAs of Gene Expression Omnibus Series (GSE) GSE146463, GSE92322 and GSE86202 datasets. Hsa-miR-1248 and hsa-miR-1290 were up regulated and related to glioblastoma poor prognosis. Targets of these microRNAs including ARHGEF7, CELA2b, RNF11, YPEL1 and ZNF37a were also screened via expression and survival data. Gene targets function were mainly enriched in signal transduction, cell plasma membrane, ATP binding and calcium signaling pathway.

Conclusions: A circRNA molecular sponge regulatory network including hsa-miR-1248 and hsa-miR-1290 has been established. In this network, hsa_circ_0001073, hsa_circ_0070700, hsa_circ_0000219, hsa-miR-1248, hsa-miR-1290, and RNF11 may have the potential being emerging glioblastoma therapeutic targets. However, their function and significance for glioblastoma need further experiments to verify.

Keywords: Glioblastoma; bioinformatics analysis; circular RNA (circRNA); sponge; RNF11

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Introduction

Glioma is most common intracranial tumor in adults. And among different types of glioma, glioblastoma multiforme (GBM) is the most malignant with rapid progress and poor prognosis. Through multi omics and epigenetics studies of glioma, researchers found enormous gene differences and key genetic changes between glioma and normal brain: such as TP53 and IDH mutations. Some of these differentially expressed genes are oncogenes, which promote the occurrence, development, metastasis, and invasion of tumors; while others tumor suppressor genes, which inhibit the proliferation and promote apoptosis. However, the biological significance and regulatory mechanism of these genes are not totally clear.

Circular RNA (circRNA) attribute to noncoding RNA. Due to back split mechanism, the sheared linear RNA will shape closed-loop structure which can contain several parts of sequences. Some circRNA sequences may contain exons or introns. The closed-loop structure maintains its stability not degraded by RNA exonuclease. Rybak-Wolf *et al.* (1) discovered that mammalian brain tissue is rich of circRNAs. However, circRNAs' functions in human brain still remain unclear. Some scientists have discovered that circRNA can regulate genes expression. The mechanisms include "molecular-sponge" (2-4), direct translation (5) and absorbing RNA binding proteins (RBD) (6,7).

In recent years, Hansen and his colleagues proposed a "molecular sponge" regulation mechanism between RNAs (4,8). According to the base complementary pairing principle, the certain nucleotides arrangement of circRNA or microRNA can combine with other RNAs (messenger RNA, message RNA, mRNA). The full length of circRNA or microRNA sequences are often much longer than the length of binding sites. CircRNA and microRNA molecule always have multiple binding sites. Therefore, they can absorb many other RNAs (such as microRNA or mRNA), which is like the absorption of water by sponge. In essence, it is also a competitive endogenous RNA (ceRNA) mechanism (2). This effect may come into extensive and powerful regulatory of downstream pathways, so that affect a variety of biological processes such as tumor proliferation or invasion.

This study analyzed the sequencing and array data of multiple GBM circRNA datasets in Gene Expression Omnibus (GEO) database and other data in several large international and Chinese databases. We selected the common differentially expressed circRNA and microRNA to construct the circRNA-microRNA- gene network,

in order to explore new targets for primary GBM comprehensively and accurately.

Methods

Overall design

This study firstly searched and screened differentially expressed circRNAs (DECs) from GEO datasets. Then, a circRNA-microRNA interaction database was used to predict potential microRNA targets. These potential microRNAs would be screened by microRNA expression database to ensure the expression trend accords with "ceRNA theory". After that the screened microRNAs' probable gene targets would be calculated by a microRNA-gene interaction database. At the same time, we extracted the differentially expressed genes (DEGs) of glioblastoma compared with normal brain tissue from The Cancer Genome Atlas (TCGA) database. Next, we figured out the specific TCGA DEGs in microRNA targets whose expression accord with "ceRNA theory" equally. Last, both potential microRNAs and their DEG targets were re-screened by survival analysis to establish a possible "Molecular Sponge Network". The function enrichment analysis of DEGs with prognosis significant was also induced by Database for Annotation, Visualization and Integrated Discovery (DAVID) and Kyoto Encyclopedia of Genes and Genomes (KEGG). The analysis process is shown in *Figure 1*. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

Differentially expressed circRNAs (DEC) data selection

We screened the Gene Expression Omnibus (GEO, https://www.ncbi.nlm.nih.gov/gds/) database by setting the search strategy as "circRNA" and "glioblastoma". The inclusion criteria were as follows: (I) datasets contain primary glioblastoma data; (II) circRNA data is essential; (III) both GPL probe sequence files and raw data of sample sequencing are available. The exclusion criteria were: (I) data contain recurrent glioblastoma samples; (II) data of children samples; (III) no circRNA data or data not available. After dataset inclusion, differentially expressed circRNAs of datasets were extracted. Considering the different identification methods of sequencing platform, converting the circRNA identities from different GEO datasets is imperative for further analysis. CircAtlas 2.0 website (http://circatlas.biols.ac.cn/) integrates multiple
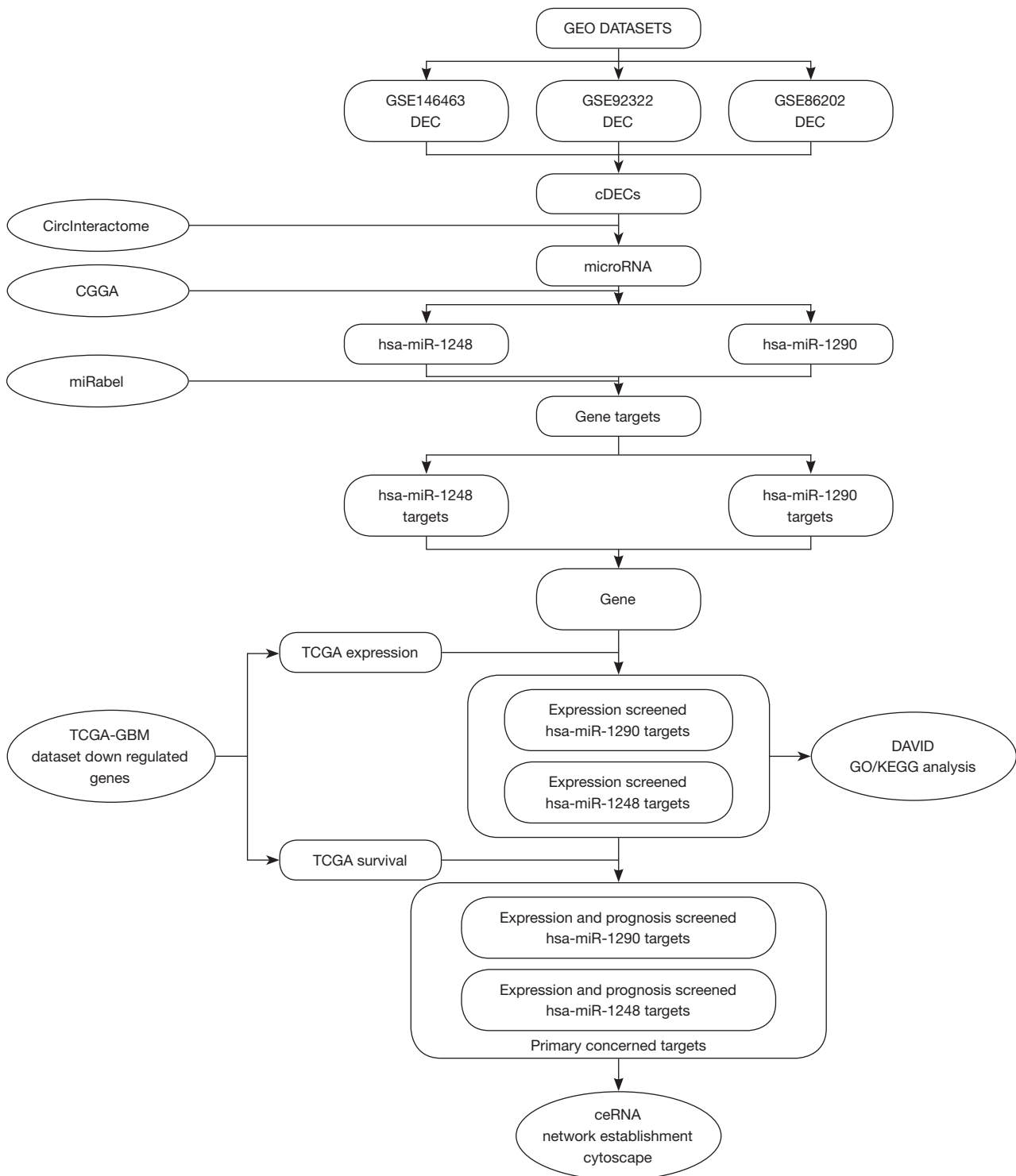
Figure 1 Flow chart of research design. CGGA, Chinese Glioma Genome Atlas; DEC, differentially expressed circRNAs; GBM, glioblastoma multiforme; GEO, Gene Expression Omnibus.

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databases and converts among the circRNA chromosome location, circAltas, circBase, circRNAdb, deepbase2, circpedia2 and other databases IDs. We choose circBase ID format to annotate GSE86202 and GSE92322 DECs. Arrystar platform probe annotation was used for GSE146463 DEC converting.

Prediction and screening of circRNA targets

(I) CircInteractome calculate potential microRNAs targets of circRNA. In order to further explore the microRNAs regulated by circRNAs, we ran the CircInteractome database and use DECs' circBase ID (https://circinteractome.irp.nia.nih.gov/) (9) to predict circRNA microRNA interaction.

(II) Screening. We used Chinese Glioma Gene Atlas mapping program (CGGA, http://www.cgga.org.cn/analyse) to screen the microRNAs. CGGA is a glioma and GBM mRNA and microRNA expression, prognosis and methylation database developed by Jiang Tao team of Beijing Tiantan Hospital (10). When screening, the first step is to analyze the microRNA distribution in different grades of glioma. According to "ceRNA theory", microRNA expression trend must be opposite to circRNA, which means down-regulated circRNA must correspond to up-regulated microRNA, vice versa. MicroRNAs expression data were from CGGA _array_198 chip, which contains 198 different glioma patients' microRNA data. MicroRNAs expression difference must be significant in both overall glioma and glioblastoma (WHO grade IV). After expression screening, the microRNA prognosis analysis was also taken into consideration.

After screening, the primary circRNA-microRNA interactions were determined. The sequence length, binding site of DECs and microRNA targets were extracted from circBase and CircInteractome databases to visualize the binding sites between circRNAs and microRNAs.

Prediction and screening of microRNA targets

MiRabel (http://bioinfo.univ-rouen.fr/mirabel/) (11) is a powerful online interaction tool integrating gene targets data from MiRanda (http://www.microrna.org/microrna/home.do), PITA (http://genie.weizmann.ac.il/pubs/mir07/mir07_data.html), SVmicro (http://compgenomics.utsa.edu/svmicro.html), TargetScan (http://www.targetscan.org/vert_80/) prediction tools and miRwalk database (http://

mirwalk.umm.uni-heidelberg.de/) and microRNA data from miRbase (https://www.mirbase.org/) to automatically predict possible targets. The testing threshold was set as $P<0.05$. The result would be screened by PITA, miRanda, SVMicro and Targetscan scores. Only all predicting scores of these four databases were available, the predicted targets could be selected.

Screening of genes by TCGA

The microRNA targets need not only screened by algorithm but also tested by real-world data. The Cancer Genome Atlas (TCGA, https://portal.gdc.cancer.gov/) is the largest and authoritative database of tumor research. Containing enormous clinical and sequencing data of various tumor samples, TCGA could filter the significant difference of gene targets commendably. Primary glioblastoma samples sequencing data and corresponding clinical information of TCGA-GBM project was downloaded, extracted, and analyzed. Differentially expressed genes (DEGs) heat-map and volcano map were also calculated. Then genes predicted by miRabel were rescreened by TCGA-GBM DEGs and survival data.

At last, in order to accord with "ceRNA" theory, screened genes could be divided into two circumstances: (I) if microRNAs were up-regulated and with poor prognosis, the gene targets of microRNAs should be down-regulated with good prognosis; (II) if microRNAs were up-regulated with good prognosis, the gene targets of microRNAs should be down-regulated with poor prognosis.

Functional enrichment analysis

Database for annotation, visualization and integrated discovery (DAVID, https://david.ncifcrf.gov/) V6.8 database is used for gene ontology (GO) analysis and Kyoto Encyclopedia of genes and genomes (KEGG) analysis. First, the selected gene list is identified as "OFFICIAL_GENE_SYMBOL"; "Homo Sapiens" and "GENE LIST" for further analysis. Then, the "Analyze above gene list with one of DAVID tools" and "Functional annotation tool" were applied to get the annotation summary results of "GO term-BP", "GO term-CC", "GO term-MF" and "KEGG-pathways".

Construction of molecular sponge network

The DECs, screened microRNAs, and screened genes'

Table 1 DECs of GEO datasets

Dataset ID	CircRNAs (n)	Sample (n)			DECs (n)			Annotated DECs (n)		Total
		T	N	Total	Up	Down	Total	Up	Down	
GSE86202	37,084	3	3	6	9	149	158	7	60	67
GSE92322	59,386	5	5	10	11	1,995	2,006	0	1,516	1,516
GSE146463	12,660	8	3	11	822	539	1,361	737	389	1,126

DEC, differentially expressed circRNAs; GEO, Gene Expression Omnibus; GSE, Gene Expression Omnibus Series; ID, identity.

potential molecular sponge interactions were visualized by Cytoscape 3.9.0 software. Gene targets with prognosis significance were labeled specially.

Statistical analysis

Test level of DECs and DEGs were set as |logFoldChange (logFC)|>1 and P<0.05 by "edgeR" with R 4.03 software (12). Log-rank test, univariate Cox proportional hazards regression, P value, 95% confidence interval (CI) and hazard ratio (HR) were used for survival assessment. HR >1 means high expression of gene associated with worse prognosis and 0< HR <1 referred to better prognosis. P<0.05 was considered as statistically significant.

P<0.05 is also set as the significant threshold for GO and KEGG enrichment analysis.

Visualization

The Kaplan Meier curve was induced by "survival" and "surviviner" packages. The "ggplot2" package output graphical results. DEC for each GEO dataset was visualized as heat-map and volcano-plot. DECs were also induced intersection to discover common DECs (cDECs) by Venn plot online server (http://bioinformatics.psb.ugent.be/webtools/Venn/). The visualization of binding can be fulfilled by bioinformatics online mapping tool (http://www.bioinformatics.com.cn).

Results

Acquisition of differentially expressed circRNAs

The keywords "GBM" and "circRNA" were searched in the GEO database. After screening the results, we finally got four datasets: GSE146463 by Bronisz et al. (13), GSE92322 by Zhu et al. (14), GSE86202 by Yuan et al. (15), and

GSE153692. Because of being unable to be recognized by circAtlas conversion system, GSE153692 dataset is excluded from analysis. All differentially expressed circRNAs were extracted by "edgeR" package. All FDR values were 0.807043219 in GSE86202. And 752 FDR >0.05 were detected in GSE92322. And 1,672 DEC FDR >0.05 in GSE146463 (available online: https://cdn.amegroups.cn/static/public/tcr-21-2597-01.zip). If we test with the FDR<0.05 level, large number of potential circRNAs might be ignored. So we set |logFC|>1, P<0.05 as filter condition. Excluding circRNAs not being annotated with circBase ID, 737 up-regulated and 389 down-regulated DECs of GSE146463, 0 up-regulated and 1,516 down-regulated DECs of GSE92322 and 7 up-regulated and 60 down-regulated DECs of GSE86202 were obtained. No up-regulated common DEC (cDEC) was discovered among the three datasets. There were 42 down-regulated cDECs. GEO datasets profile, and cDECs' details were shown in *Tables 1,2* and *Figure 2*.

CircInteractome predicts circRNA microRNA targets

Calculated by the CircInteractome database, 761 microRNAs were predicted. Details were listed in *Table 3*.

Screening of microRNAs in CGGA database

Integrated with microRNA expression, distribution, prognosis analysis and glioma WHO grades, hsa-miR-1248 and hsa-miR-1290 were screened of up-regulated and related to whole glioma (P=0.00016 for hsa-miR-1248 and P=0.02 for hsa-miR-1290) and GBM patients (P=0.0066 for hsa-miR-1248 and P=0.0075 for hsa-miR-1290) poor prognosis. Nevertheless, hsa-miR-330-5p and hsa-miR-874 associated with a worse prognosis in whole glioma (P<0.05) but better prognosis in glioblastoma (P<0.05) seemed paradox. So they were excluded. See *Table 4* and *Figure 3*.

Table 2 Common differential expressed circRNAs

Intersection	DEC number	DEC ID
GSE146463-down & GSE86202-down	1	hsa_circ_0002096
GSE146463-down & GSE92322-down	33	hsa_circ_0005654
		hsa_circ_0070113
		hsa_circ_0035957
		hsa_circ_0006956
		hsa_circ_0079557
		hsa_circ_0034182
		hsa_circ_0004619
		hsa_circ_0000219
		hsa_circ_0058495
		hsa_circ_0007848
		hsa_circ_0003390
		hsa_circ_0005566
		hsa_circ_0001073
		hsa_circ_0007771
		hsa_circ_0006499
		hsa_circ_0047880
		hsa_circ_0030788
		hsa_circ_0006931
		hsa_circ_0009000
		hsa_circ_0006580
		hsa_circ_0008021
		hsa_circ_0009064
		hsa_circ_0019799
		hsa_circ_0001792
		hsa_circ_0000932
		hsa_circ_0051348
		hsa_circ_0075648
		hsa_circ_0037516
		hsa_circ_0030787
		hsa_circ_0023858
		hsa_circ_0005535
		hsa_circ_0004538
		hsa_circ_0008390

Table 2 (*continued*)

Table 2 (*continued*)

Intersection	DEC number	DEC ID
GSE86202-down & GSE92322-down	8	hsa_circ_0002590
		hsa_circ_0008456
		hsa_circ_0070700
		hsa_circ_0032249
		hsa_circ_0069249
		hsa_circ_0047391
		hsa_circ_0069492
		hsa_circ_0024366

DEC, differentially expressed circRNAs; GSE, Gene Expression Omnibus Series; &, find intersection; ID, identity.

Prediction of microRNA downstream targets

We search hsa-miR-1248, and hsa-miR-1290 potential targets in miRabel database. 2,690 gene targets of hsa-miR-1248, and 2830 gene targets of hsa-miR-1290 were predicted (P<0.05). However, whether these genes targets being significant in GBM and accords with "ceRNA" trends was unclear and worth further exploration. Only down-regulated gene targets in GBM tissues would be screened.

Gene targets screening in TCGA-GBM database

After entering TCGA database, we select the "Case" tab in "Exploration", and "Brain" as the "Primary site", "TCGA" in "Program", "GBM" in the "Project", "Gliomas" in the "Disease type", and "primary tumor" in sample type. Through "repository" and "transcript profiling" option, the TCGA-GBM data were download. Strawberry Perl (64-bit) 5.30.0.1 software and "edgeR" package were induced to get DEGs and establish expression matrix.

The targets of hsa-miR-1248 andhsa-miR-1290 were intersected with significantly down-regulated genes in TCGA-GBM. 643 hsa-miR-1248 targets and 570hsa-miR-1290 targets were screened with expression level. Finally, 5 genes were significantly associated with better prognosis: ARHGEF7 (log-rank P=0.036, HR =0.681, 95% CI: 0.475–0.976); CELA2b (log-rank P=0.025, HR =0.662, 95% CI: 0.461–0.949); RNF11 (log-rank P=0.026, HR =0.664, 95% CI: 0.462–0.953); YPEL1 (log-rank P=0.021, HR =0.649, 95% CI: 0.45–0.936) and ZNF37a (log-rank P=0.029, HR =0.668, 95% CI: 0.465–0.96). Details were listed in *Table 5* and *Figures 4,5*.
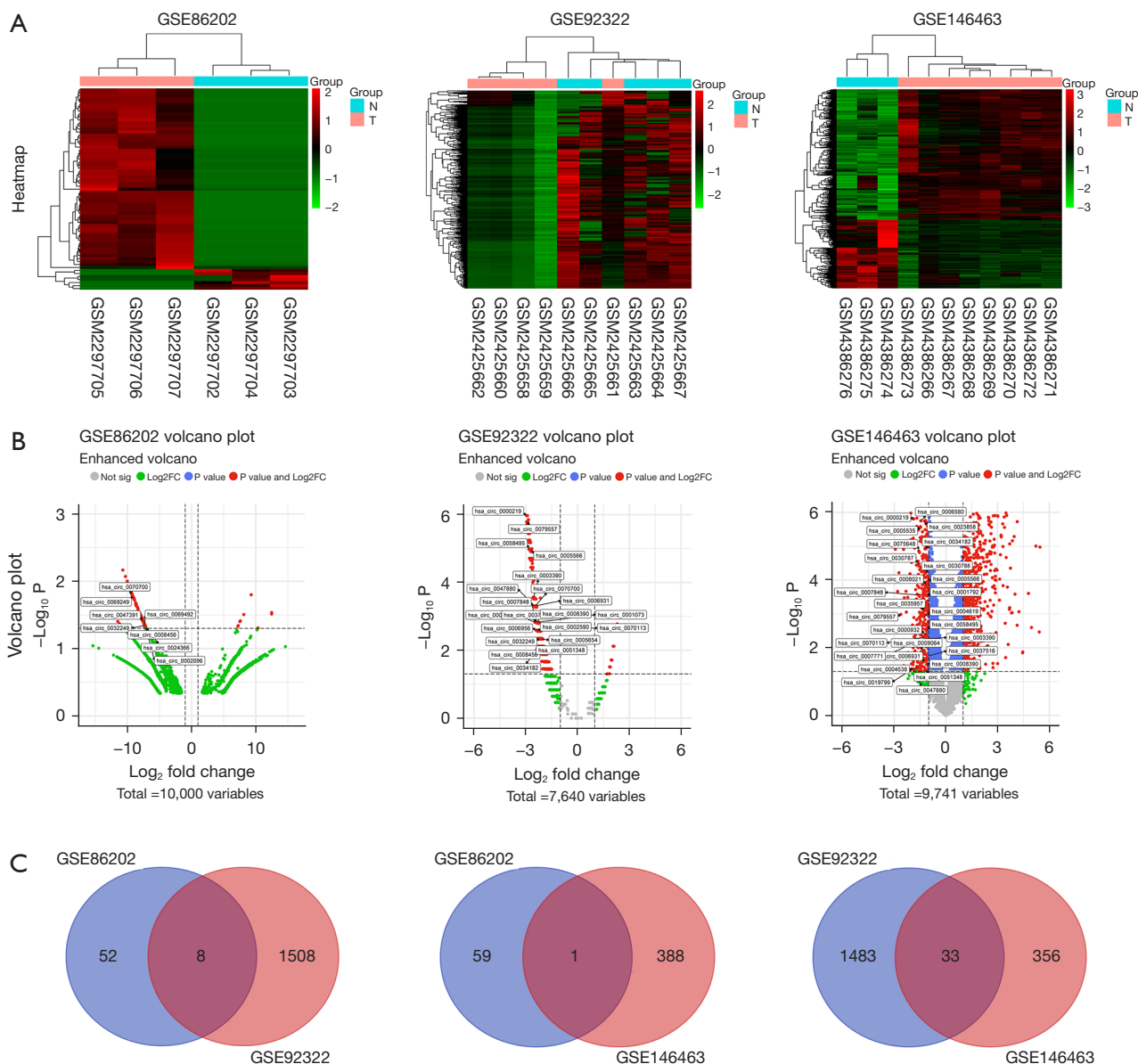
Figure 2 General DEC information of GSE86202, GSE146463 and GSE92322 datasets. (A) Heat maps show the distribution of DEC in each group and sample. (B) Volcano maps showed the significant up-regulated and down-regulated DEC and part identities. (C) Venn plot showed the common DECs of different groups. Note that there were no common up-regulated DECs for the three datasets. All common DECs were down regulated in GBM tissue compared to normal. DEC, differentially expressed circRNAs; GBM, glioblastoma multiforme; GSE, Gene Expression Omnibus Series.

Function enrichment

All expression screened targets were analyzed by DAVID from gene ontology (GO) and KEGG pathways. GO analysis included three aspects: biological process (BP), cellular components (CC), molecular function (MF).

Both GO and KEGG pathway analysis indicated that hsa-miR-1248 targets were enriched in 55 pathways, 31 pathways with significant (P<0.05), and hsa-miR-1290 targets enriched in 68 pathways, 49 pathways statistically significant (P<0.05). Sort by counts, genes were most enriched in signal transduction (counts =100, P<0.01), cell

Table 3 MicroRNA interacted with cDECs

circ ID	MicroRNA number (n)
hsa_circ_0002096	19
hsa_circ_0005654	35
hsa_circ_0070113	18
hsa_circ_0035957	24
hsa_circ_0006956	15
hsa_circ_0079557	19
hsa_circ_0034182	7
hsa_circ_0004619	14
hsa_circ_0000219	39
hsa_circ_0058495	34
hsa_circ_0007848	22
hsa_circ_0003390	26
hsa_circ_0005566	11
hsa_circ_0001073	15
hsa_circ_0002590	17
hsa_circ_0008456	3
hsa_circ_0070700	16
hsa_circ_0032249	16
hsa_circ_0007771	25
hsa_circ_0006499	24
hsa_circ_0047880	6
hsa_circ_0030788	16
hsa_circ_0006931	16
hsa_circ_0009000	10
hsa_circ_0006580	26
hsa_circ_0008021	7
hsa_circ_0009064	12
hsa_circ_0019799	6
hsa_circ_0001792	11
hsa_circ_0000932	6
hsa_circ_0051348	14
hsa_circ_0075648	47
hsa_circ_0037516	13
hsa_circ_0030787	13
hsa_circ_0023858	43

Table 3 (*continued*)

Table 3 (*continued*)

circ ID	MicroRNA number (n)
hsa_circ_0005535	19
hsa_circ_0004538	7
hsa_circ_0008390	15
hsa_circ_0069249	23
hsa_circ_0047391	21
hsa_circ_0069492	26
hsa_circ_0024366	5
total	761

cDECs, common differentially expressed circRNAs; ID, identity.

plasma membrane (counts =328, P<0.01) and ATP binding (counts =112, P<0.01) of GO and calcium signaling pathway of KEGG (counts =41, P<0.01). The prognosis associated gene targets were also checked in theses GO and KEGG pathways. For instance, ARGHEF7 was mainly enriched in signal transduction and positive regulation of apoptotic process and performed function of guanyl-nucleotide exchange and protein kinase binding. ARGHEF were also involved in regulation of actin cytoskeleton pathway. RNF11 was associated with zinc ion binding function. CELA2B was involved in pancreatic secretion and protein digestion-absorption. Details were shown in *Figure 6* and available online: https://cdn.amegroups.cn/static/public/tcr-21-2597-01.zip.

Construction of molecular sponge network of circRNA-microRNA-genes in GBM

After the extraction and screening of above databases, the eligible circRNA-microRNA-genes sponge network was visualized by Cytoscape (version 3.9.0). Hsa_circ_0000219, hsa_circ_0070700, hsa_circ_0001073, hsa-miR-1248, hsa-miR-1290 and potential downstream gene targets were particularly remarked with different shapes and colors. ARHGEF7, CELA2b, RNF11, YPEL1 and ZNF37a associated with GBM better prognosis were highlighted (*Figure 7*).

Discussion

In recent years, microRNA's molecular sponge role to mRNA has been verified in glioma. Nevertheless, there is

Table 4 MicroRNAs' screening of CGGA

circ or miR ID	Start	End	CGGA expression	CGGA survival
hsa_circ_0070700	1	300	–	–
hsa-miR-1248	199	205	Up	Highly expressed with worse prognosis
hsa_circ_0000219	1	788	–	–
hsa-miR-1248	151	157	Up	Highly expressed with worse prognosis
hsa_circ_0001073	1	473	–	–
hsa-miR-1290	326	332	Up	Highly expressed with worse prognosis

circ, circular RNA; miR, microRNA; CGGA, Chinese Glioma Genome Atlas; ID, identity.

still lack of research on "circRNA-microRNA-gene" sponge network in glioma and GBM. TCGA includes enormous gene and microRNA but no circRNA expression and survival data. GEO only contains circRNA expression data. As we all know, GEO data comes from different platforms and datasets. During the ID conversion, some circRNAs had already lost because they could not be annotated as circBase IDs. We realized P value was less stringent than the FDR test. But if we insist FDR <0.05 filter standard, we would lose the whole GSE86202 dataset. So we used other conditions to filter. LogFC represents the fold change of differential gene expression, which can directly reflect the expression of circRNA. As an additional condition, more significant differential genes can be retained after logFC screening. And the shortcomings of not being tested by FDR values will partially be improved.

Moreover, single dataset analysis is difficult to ensure the data quality and the results reliability. Therefore, the joint analysis of multiple datasets could highly elevate targets screening efficiency. For thoroughly analyzing, we have included all current circRNA datasets of primary GBM. The circRNA annotation appeared diverse because of the difference between sequencing platforms and companies. For most circRNA-microRNA interaction databases using circBase ID, we also adopt circBase ID for circRNA annotation to conduct further research. However, some circRNAs cannot be identified because they do not have circBase ID. The loss of circRNAs and survival data may bring biases to results. And current reality of insufficient glioblastoma datasets makes it difficult to select more samples. The reason for selecting CGGA database to screen microRNAs is also simple: (I) TCGA microRNA only contains low grade glioma (LGG) data but lack of glioblastoma microRNA data; (II) CGGA is the first database of Chinese population. So the screening of CGGA
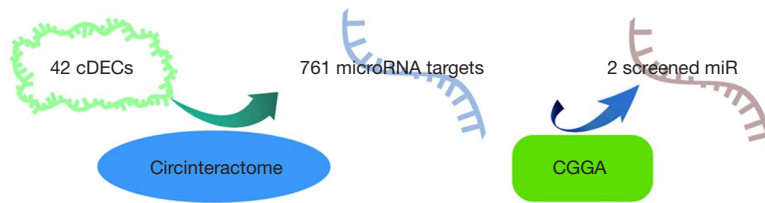
to microRNA targets may come to conclusions fit for Chinese domestic population. For microRNA targets, we compare current large microRNA gene interaction databases including miRabel, miRanda, miRdb and RNA22 and finally choose miRabel database. MiRabel contains emerging data updated in 2020 and adpot multiple algorithms of PITA, miRanda, SVmicr0 and Targetscan. So the results can be guaranteed comprehensively and reliably (11).

Most of all, the meaning of the sponge network needs to be comprehended. In current published literature, the research rarely reported these circRNAs, microRNAs or genes on glioma or glioblastoma.
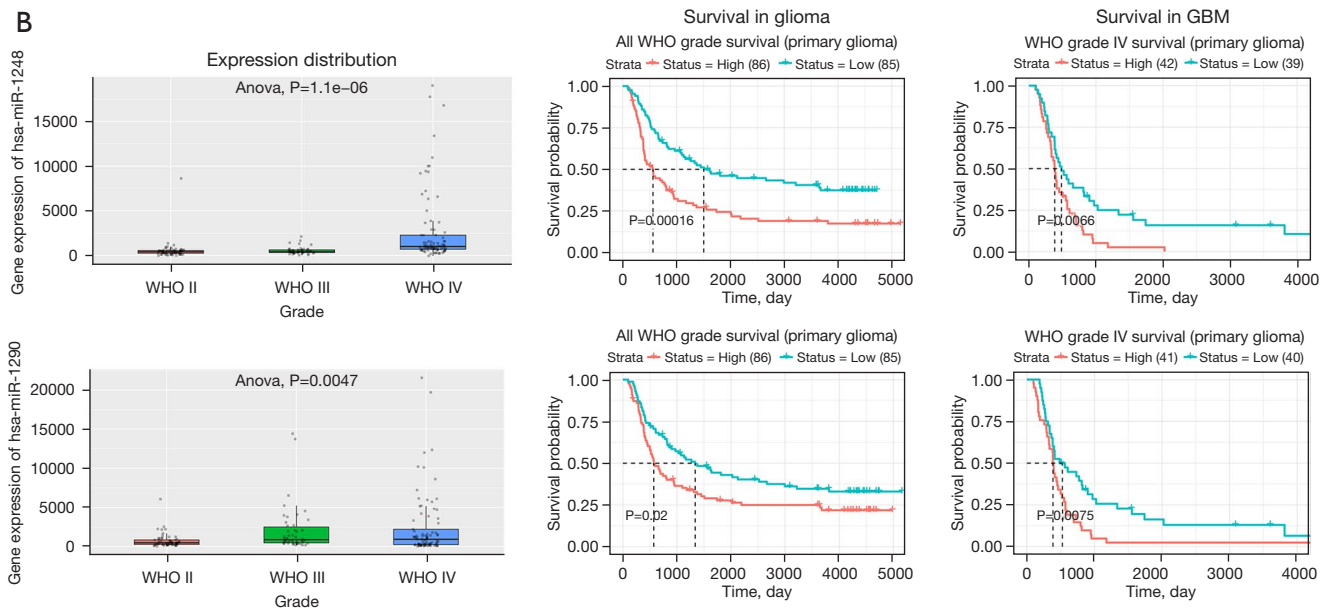
FAM188a is the host gene of hsa_circ_0000219 and also called MINDY3 (MINDY Lysine 48 Deubiquitinase 3), CARP or C10orf97. MINDY3 can activate Caspase-apoptosis and has been proven suppressor of the gastric cancer and non-small-cell lung cancer (16-18). ANK2 is the host gene of hsa_circ_0070700 whose full name is Ankyrin2. ANK2 mainly links to the integral membrane proteins and is essential for maintaining cardio-myocytes Na/Ca exchanger 1 stability (19-21). ACVR2A is the host gene of hsa_circ_0001073 associate with preeclampsia (22,23). The predicted microRNAs' functions are highly involved in multiple tumor regulation. In particular, hsa-miR-1248 and hsa-miR-1290 show obvious oncology-genesis characteristics (24-26). However, their potential regulate function in GBM needs further research.

ARHGEF7 (Rho guanine nucleotide exchange factor 7, AK-interacting exchange factor beta) is a G protein that activates the Ras like family of Rho proteins. It plays important roles in tumor migration and metastasis (27,28). CELA2b belongs to human elastase gene of pancreatic secretion and protein digestion. However, no current reports show CELA2b can degrade desmin, vimentin or actin of tumor tissue. YPEL1 is conserved and expressed
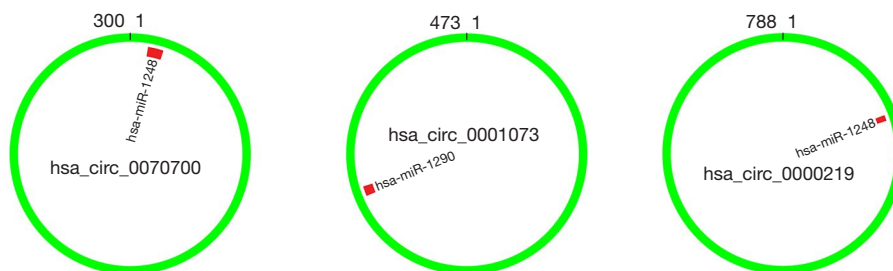
Figure 3 Prediction and screening of miRs. (A) Overall expression and survival trend screening process of miRs. (B) CGGA results of hsa-miR-1248 and hsa-miR-1290. The bar graphs represent miR expression level of different grades of glioma. Kaplan-Meier survival curves were calculated with all glioma and GBM by expression strata respectively. P<0.05 is deemed as significance. (C) Binding simulation between circRNAs and miRs. CGGA, Chinese Glioma Genome Atlas; miR, microRNA; GBM, glioblastoma multiforme.

in the first half of early embryos and participates with regulating cell morphology, behavior, and development of craniofacial complex (29). ZNF37a (zinc finger protein 37a) is mainly involved in herpesvirus infection and transcriptional regulation. At present, the specific roles of these genes in GBM are not clear.

GO and KEGG is essentially a hyper-geometric distribution test for screened targets. As the effect of prior knowledge limitations cannot be ignored, the GO and KEGG results may exist bias. So we cannot deem that the gene targets and their molecular sponge axis are meaningless in glioma. More experiments are urgently

Table 5 miR screened by TCGA-GBM expression and survival data

miR ID	Targets	Screened by expression level	Screened by survival analysis	
			Good prognosis	Bad prognosis
hsa-miR-1248	2,690	643	5	29
hsa-miR-1290	2,830	570	3	21

TCGA, The Cancer Genome Atlas; GBM, glioblastoma multiforme; good prognosis, highly expressed targets along with good prognosis; bad prognosis, highly expressed targets along with bad prognosis; ID, identity; miR, microRNA.
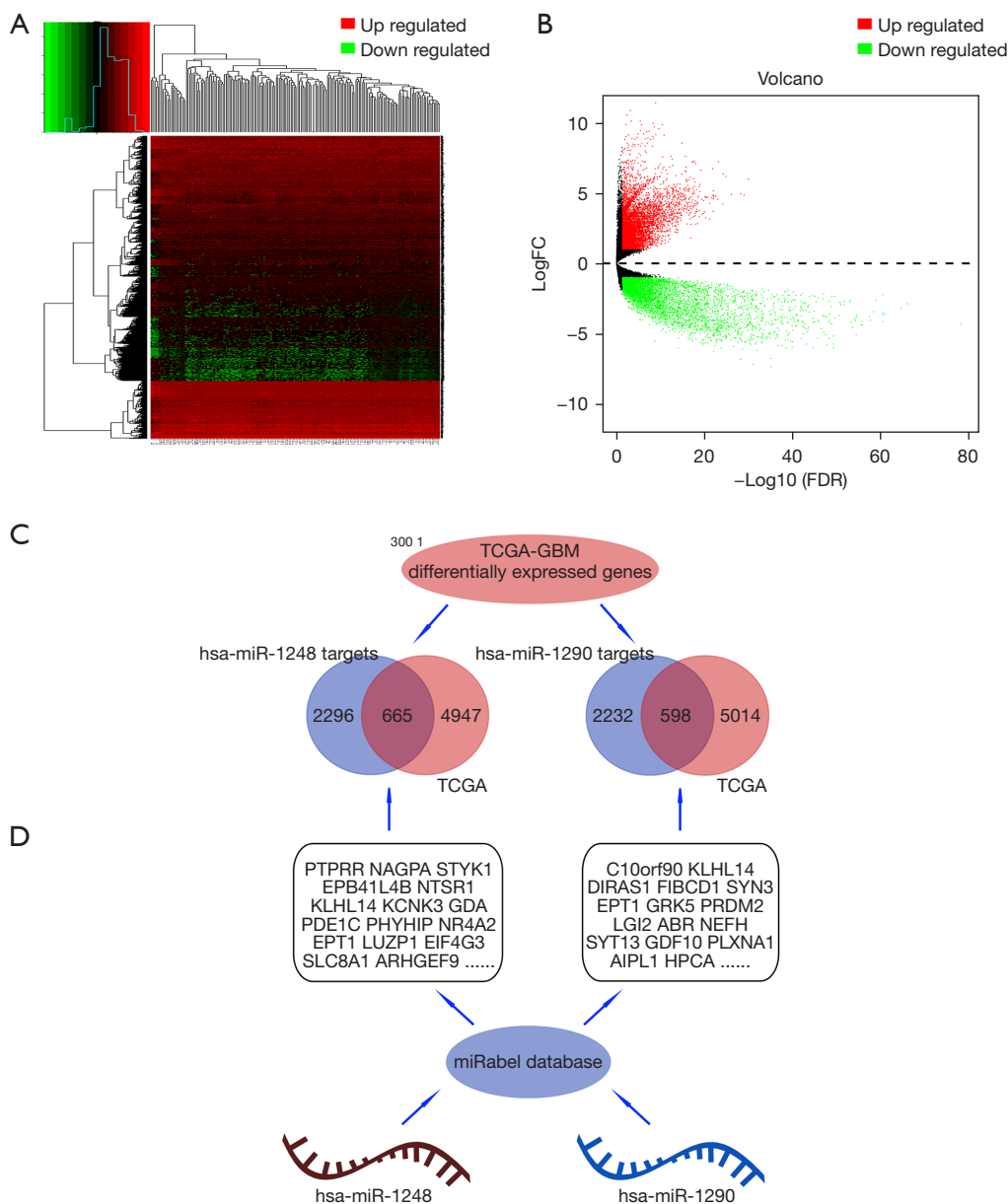

Figure 4 Prediction and screening of miRs. (A) DEGs' distribution of TCGA-GBM data analysis; (B) up and down-regulated genes of DEGs; (C) intersection of miR targets and TCGA-GBM; (D) part of potential miR targets calculated by miRabel database. DEG, differentially expressed genes; miR, microRNA; TCGA, The Cancer Genome Atlas.
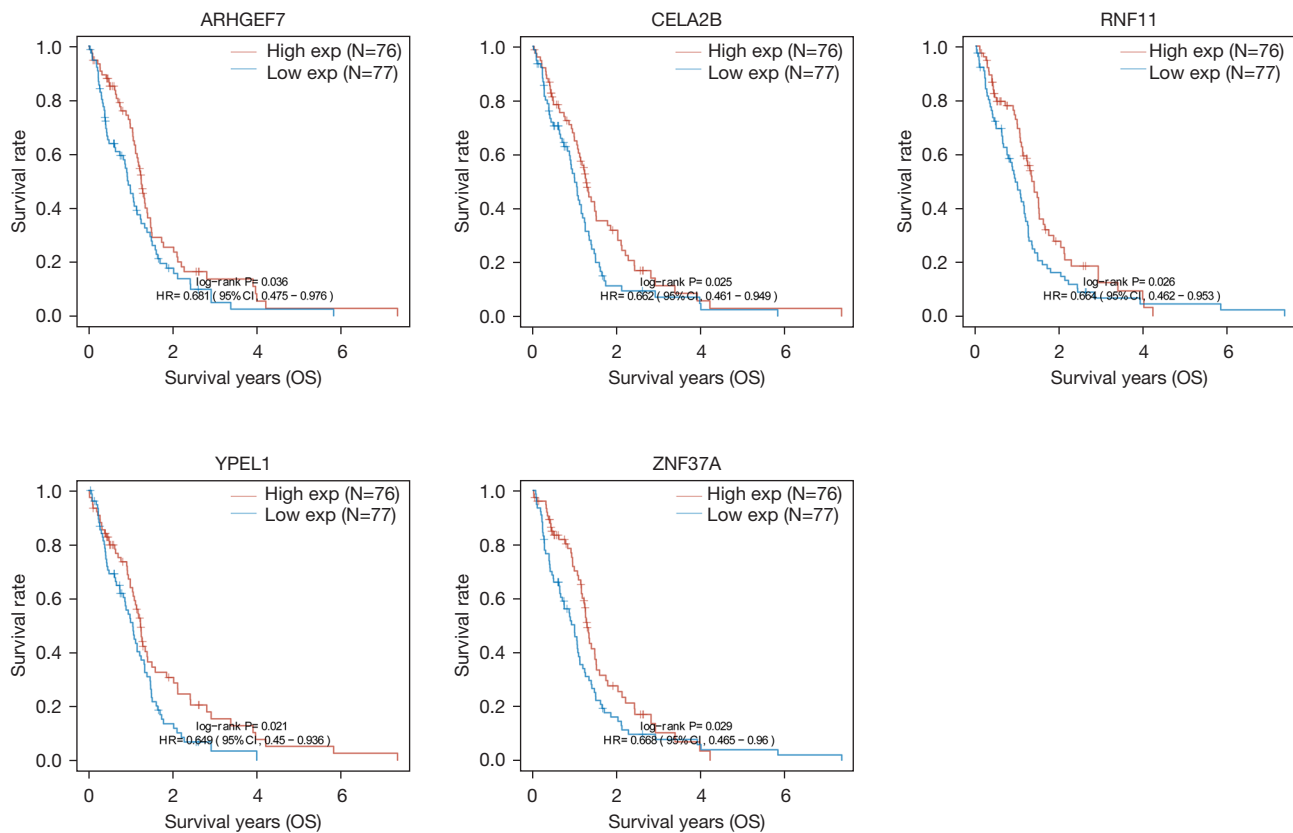
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Figure 5 miR targets with prognosis significant. OS, overall survival; miR, microRNA.

needed.

Unlike those targets previously described, RNF11 (ring finger protein 11), a ubiquitin E3 ligases, is with ubiquitin protein transferase activity and forms ubiquitin editing protein complex with TNFAIP3 (A20), ITCH and TAX1BP1 to inhibit NF–κB signaling pathway over-activation and prevent tissue damage caused by inflammatory response (30,31). It has been confirmed that NF-κB signaling pathway including the classical IKK α, IKK β pathway (32) and IKBKE, TBK1/cRel pathway (33) are activated in GBM (34). NF-κB signaling pathway can promote epithelial mesenchymal transformation (EMT) (35), angiogenesis (36) and resistance to radiotherapy (37,38). The ubiquitination process mediated by RNF11 can negatively regulate and promote the termination of NF-κB signal pathway (39), which may emerge as potential anti-tumor target. RNF11 shows antitumor properties in many tumors, such as promoting ubiquitination of TGF-β and

EGFR to inhibit hepatoma proliferation and migration (40). Overexpression of RNF11 also inhibits tumor proliferation in early breast cancer (41). Therefore, RNF11 regulation network is worth concerning in future research.

Conclusions

Through a series of bioinformatics analysis, we finally get the GBM circRNA sponge network. Hsa_circ_0000219, hsa_circ_070700 and hsa_circ_0001073 targeting miR-1248 and miR-1290 were of value to research, especially the function of RNF11 and its regulation network. These molecular sponge networks may bring important significance of fighting against GBM. Nevertheless, the conclusions of this research are merely database predictions, which need to be confirmed by further experiments *in vivo* and *in vitro*.
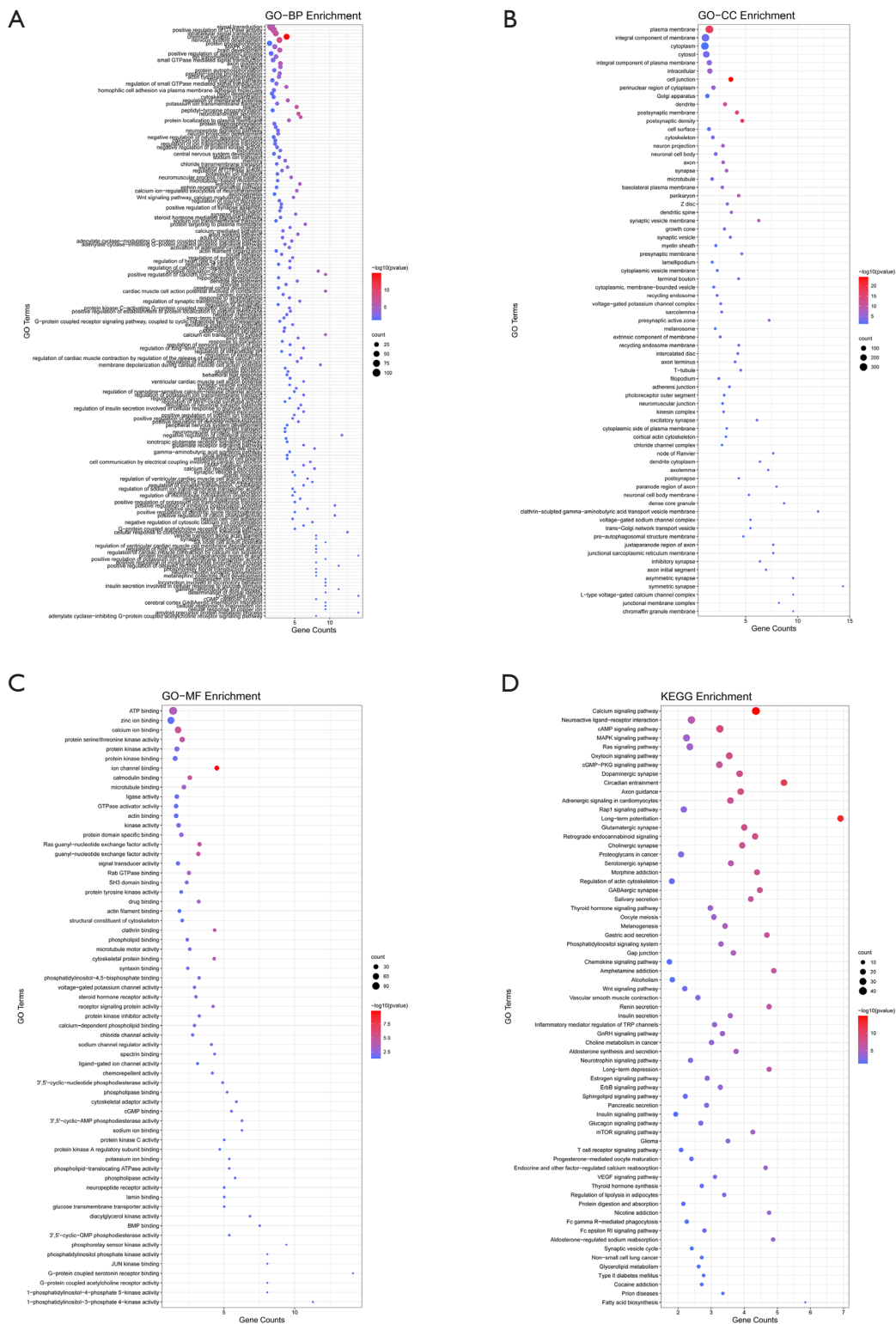
Figure 6 GO and KEGG analysis bubble plot. (A) Biological process of GO analysis; (B) cellular components of GO analysis; (C) molecular function of GO analysis; (D) KEGG analysis. Dot size represents for the count number. And different color represents for the significance. The redder means more significant, vice versa. GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes.

Figure 7 Potential ceRNA network. Plot by Cytoscape 3.9.0. Diverse shape and color were to highlight different meaning of elements, as was shown in legends. ceRNA, competitive endogenous RNA; miR, microRNA.

Footnote

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://tcr.amegroups. com/article/view/10.21037/tcr-21-2597/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

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