Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Comprehensive Analysis of Prognostic lncRNAs, miRNAs, and mRNAs Forming a Competing Endogenous RNA Network in LGG

Yiming Ding
Beijing Tiantan Hospital
Hanjie Liu
Beijing Neurosurgical Institute
Chuanbao Zhang
Beijing Tiantan Hospital
Zhaoshi Bao
Beijing Tiantan Hospital
Shuqing Yu (yushuqingttyy@163.com)
Beijing Tiantan Hospital https://orcid.org/0000-0001-8012-2398

Research

Keywords: Low grade gliomas, mRNA, lncRNA, miRNA, ceRNA network

Posted Date: September 22nd, 2020

DOI: https://doi.org/10.21203/rs.3.rs-63555/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Abstract

Background: Messenger RNA(mRNA) and Long non coding RNA (lncRNA) targets can interact through the ability to compete for microRNA binding. However, the roles of cancer specific lncRNAs in lncRNA-related ceRNA network of low grade glioma (LGG) are still unclear.

Methods: This study obtained two types of RNAs sequencing data in Solid Tissue Normal and LGG Primary Tumor from TCGA database. We used a computational method to analyse the relation between mRNAs, lncRNAs and miRNAs. The function enrichment of Go item and KEGG pathway were analyzed to predict the biological process and pathway of the screened gene. Kaplan-Meier survival analysis was used to evaluate the association with the expression levels of mRNAs, lncRNAs, and micRNAs and the overall survival of the patients. the ceRNA network of mRNA-lncRNA-miRNA was constructed with the version of cycloscape 3.5.1.

Results: 2555 DEmRNA, 218 DElncRNA. 192 DEmiRNAs were screened by using the R package. We analyzed the function enrichment of Go item and KEGG pathway of mRNAs and lncRNAs in ceRNA network. The main 10 BP items, 10 CC items, 10 MF items and 48 KEGG pathways were selected.55 survival related lncRNAs, 50 survival related miRNAs and top 10 survival most related mRNAs in LGG. Finally, 59 miRNAs, 235 mRNAs and 17 lncRNAs, a total of 313 nodes and 1046 edges, constructed the ceRNA network of mRNA-lncRNA-miRNA.

Conclusions: This study is advantageous to deeply understand the biological mechanism of ceRNA and to clarify the pathogenesis of LGG.

Background

Low grade gliomas (LGGs) are considered to be grade I or grade II by the World Health Organization (WHO). The main components are oligodendroglioma and astrocytoma, accounting for about 15% of all gliomas (1). Current treatment of LGG patients includes surgery, radiotherapy and adjuvant chemotherapy (2). The overall survival time reported recently is 13.3 years (3), but may vary depending on the molecular subtypes. Some molecular markers may be potential targets for predicting the prognosis of LGG(4). In addition, epilepsy is the most common initial symptom of LGGs on the tentorium (5). Recent studies have pointed out that epilepsy and tumor growth of LGG may have a common pathogenesis and mutual influence. This representing two aspects of the same disease (6). In this context, some genetic changes are considered to be risk factors of gliomas related epilepsy. Therefore, it is necessary to study the molecular mechanism of LGGs in order to provide evidence for the effective treatment of LGG.

Non coding RNAs (ncRNAs) are a kind of RNA lacking protein coding function. Only 2% of human transcriptome is composed of protein encoding RNA, and the remaining 98% is non coding RNA. NcRNAs have become more and more important research objects because of their special and adaptive biological role in tumor development (7). Generally, ncRNAs can be divided into two categories according to their

size: small ncRNAs and long ncRNAs (lncRNAs). Small ncRNAs include several subtypes, including microRNAs (miRNAs), ribosomal RNAs, microkernel RNAs and transfer RNA (tRNA).

LncRNA is a kind of noncoding functional RNA with a length of more than 200 nucleotides. It is situated in the nucleus and cytoplasm of eukaryotic cell and has attracted more and more attention in recent years. In fact, lncRNA plays an important regulatory role in various cell processes, especially in various types of tumors. The change of lncRNA expression has been reported to be related to the development of tumors(8), and some lncRNA has been used as biomarkers and potential targets for a variety of tumors.

MiRNA is an endogenous single stranded RNA molecule with 22 nucleotide lengths, which do not encode proteins. MiRNA can inhibit the expression of target gene by complementary binding of its seed region and microRNA response element (MREs) on mRNA. A single miRNA can regulate hundreds of target genes, and each gene can also be regulated by multiple miRNAs. The regulatory network between miRNA and target genes involves a variety of biological processes, including tumor occurrence and metastasis. In recent years, the role of miRNAs in the occurrence and development of LGGs and its regulatory mechanism has been studied in depth, and a large number of papers have been published (4).

In 2011, Salmena et al. Put forward the hypothesis of competitive endogenous RNA (ceRNA), which hypothesized the relationship between microRNA ,mRNA and microRNA, in which mRNA and ncRNA targets can interact through the ability to compete for microRNA binding (9).With the development of bioinformatics technology, more and more researchers use the methods of data analysis and mining to study the ceRNA network, including head and neck squamous cell carcinoma(10), renal cell carcinoma (11), hepatocellular carcinoma(12), lung squamous cell carcinoma(13), glioblastoma multiform(14), lung adenocarcinoma(15), chromatic cancer(16), alternative carcinoma(17) and endometrial cancer(18). In addition, some representative databases, such as miRTarBase (19), TargetScan (20) and StarBase (21), provide data and useful resources for the research of ceRNA network. The Cancer Genome Atlas (TCGA) is a public comprehensive database, which provides multiplatform genome data and clinical information of matched patients. This database promotes the development of genomics to describe the molecular landscape of cancer. The roles of cancer specific lncRNAs in lncRNA-related ceRNA network of LGG are still unclear. In this study, the expression profiles of mRNAs, lncRNAs and miRNAs in LGG were analyzed by TCGA database, and a LGG specific and lncRNA related ceRNA network was constructed. Based on the analysis of RNA survival in the ceRNA network, we analyzed the mRNAs, lncRNAs and miRNAs that have a significant impact on survival and prognosis of LGG patients. This network is expected to elucidate the interaction of RNAs-miRNAs network of LGG, and to further understand the molecular mechanism of the occurrence and development of LGG.

Methods

Patient datasets and data processing

We obtained two types of RNAs sequencing data in Solid Tissue Normal and LGG Primary Tumor from TCGA database (https://portal.gdc.cancer.gov/), Integration and extraction of the RNAs expression profiles was done by using the R package. And then, the expression values of mRNAs, lncRNAs, and micRNAs were obtained by background correction and quantile normalization. This study used sequencing data from the TCGA public database and there was no ethical review and informed consent.

Differential analysis of mRNAs, lncRNAs, and micRNAs

The differential expression of mRNAs, lncRNAs, and micRNAs was analyzed by using limma package of R software. The thresholds were |logFc (fold change)|>2 and a false discovery rate (FDR)<0.05, Volcano plots and heatmaps were drawn by using GDCRNATools package.

Functional Enrichment Analysis

We studied the functional roles of the ceRNA network in the LGG by Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses. These gene functional enrichments analyses were conducted by using the cluster Profiler package of R software. The threshold for GO and KEGG enrichment analysis was P<0.05. The GO plot package of R software was utilized to display the results of the GO and KEGG analyses.

Survival Analysis

To estimate the relationship between prognoses and RNAs expression signatures, we divided the LGG samples into high-expression and low-expression groups, and define the median as a cutoff. We used Kaplan-Meier survival analysis to evaluate the association between with the expression levels of mRNAs, lncRNAs, and micRNAs and the overall survival of the patients with the survival package in R3.6.2. P < 0.05 was considered statistically significance.

ceRNA Network Construction.

We selected mRNAs, lncRNAs, and micRNAs which related to prognosis to construct the ceRNA network. The starBase (http://starbase.sysu.edu.cn/) database was used to predict lncRNA-miRNA interactions and micRNA-mRNA interactions. Based on the DEmiRNA–DElncRNA and DEmiRNA–DEmRNA interactions, we constructed the lncRNA–miRNA–mRNA ceRNA network, at the same time the ceRNA network was visualized by using Cytoscape software(22). In this analysis, P < 0.05 was considered statistically significance. In Cytoscape, we obtain not only the identified nodes with score from calculation but the subnetwork composed of these nodes by utilizing cytoHubba. Finally, MCODE finds clusters (highly interconnected regions) in the Constructed ceRNA network In Cytoscape.

Results

Differentially expressed lncRNAs, miRNAs, and mRNAs in LGG

The expression of RNA in 450 LGG tumor tissues and 5 normal tissues was studied in TCGA database. According to this standard, we screened 804 up-regulated mRNA and 1751 down-regulated mRNA, involving 60483 genes. 140 up-regulated mRNA and 78 down-regulated lncRNA. 78 up-regulated miRNAs and 114 down-regulated miRNAs involved 2588 genes. The top 20 up-regulated mRNA, the top 20 down-regulated mRNA and their corresponding logfc, P value and FDR value (by edge) are shown in Table 1. The top 20 lncRNAs and miRNAs are shown in Table 2, Table 3. In Fig. 1B, The volcano plot showed the distribution of all differentially expressed mRNA in log FDR and logFC. All mRNAs expression levels were standardized to the sample mean. It can be seen from Table 1 and Fig. 1B that the difference of down-regulated mRNA is more remarkable than up-regulated mRNA. Figures 1A, 1B and 1C show heatmap and volcano plot of differences in lncRNAs, miRNAs, and mRNAs expression. The proportion of mRNA, lncRNA and pseudogene in RNA can be seen in the Fig. 1D.

Table 1

Differentially expressed mRNAs in LGG

Top 20 down-regulated mRNAs				***Top 20 up-regulated mRNAs***			
Symbol	logFC	PValue	FDR	Symbol	logFC	PValue	FDR
ANKRD34A	-3.251	5.310E-32	8.370E-28	MAPRE1	1.525	4.377E-26	6.272E-23
FABP3	-2.844	8.989E-30	7.084E-26	DPYSL3	2.279	1.014E-24	7.611E-22
AKAP5	-3.511	1.586E-28	8.048E-25	ANTXR1	2.443	7.741E-24	4.693E-21
PTER	-4.827	2.465E-28	8.04762E-25	VANGL2	2.680	1.340E-22	5.150E-20
PTPRR	-4.796	2.553E-28	8.048E-25	DDR1	1.859	5.236E-22	1.684E-19
DGKE	-3.175	5.208E-28	1.368E-24	SOX2	2.549	4.080E-20	7.749E-18
ZCCHC12	-4.859	2.095E-27	4.321E-24	RPLP0	2.263	2.745E-19	4.120E-17
SH2D5	-4.702	2.193E-27	4.321E-24	44076	1.338	1.128E-18	1.394E-16
CES4A	-4.081	2.571E-27	4.504E-24	SPARC	3.344	2.806E-18	3.093E-16
SAMD12	-3.237	9.999E-27	1.576E-23	TCF12	2.970	4.975E-18	5.091E-16
ISLR2	-4.739	5.800E-26	7.618E-23	APH1A	1.018	7.585E-18	7.472E-16
RTN4RL1	-4.456	6.792E-26	8.235E-23	CDK2AP1	1.610	4.028E-17	3.324E-15
CPNE7	-4.682	1.09872E-25	1.237E-22	EEF1A1	1.645	1.648E-15	9.992E-14
PRRT3	-1.959	1.800E-25	1.891E-22	MAVS	1.017	1.925E-15	1.149E-13
HPRT1	-2.734	3.225E-25	3.074E-22	LIX1L	1.653	1.959E-15	1.165E-13
LRRC7	-3.628	3.315E-25	3.074E-22	TXNIP	2.113	2.594E-15	1.498E-13
KIAA1324	-3.456	4.569E-25	3.937E-22	BCAN	3.247	5.842E-15	3.197E-13

Top 20 down-regulated mRNAs				*Top 20 up-regulated mRNAs*			
RASAL1	-5.757	4.746E-25	3.937E-22	NES	3.069	8.806E-15	4.673E-13
SMIM10L2B	-3.404	8.695E-25	6.853E-22	RELA	1.049	1.054E-14	5.517E-13
GLT1D1	-4.353	3.588E-24	2.464E-21	RACK1	1.731	1.104E-14	5.708E-13

Table 2

Differentially expressed lncRNAs in LGG

Top 20 down-regulated lncRNAs				***Top 20 up-regulated lncRNAs***			
Symbol	logFC	PValue	FDR	Symbol	logFC	PValue	FDR
SLC26A4-AS1	-5.416	4.505E-22	1.479E-19	AC093010.3	2.393	3.505E-14	1.642E-12
PART1	-4.040	6.598E-21	1.486E-18	LRRC75A-AS1	1.659	3.225E-10	6.805E-09
ATP2B1-AS1	-2.385	3.408E-19	5.020E-17	PITPNA-AS1	1.459	5.222E-10	1.063E-08
SNAI3-AS1	-1.853	1.952E-16	1.405E-14	AC010186.3	2.075	2.743E-09	4.740E-08
AC016831.1	-2.894	3.019E-16	2.115E-14	UBL7-AS1	1.182	6.393E-09	1.022E-07
PRKAG2-AS1	-2.503	2.344E-15	1.374E-13	AL391069.3	2.385	7.037E-09	1.114E-07
AL049796.1	-1.987	7.905E-15	4.223E-13	AL121603.2	1.785	1.862E-08	2.703E-07
LINC00957	-3.115	1.065E-14	5.557E-13	DLEU1	1.982	2.027E-08	2.915E-07
RFPL1S	-3.441	6.604E-13	2.382E-11	AC021188.1	3.031	3.532E-08	4.824E-07
AL365361.1	-3.080	8.084E-12	2.386E-10	C21orf62-AS1	1.471	4.482E-08	5.947E-07
DLX6-AS1	-3.773	1.544E-11	4.240E-10	GLYCTK-AS1	4.248	5.735E-08	7.379E-07
MIR600HG	-1.532	1.752E-11	4.746E-10	AC019080.5	1.618	8.405E-08	1.040E-06
LINC00982	-2.763	3.187E-11	8.223E-10	AC025164.2	2.021	9.990E-08	1.213E-06
AC004656.1	-2.027	3.525E-11	8.977E-10	TFAP2A-AS1	4.887	1.920E-07	2.188E-06
MIR22HG	-1.778	1.008E-10	2.329E-09	ASB16-AS1	1.194	2.505E-07	2.776E-06
LINC00294	-1.308	2.129E-10	4.629E-09	LINC00511	2.412	3.480E-07	3.729E-06
NUP50-AS1	-1.241	1.018E-09	1.938E-08	ZEB1-AS1	1.638	3.968E-07	4.192E-06

Top 20 down-regulated lncRNAs				*Top 20 up-regulated lncRNAs*			
AC015712.2	-3.049	1.107E-09	2.097E-08	AL513165.1	1.231	5.219E-07	5.405E-06
AC114811.2	-1.781	1.429E-09	2.640E-08	RNF157-AS1	1.999	6.027E-07	6.141E-06
HCG11	-2.225	1.598E-09	2.908E-08	WEE2-AS1	2.034	6.424E-07	6.465E-06

Table 3

Differentially expressed miRNAs in LGG

Top 20 down-regulated miRNAs				***Top 20 up-regulated miRNAs***			
Symbol	logFC	PValue	FDR	Symbol	logFC	PValue	FDR
hsa-miR-769-5p	-1.893	1.698E-11	1.385E-09	hsa-miR-497-5p	2.379	2.265E-14	8.151E-12
hsa-miR-769-3p	-2.250	1.003E-10	6.047E-09	hsa-miR-135a-3p	6.086	3.863E-14	8.151E-12
hsa-miR-433-3p	-3.620	5.582E-10	2.617E-08	hsa-miR-181a-2-3p	2.808	1.329E-13	1.869E-11
hsa-miR-432-5p	-3.079	1.532E-09	4.972E-08	hsa-miR-195-3p	2.883	2.095E-13	2.211E-11
hsa-miR-132-3p	-2.173	1.871E-09	5.639E-08	hsa-miR-629-3p	3.085	1.969E-11	1.385E-09
hsa-miR-132-5p	-1.992	2.219E-09	6.243E-08	hsa-miR-93-5p	1.964	3.672E-10	1.937E-08
hsa-miR-485-3p	-3.303	2.611E-09	6.888E-08	hsa-miR-3074-5p	2.972	7.807E-10	3.295E-08
hsa-miR-329-3p	-3.229	3.322E-09	8.247E-08	hsa-miR-629-5p	2.141	1.382E-09	4.972E-08
hsa-miR-889-3p	-3.040	9.895E-09	2.320E-07	hsa-miR-130b-5p	2.502	1.498E-09	4.972E-08
hsa-miR-218-5p	-3.549	2.269E-08	4.787E-07	hsa-miR-195-5p	2.000	3.082E-08	5.656E-07
hsa-miR-3200-3p	-1.950	2.175E-08	4.787E-07	hsa-miR-9-5p	2.240	6.263E-08	8.621E-07
hsa-miR-380-5p	-2.967	2.772E-08	5.317E-07	hsa-miR-99a-3p	1.844	8.806E-08	1.119E-06
hsa-miR-487b-3p	-2.900	2.684E-08	5.317E-07	hsa-miR-20a-3p	2.440	2.760E-07	2.532E-06
hsa-miR-1224-5p	-3.333	3.704E-08	6.450E-07	hsa-miR-19b-1-5p	1.969	3.241E-07	2.910E-06
hsa-miR-139-5p	-2.901	3.821E-08	6.450E-07	hsa-miR-92b-5p	2.812	6.546E-07	5.416E-06
hsa-miR-766-3p	-2.171	4.616E-08	7.493E-07	hsa-miR-28-3p	1.471	7.239E-07	5.875E-06
hsa-miR-369-3p	-2.722	4.829E-08	7.547E-07	hsa-miR-26b-3p	1.391	2.199E-06	1.497E-05

Top 20 down-regulated miRNAs				*Top 20 up-regulated miRNAs*			
hsa-miR-874-3p	-1.888	5.056E-08	7.620E-07	hsa-miR-505-3p	1.517	2.361E-06	1.557E-05
hsa-miR-7-5p	-3.651	5.525E-08	8.039E-07	hsa-miR-92a-3p	1.436	4.215E-06	2.578E-05
hsa-miR-370-3p	-2.681	6.333E-08	8.621E-07	hsa-miR-92b-3p	1.874	1.103E-05	6.126E-05

Go analysis and KEGG analysis

In order to further predict the biological process and pathway of the screened gene, we analyzed the function enrichment of Go item and KEGG pathway of mRNAs and lncRNAs in ceRNA network. The main 10 BP items, 10 CC items and 10 MF items were selected(Fig. 2A). Biological process (BP) mainly includes regulation of neuron project development, axogenesis, signal release, regulation of chemical synaptic transmission, regulation of trans-synaptic signaling, position regulation of neuron differentiation; cell composition (CC) mainly includes postsynapse, presynapse, axon part, neuron to neuron Synapse, asymmetric synapse, postsynaptic density, postsynaptic specialization, total axon and postsynaptic membrane; molecular function (MF) mainly includes metal transmembrane transporter activity, ion channel activity, substrate-specific channel activity, phosphoid binding, gated channel activity and ion gated channel activity. In the ceRNA network, 48 KEGG pathways were significantly enriched by DemRNAs, including cAMP signaling pathway, calcium signaling pathway, axon guidance, glutamic synapse, dopaminergic synapse, streamlined synapse and GABAergic synapse(Fig. 2B-2E). The GO enrichment networks of BP, CC and MF for these genes are shown in Fig. 1E.

Survival analysis of mRNAs, lncRNAs and miRNAs in LGG

The relationship between the expression of differential lncRNAs, miRNAs and mRNAs and OS in LGG patients was examed in detail. We detected 55 survival related lncRNAs, 50 survival related miRNAs and top 10 survival most related mRNAs in LGG ($P < 0.05$). Figure* shows the 10 top important survival related genes. The most significant mRNA was TOLLIP, FBLN7, ODF2L, RHBDL1, SNAP91, VASN, LMO4, CAMK2G, AURKB, PTBP1(Fig. 3A). Pearson correlation analysis showed that HNRNPA1 and LRRC75A-AS1 were the highest R-values ($R = 0.855$, $p = 2.59e\text{-}131$) (Fig. 3B).

Construction of a ceRNA network

The targets of all differential mRNAs, lncRNAs and miRNAs were predicted. Through calculation, we selected 59 DemiRNAs, 235 DemRNAs and 17 DelncRNAs, a total of 313 nodes and 1046 edges, and constructed the ceRNA network of mRNA-lncRNA-miRNA with the version of cycloscape 3.5.1(Fig. 4A).

The node connection in the network can reflect the interaction between RNA, and the stronger the connection, the more important the biological function of the RNA in the network. Through the analysis of the maximum clique centrality (MCC) method, the top ten hub miRNAs are calculated as shown in Fig. 4C. Through the MCODE tool, we find highly interconnected regions in the constructed ceRNA network (Fig. 4B)

Discussion

With more and more research on lncRNA and miRNA in various cancer fields, it has been found that lncRNA can be used as miRNA sponge to regulate mRNA, and more and more attention has been paid to ceRNA. Kai Ma et al. Constructed the ceRNA network (23) of pulmonary artistic hypertension. It is becoming more and more clear that many complex diseases, especially cancer, seldom can be attributed to one or several genomic variations alone (24). The study of ceRNA network is helpful to systematically understand the relevant processes of tumor occurrence, development, metastasis, prognosis, etc. In this study, large queues from TCGA database were used to identify the DElncRNAs, DEmRNAs and DEmiRNAs between LGG and normal tissues, so as to construct a prognosis related ceRNA network.

Through the Go analysis of DElncRNAs, DEmRNAs and DEmiRNAs, it was found that the differentially expressed genes in BP mostly led to the development of neurons, the formation of synapses and the release of synaptic signals. In CC, most of the differentially expressed genes constitute synaptic structure, while in MF, most of them are enriched in ion channel activity. These results show that the formation of LGG is inseparable from the formation of neuron structure, which seems to be closely linked to the development of epilepsy, and these conclusions need further experimental verification. Several key genes related to Go analysis are shown in Go circle plot map, among which several members of GABAR family appear in the results, which further verify the previous point of view. In KEGG analysis, cAMP signaling pathway is the most significant expression, which regulates many biological processes, such as cell migration, differentiation, proliferation and apoptosis (25). This seems to play an important role in the development of LGG. The axon guidance, glutamatergic synapse and GABAergic synapse signaling pathway indicate the cause of LGG epilepsy. Yinian Zhang et al found that glutamatergic synapse pathway is an important signal pathway related to epilepsy (26). We show the above important signal pathways, which will be helpful in the future development of LGG and epilepsy research. KM analysis of mRNA, lncrna and miRNA is helpful for molecular typing and targeted therapy of LGG.

The results of prognostic analysis showed that dlg2, GRIN1, HTR2A, kcnj3, KCNJ9, kif3c, SOCS3 and linc00599 were verified respectively (27, 28, 29, 30). In the constructed ceRNA network, we found 10 central key nodes through cytohubba: hsa-miR-320d,hsa-miR-30a-5p,hsa-miR-320c,hsa-miR-30b-5p,hsa-miR-30d-5p,hsa-miR-30e-5p,hsa-miR-320a,hsa-miR-137,has-miR-320b,has-miR-30c-5p.Hsa-mir-137 seems to be a key target in glioma cells, which has been reported in some studies.

Chen et al. found that miR-137down-regulated in glioma samples and glioma cells by qRT-PCR. They demonstrate that miR-137 deregulation is common in glioma, and restoration of its function inhibits cell proliferation and invasion, suggesting that miR-137 may act as a tumour suppressor (31). Deng et al. discovered miR-137 was significantly down regulated in astrocytoma tissues, and its expression level was inversely correlated with the clinical stage. They verified that miR-137 acts as a tumor suppressor in astrocytoma by targeting RASGRF1(32). Li et al. demonstrate that miR-137 expression is significantly downregulated in a cohort of 35 oligodendroglial tumors and nine glioma cell lines compared with normal brains. And miR-137 regulates growth of glioma cells and targets CSE1L(33). Sun et al. confirmed over expression of miR-137 in vitro by chemically synthesized miR-137 mimics suppressed the proliferation, inhibited cell cycle arrest in the G1/G0 phase, and induced cell apoptosis. Their findings indicate that miR-137 inhibits the growth of gliomas cells by directly targeting Rac1(34). In addition,Wang et al. presented the evidence that miR-30a-5p is overexpressed in glioma cell lines and glioma samples compared to the normal brain tissues, and its expression level is positively correlated with tumor grade of malignancy(35). In the core sub-network，the high Cytochrome P450, family 1, subfamily B, polypeptide 1 (CYP1B1) expression is a key role in glioma. CYP1B1 is involved in the xenobiotic detoxification metabolism and possibly activation of numerous procarcinogens and promutagens(36). Yu et al. demonstrate that extrasynaptic glutamate could in situ affect the arachidonic acid(AA) metabolism via brain CYP1B1, that can change the neuron-astrocyte reciprocal signaling(37). Besides，Wnt/beta-catenin signaling regulates endothelial metabolic barrier function through Cyp1b1 transcription (38). There are few studies on relative lnrna, and it is only reported in the papillary gyroid carcinoma that Overexpression of long noncoding RNA SLC26A4-AS1 inhibits the epithelial-mesenchymal transition via the MAPK pathway (39).

At present, lack of research on ceRNA in LGG may be due to the limitations of in vitro experiments. In addition, this study has some limitations, which need to be further verified in vitro and in vivo. The results and conclusions of this study can be used as the basis for the establishment of mechanical hypothesis and for further experiments of clinical samples and cell lines. We hope that the systematic analysis of the interaction of ceRNA will help us to gain a better understanding of the molecular pathogenesis of LGG.

Conclusion

In conclusion, we identified the differentially expressed mRNAs, lncRNAs and miRNAs by utilizing TCGA database, defined the relationship between them, and constructed a ceRNA network. According to comprehensive analysis, the key RNAs in the network can be selected as potential biomarkers for diagnosis and prognosis in LGG. This study is advantageous to deeply understand the biological mechanism of ceRNA and to clarify the pathogenesis of LGG.

Abbreviations

mRNA: Messenger RNA; lncRNA: Long non coding RNA; ncRNAs: Non coding RNAs; miRNAs: microRNAs; ceRNA: competitive endogenous RNA; LGGs: Low grade gliomas; WHO : World Health Organization;

TCGA: The Cancer Genome Atlas; GO: Gene Ontology; KEGG: Kyoto Encyclopedia of Genes and Genomes; BP: Biological process; CC: cell composition; MF: molecular function.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and material

The datasets used during the current study are available from TCGA.

Competing interests

The authors declare that they have no competing interests

Funding

The study was supported by General Technological Program of peking Education Commission (KM201810025023)

Authors' contributions

YM Ding and HJ Liu performed the data analyses and wrote the manuscript. ZS Bao and CB Zhang contributed significantly in manuscript revision. SQ Yu conceived and designed the study. All authors read and approved the final manuscript and agree to be accountable for all aspects of the research in ensuring that the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Acknowledgements

Not applicable.

References

1. Olar A, Sulman EP. Molecular markers in low-grade glioma-toward tumor reclassification. Semin Radiat Oncol. 2015;25:155–63.
2. Shaw EG, Wang M, Coons SW, et al. Randomized trial of radiation therapy plus procarbazine, lomustine, and vincristine chemotherapy for supratentorial adult low-grade glioma: initial results of RTOG 9802. J Clin Oncol. 2012;30:3065–70.
3. Buckner JC, Shaw EG, Pugh SL, et al. Radiation plus procarbazine, CCNU, and vincristine in low-grade glioma. N Engl J Med. 2016;374:1344–55.
4. Ju Cheol Son1, Hyoung Oh Jeong1. Park D, et al. miR-10a and miR-204 as a Potential Prognostic Indicator in Low-Grade Gliomas. J Cancer Informatics. 2017;16:1–6.
5. Pallud J, Audureau E, Blonski M, et al. Epileptic seizures in diffuse low-grade gliomas in adults. Brain. 2014;137:449–62.
6. Huberfeld G, echt V. C.J. Seizures and gliomas-towards a single therapeutic approach. Nat Rev Neurol. 2016;12:204–16.
7. Ling H, Fabbri M, Calin GA. MicroRNAs and other non-coding RNAs as targets for anticancer drug development. Nat Rev Drug Discov. 2013;12:847.
8. Kwok ZH, Tay Y. Long noncoding RNAs: Lincs between human health and disease. Biochem Soc Trans. 2017;45:805–12.
9. Salmena L, Poliseno L, Tay Y, et al. A ceRNA hypothesis: the Rosetta stone of a hidden RNA. language? Cell. 2011;146:353–8.
10. Fang X-N, Yin M1, Li H, et al. Comprehensive analysis of competitive endogenous RNAs network associated with head and neck squamous cell carcinoma. J Scientific Reports. 2018; 8:10544.
11. Beibei Liu T, Ma Q, Li, et al. Identification of a lncRNA–associated competing endogenous RNA–regulated network in clear cell renal cell carcinoma. Mol Med Rep. 2019;20:485–94.
12. Lina P, Wena D, Li Q, et al. Genome-Wide Analysis of Prognostic lncRNAs, miRNAs, and mRNAs Forming a Competing Endogenous RNA Network in Hepatocellular Carcinoma. Cell Physiol Biochem. 2018;48:1953–67.
13. Zhang YYao,Tingting,Lingyu Qi, et al. Competitive Endogenous RNA Network Construction and Comparison of Lung Squamous Cell Carcinoma in Smokers and Nonsmokers.J Disease Markers. 2019;5292787.
14. Shengrong L, Li G. Comprehensive analysis of a long non–coding RNA–mediated competitive endogenous RNA network in glioblastoma multiforme. J Experimental Therapeutic Medicine. 2019;18:1081–90.
15. Xiaojuan Wang Y, Ding B, Da, et al. Identification of potential prognostic long non–coding RNA signatures based on a competing endogenous RNA network in lung adenocarcinoma. J Oncology Reports. 2018;40:3199–212.
16. Qiaowei F. Bingrong liu. Comprehensive analysis of a long noncoding rna-associated competing endogenous RNA network in colorectal cancer. J Oncot argets Therapy. 2018;11:2453–66.

17. Liang W, Sun F. Competing Endogenous RNA Network Analysis Reveals Pivotal ceRNAs in Adrenocortical Carcinoma. Front Endocrinol (Lausanne). 2019;10:301.
18. Wang Y, Huang T, Sun X, et al. Identification of a potential prognostic lncRNA-miRNA-mRNA signature in endometrial cancer based on the competing endogenous RNA network. J Cell Biochem. 2019;20:18845–53.
19. Chou CH, et al. MiRTarBase: updates to the experimentally validated miRNA-target interactions database. Nucleic Acids Res. 2016;44:D239.
20. Fromm B, et al. A Uniform System for the Annotation of Vertebrate microRNA Genes and the Evolution of the Human microRNAome. Annu Rev Genet. 2015;49:213–42.
21. Li JH, Liu S, Zhou H, et al. Star Basev2.0: decoding miRNA-ceRNA, miRNA-ncRNA and protein-RNA interaction networks from large-scale CLIP-Seq data. Nucleic Acids Res. 2014;42:92–7.
22. Shannon P, Markiel A, Ozier O, et al. Cytoscape: A Software Environment for Integrated Models of Biomolecular Interaction Networks. Genome Res. 2003;13:2498–504.
23. Ma K, Zhao Q, Li S, et al. Competing endogenous RNA network in pulmonary arterial hypertension. Int J Cardiol. 2014;172:e527–8.
24. Nibbe RK, Chowdhury SA, Koyuturk M, et al. Protein-protein interaction networks and subnetworks in the biology of disease. Wiley Interdiscip Rev Syst Biol Med. 2011;3:357–67.
25. Zhang F, Zhang L, Qi Y, et al. Mitochondrial cAMP signaling. Cell Mol Life Sci. 2016;73:4577–90.
26. Zhang Y, Dong H, Duan L, et al. SLC1A2 Mediates Refractory Temporal Lobe Epilepsy with an Initial Precipitating Injury by Targeting the Glutamatergic Synapse Pathway. IUBMB Life. 2019;71:213–22.
27. Justin B-K, Hsu T-H, Chang GA, Lee, et al. Identification of potential biomarkers related to glioma survival by gene expression profile analysis. J BMC Medical Genomics. 2019;11:34.
28. Fu Q, Li S, Zhou Q, et al. Low LINC00599 expression is a poor prognostic factor in glioma. J Bioscience Reports. 2019;39:BSR20190232.
29. Yang Gao. Liangdong Li. Hui Zheng, et al. KIF3C is associated with favorable prognosis in glioma patients and may be regulated by PI3K/AKT/mTOR pathway. Journal of Neuro-Oncology. 2020;146:513–521.
30. Zhang B, Wu Q, Xu R, et al. The promising novel biomarkers and candidate small molecule drugs in lower-grade glioma: Evidence from bioinformatics analysis of high-throughput data. J Cell Biochem. 2019;120:15106–18.
31. Chen Lingchao W, Hanbing XW, et al. miR-137 is frequently down-regulated in glioblastoma and is a negative regulator of Cox-2. Eur J Cancer. 2012;48:3104–11.
32. Deng Danni X, Lian S, Naiyuan, et al. MiR-137 acts as a tumor suppressor in astrocytoma by targeting RASGRF1. Tumour Biol. 2016;37:3331–40.
33. Li KKa-Wai, Ling Y, Chung-Sean PJ, et al. MIR-137 suppresses growth and invasion, is downregulated in oligodendroglial tumors and targets CSE1L. Brain Pathol. 2013;23:426–39.

34. Guan S, Ying C, Lei S, et al. Overexpressed miRNA-137 inhibits human glioma cells growth by targeting Rac1. Cancer Biother Radiopharm. 2013;28:327–34.
35. Wang Kun J, Zhifan Z, Jian, et al. Analysis of hsa-miR-30a-5p expression in human gliomas. Pathol Oncol Res. 2013;19:405–11.
36. Rieder CR, Parsons RB, Fitch NJ, et al. Human brain cytochrome P450 1B1: immunohistochemical localization in human temporal lobe and induction by dimethylbenz(a)anthracene in astrocytoma cell line (MOG-G-CCM). Neurosci Lett. 2000;278:177–80.
37. Yu Xuming Wu, Juan Hu, Mingbai, et al. Glutamate affects the CYP1B1- and CYP2U1-mediated hydroxylation of arachidonic acid metabolism via astrocytic mGlu5 receptor. Int J Biochem Cell Biol. 2019;110:111–21.
38. Ziegler Nicole A, Khader F, Beate, et al. Beta-Catenin Is Required for Endothelial Cyp1b1 Regulation Influencing Metabolic Barrier Function. J Neurosci. 2016;36:8921–35.
39. Wang DP, Tang XZ, Liang QK, et al. Overexpression of long noncoding RNA SLC26A4-AS1 inhibits the epithelial-mesenchymal transition via the MAPK pathway in papillary thyroid carcinoma. J Cell Physiol. 2020;235:2403–13.

Figures

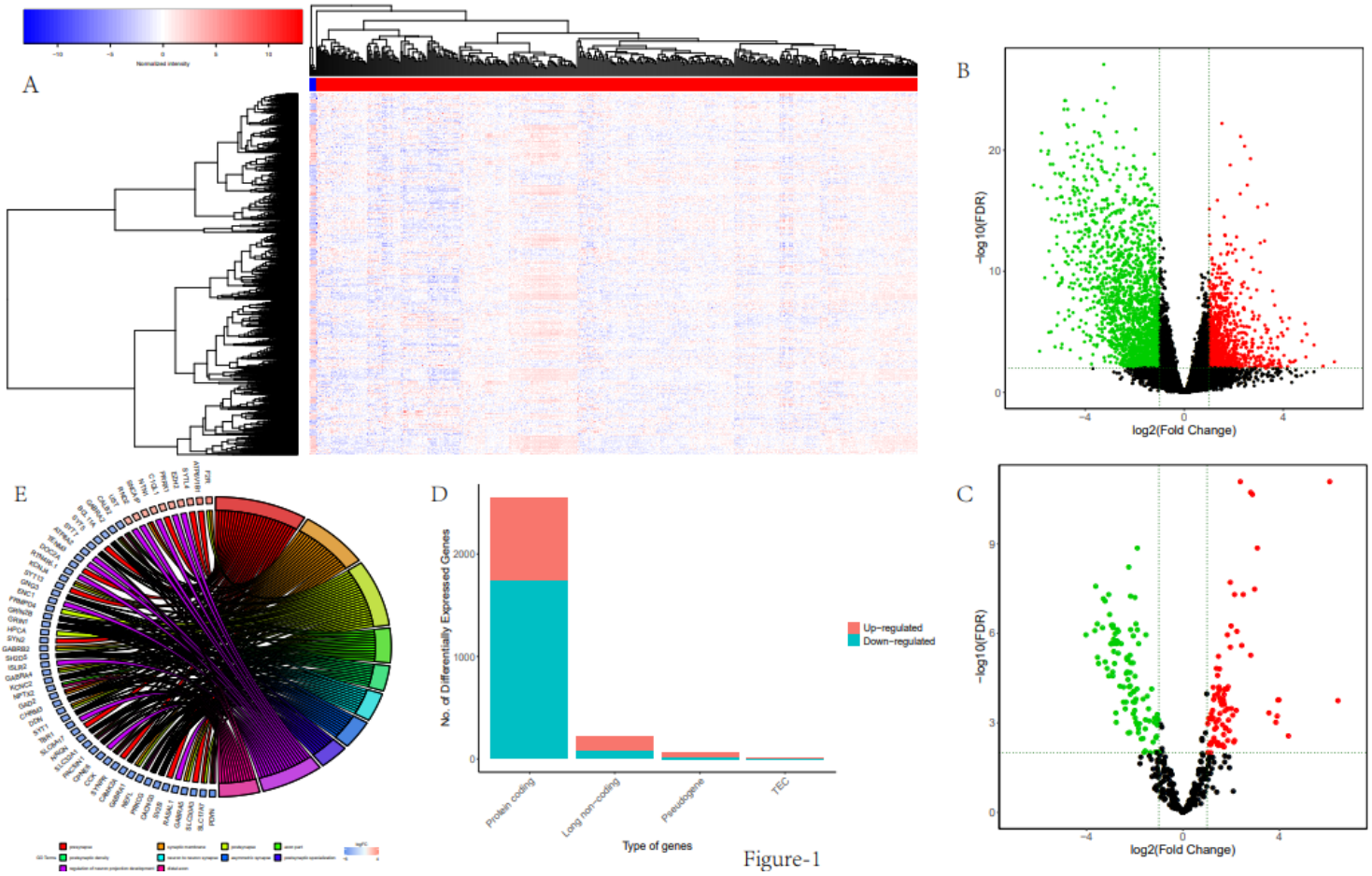

Figure 1

A Clustered heat maps of the differentially expressed RNAs in LGG; B Volcano plot of differentially expressed RNAs in LGG; C Volcano plot of differentially expressed miRNAs in LGG; D Type of differentially expressed RNAs; E The GO enrichment networks of BP, CC and MF for genes

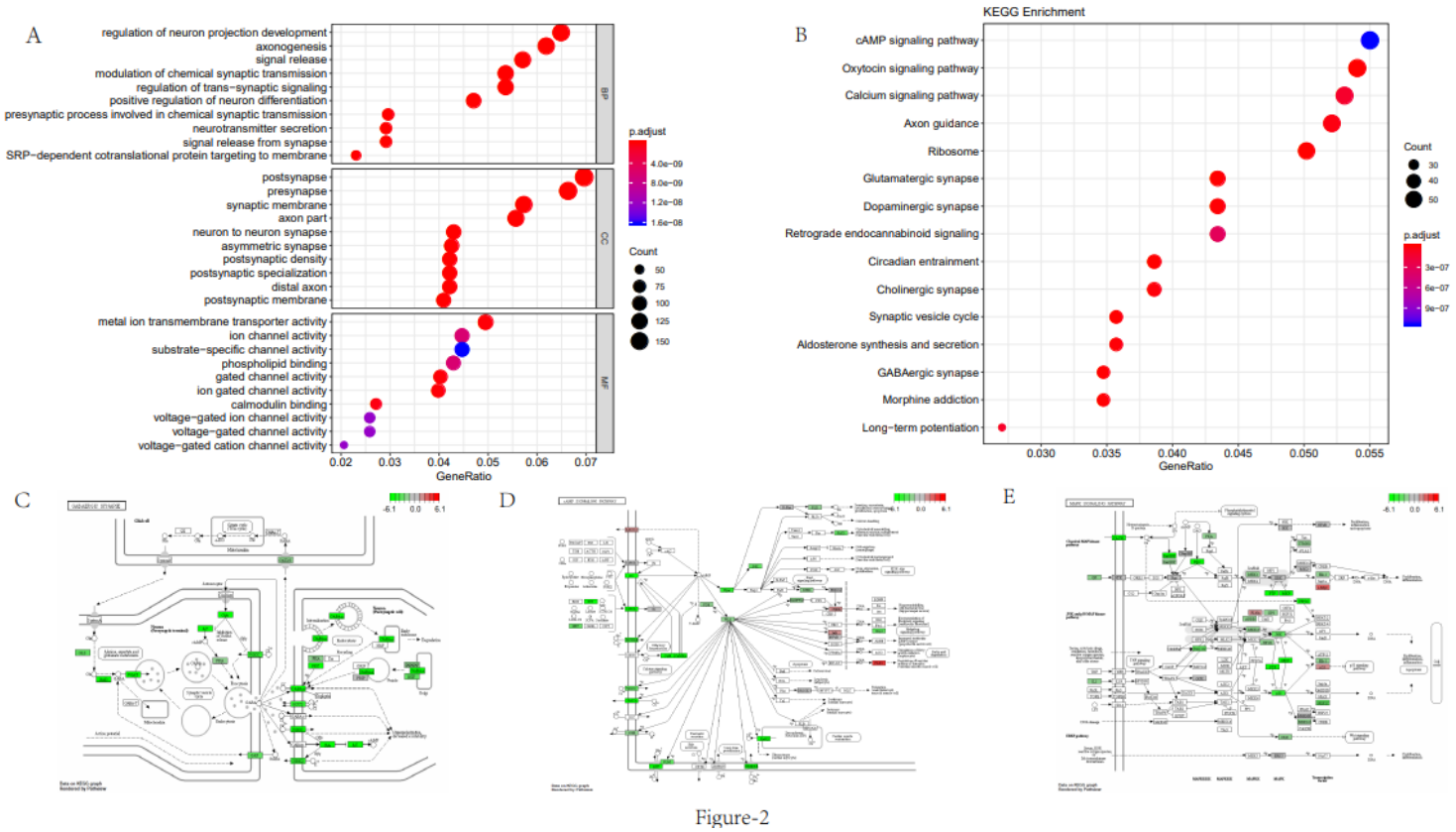

Figure-2

Figure 2

A Differentially expressed mRNAs results of GO analysis; B Differentially expressed mRNAs results of KEGG analysis; KEGG pathways enriched by the differentially expressed mRNAs in ceRNA network. (C) GABAergic synapse signaling pathway; (D)cAMP signaling pathway; (E) MAPK signaling pathway;

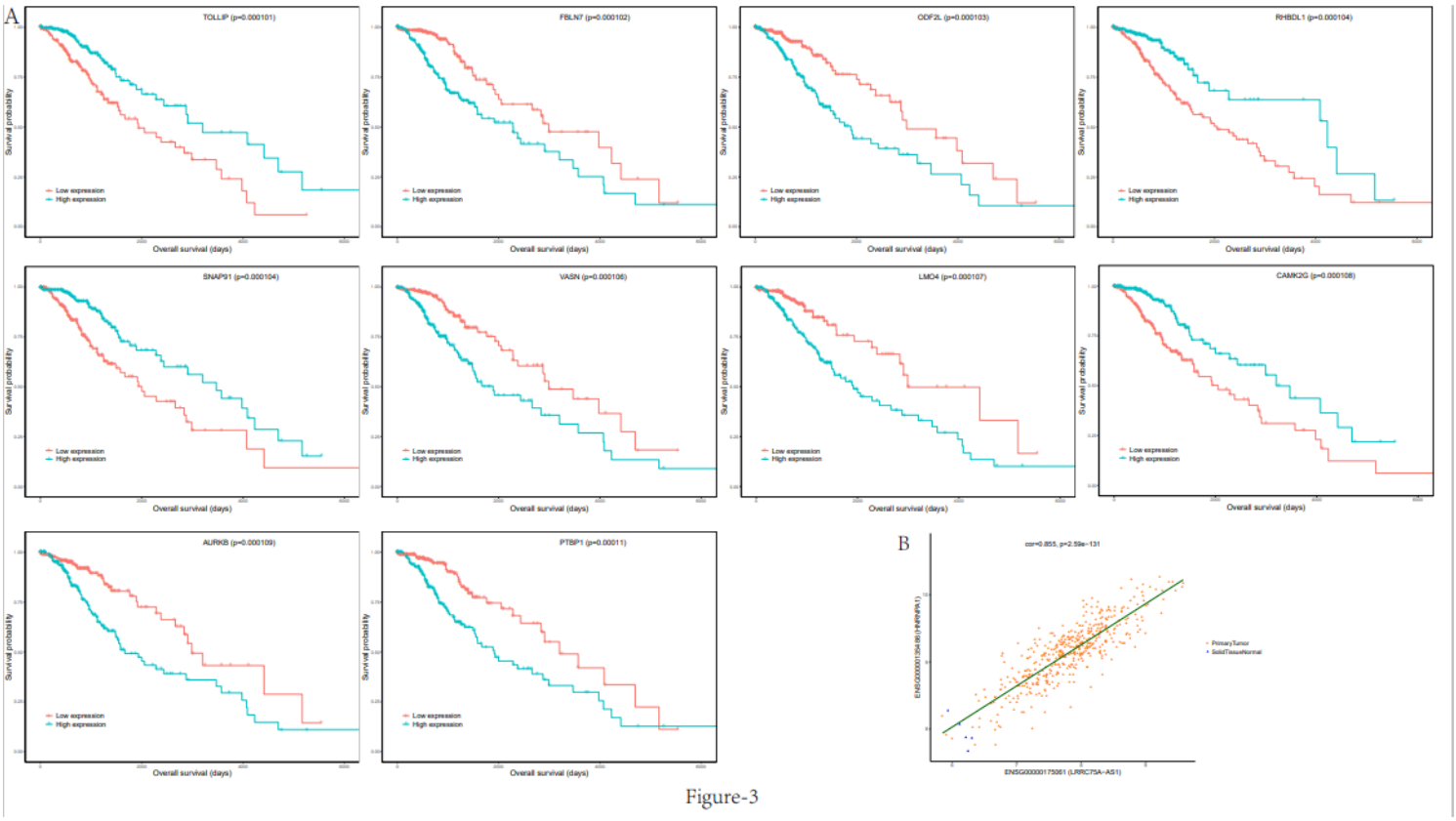

Figure-3

Figure 3

A Kaplan Meier survival analysis for 10 identified differentially expressed mRNAs;B Linear regression of HNRNPA1 and LRRC75A-AS1 expression level.

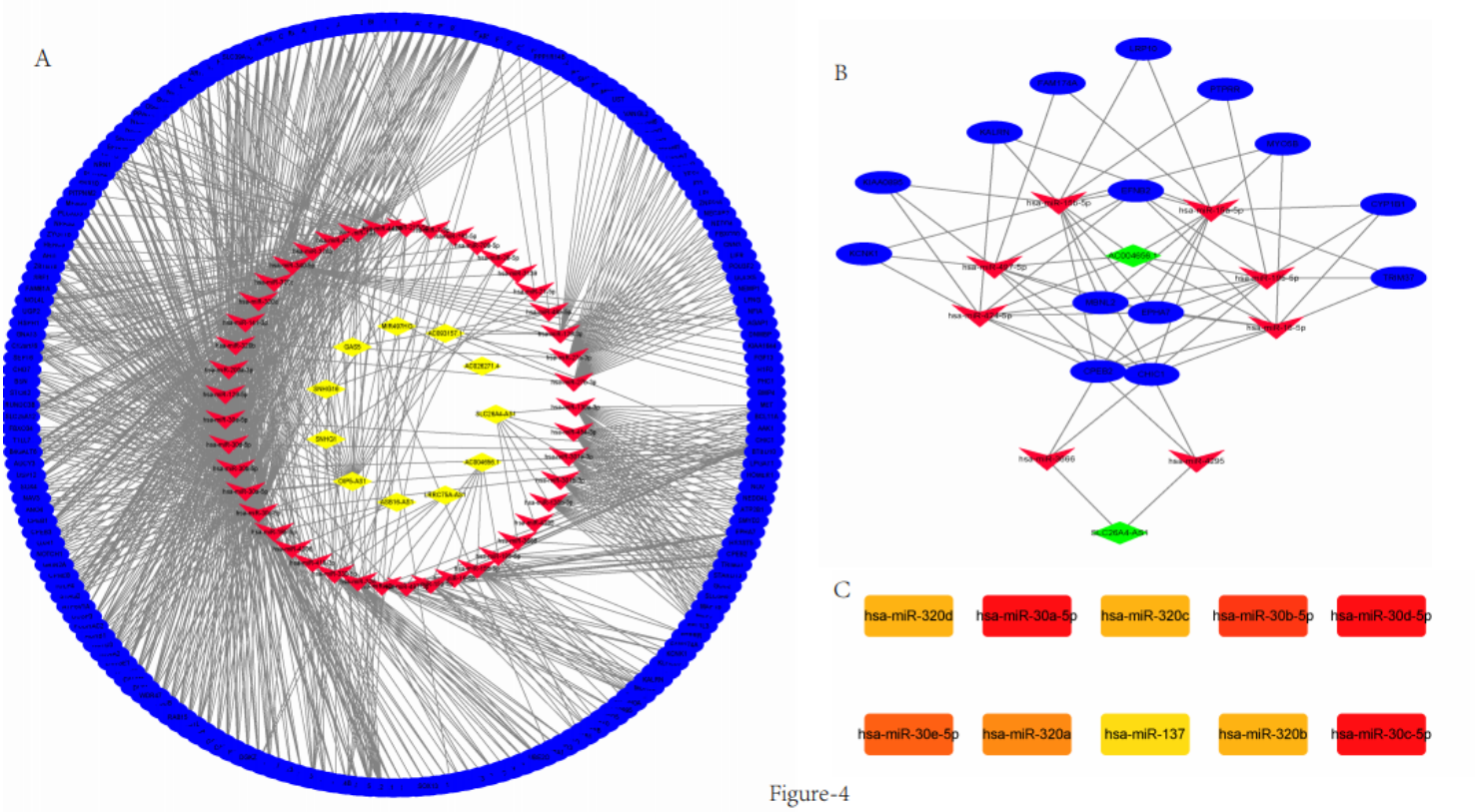

Figure-4

Figure 4

A Construction of ceRNA network. The blue ellipse represent differentially expressed mRNAs; the yellow diamonds represent differentially expressed lncRNAs; the red v-shaped represent differentially expressed miRNAs; B The highly interconnected regions in ceRNA network by MCODE; C The top ten hub miRNAs are calculated by cytohubba