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RESEARCH ARTICLE

Construction of lncRNA-associated ceRNA networks to identify prognostic lncRNA biomarkers for glioblastoma

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Abstract

Long noncoding RNAs (lncRNAs) serve as competitive endogenous RNAs (ceRNAs) that play significant regulatory roles in the pathogenesis of tumors. However, the role of lncRNAs, especially the lncRNA-related ceRNA regulatory network, in glioblastoma (GBM) has not been fully elucidated. The goal of the current study was to construct lncRNA-microRNA-mRNA-related ceRNA networks for further investigation of their mechanism of action in GBM. We downloaded data from The Cancer Genome Atlas (TCGA) and the Gene Expression Omnibus (GEO) databases and identified differential lncRNAs, microRNAs (miRNAs), and messenger RNAs (mRNAs) associated with GBM. A ceRNA network was constructed and analyzed to examine the relationship between lncRNAs and patients' overall survival. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGGs) were used to analyze the related mRNAs to indirectly explain the mechanism of action of lncRNAs. The potential effective drugs for the treatment of GBM were identified using the connectivity map (CMap). After integrated analysis, we obtained a total of 210 differentially expressed lncRNAs, 90 differentially expressed miRNAs, and 2508 differentially expressed mRNAs (DEmRNAs) from the TCGA and GEO databases. Using these differential genes, we constructed a lncRNA-associated ceRNA network. Six lncRNAs in the ceRNA network were associated with the overall survival of patients with GBM. Through KEGG analysis, it was found that the DEmRNAs involved in the network are related to cancer-associated pathways, for instance, mitogen-activated protein kinase and Ras signaling pathways. CMap analysis revealed four small-molecule compounds that could be used as drugs for the treatment of GBM. In this study, a multi-database joint analysis was used to construct a lncRNA-related ceRNA network to help identify the regulatory functions of lncRNAs in the pathogenesis of GBM.

KEYWORDS

ceRNA network, CMap, DElncRNA, DEmiRNA, DEmRNA, glioblastoma

1 | INTRODUCTION

Glioblastoma (GBM) is a highly malignant intracranial brain tumor in the central nervous system and has a high mortality rate.[1] Despite the aggressiveness of current treatments, their effects on GBM are limited, which may be because of the insufficient understanding of its pathogenesis. Genetics play a significant regulatory role in the occurrence and development of cancers. Owing to the advent of high-throughput sequencing technology, several long noncoding RNAs (lncRNAs) have been discovered, some of which appear to have a significant regulatory function in the pathogenesis of GBM.[2]

lncRNAs, which are more than 200 nucleotides in length and do not encode proteins, have been shown to have important regulatory functions in various diseases, including GBM.[3] For example, knockdown of the lncRNA HMMR-AS1 inhibits the proliferation of GBM and increases its sensitivity to chemotherap.[4] High expression of the oncogene *HOTAIRM1* can promote the proliferation and migration of GBM cells, which can be achieved through the regulation of *HOXA1*.[5] *MNX1-AS1* antagonizes *miR-4443* through competitive inhibition and thereby increases GBM proliferation and migration.[6] The competitive endogenous RNA (ceRNA) hypothesis suggests that lncRNAs can bind to microRNA (miRNA) response elements through sponging and influence their regulation of downstream gene expression.[7] Therefore, the ceRNA hypothesis plays a significant regulatory function in the pathological process of various cancers.

There have been some reports on lncRNA-related ceRNA networks in GBM; however, to the best of our knowledge, this study is the first study that involves the analysis of large-scale samples, especially through integrated analysis of multiple databases. In addition, this study analyzed the overall survival of differentially expressed lncRNAs (DElncRNAs) in patients with GBM and employed differentially expressed messenger RNAs (DEmRNAs) to identify four small-molecule compounds that may be potential drugs for the treatment of GBM. Therefore, we believe that the lncRNA-related ceRNA networks constructed in this study will contribute to the study of the role of DElncRNAs in the pathogenesis of GBM.

2 | EXPERIMENTAL METHODS AND DESIGN

2.1 | Data collection

Series matrix files of the GSE63319 and GSE103227 datasets were downloaded from the Gene Expression Omnibus database (https://www.ncbi.nlm.nih.gov/geo/). The GSE63319 dataset included miRNA microarray data of 11 GBM tissues and four epileptic tissues and was based on GPL16384 Affymetrix Multispecies miRNA-3 Array (Affymetrix, Inc, Santa Clara, CA).[8] The GSE103227 dataset, which comprised gene expression microarray data and lncRNA microarray data, was based on GPL16956 Platforms (Agilent-045997 Arraystar human lncRNA microarray V3 (Probe Name Version), Arraystar Inc, Rockville, MD) and included data from five GBM and five normal brain tissues.[9] The RNA-seq data expression profiles of GBM were obtained from The Cancer Genome Atlas (TCGA) public database (http://cancergenome.nih.gov/) (as of December, 2018) using the Data Transfer Tool, which included data of 169 GBM and five non-tumoral brain samples, for DEmRNA and DElncRNA analysis, and the clinical information of the cases was also collected. All glioma specimens were obtained from patients with gliomas that were removed during surgery. The glioma tissue samples were immediately frozen in liquid nitrogen and stored at −80℃ until they were used. According to the classification rules issued by WHO in 2007, neuropathologists divided these glioma tissue samples into two groups: grade III (n = 8) and grade IV (n = 22). Nontumor brain tissue was used as the negative control group (n = 5). Informed consent was obtained from patients to collect tissue samples for the study, and the study was reviewed and approved by the Ethics Committee of the First Affiliated Hospital of Harbin Medical University.

2.2 | Identification of DEmRNAs, DEmiRNAs, and DElncRNAs from Gene Expression Omnibus or TCGA

We used the limma package in R to identify DEmRNAs, DElncRNAs, and DEmiRNAs that were differentially expressed between GBM and non-tumoral brain tissues in the GSE63319 and GSE103227 datasets, with thresholds set at log fold change (logFC) > 1 and logFC < −1, while differences with a $P < .05$ were considered significant.[10] Using edgeR Bioconductor package in the R software,[11] with thresholds of logFC > 1 and logFC < −1 with FDR < 0.05, we identified the DElncRNAs and the DEmRNAs in the data of 169 GBM and five non-tumoral brain samples obtained from the TCGA database. Integrated bioinformatical analyses were conducted using online tools (http://bioinfogp.cnb.csic.es/tools/venny/).

2.3 | ceRNA network construction

First, we compared the dysregulated lncRNAs between Gene Expression Omnibus (GEO) and TCGA GBM samples and only retained the data of overlapping DElncRNAs. Then, we used the starBase v3.0 to convert the names of the

DEmiRNAs into mature miRNA names. Next, the relationships between the lncRNAs and miRNAs were defined using the miRcode database (http://www.mircode.org). To further investigate the roles of DEmiRNAs in GBM, we used the following three databases to predict the target genes of the miRNAs:miRTarBase, TargetScan, and miRDB.[12] Then, to further ensure the reliability of the constructed ceRNA network, we compared the predicted target miRNAs and mRNAs with the dysregulated miRNAs and mRNAs in the TCGA or GEO GBM samples and retained only the data of the overlapping miRNAs and mRNAs and their interaction pairs for further analysis. Finally, Cytoscape (version3.6.1) was employed to construct the lncRNA-miRNA-mRNA network for visualization.

2.4 | Functional annotation and pathway analysis and survival analysis

ClusterProfiler, an R package, is one of the most common tools used for the analysis and classification of biological terms as well as enrichment analysis of genes.[13] We used ClusterProfiler to perform functional annotation of Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genome (KEGG) for the mRNAs involved in the constructed lncRNA-related network, where a $P < .05$ was set as the cut-off criteria. To screen prognostic signature DElncRNAs, the R package "survival" was used to analyze the correlation between lncRNAs and the survival time in the clinical data of GBM obtained from TCGA.[14] $P<.05$ were considered as the cut-off value.

2.5 | Construction of protein-protein interaction networks

To further understand the protein-protein interactions in DEmRNAs, a protein-protein interaction (PPI) network was constructed by employing the Search Tool for the Retrieval of Interacting Gene (STRING) online database (https://string-db.org/),[10] accompanied by the Cytoscape software (version3.6.1). The datasets with a combined score of greater than 0.4 were incorporated into the PPI network.

2.6 | CMap for DEmRNAs analysis of drug molecules for GBM

To unearth small-molecule drugs for treating GBM, the connectivity map (CMap) database, a drug development tool (https://portals.broadinstitute.org/CMap/), was employed. This tool was used to identify the therapeutic effect of drugs by using DEmRNAs.[14] DEmRNAs, which contained two lists of upregulated and downregulated genes, were uploaded into the CMap web tool for CMap analysis. The negatively related molecular agents ($P < .01$ and enrichment <0) for anti-GBM were then identified.

2.7 | RNA isolation and reverse transcription-quantitative polymerase chain reaction analysis

Total RNA was isolated from the glioma tissues and nontumor brain tissues using Tri®-Reagent (Sigma) according to the manufacturer's instructions. The quality and quantity of the RNA were detected by 260/280 nm absorbance using the NanoDrop One spectrophotometer (Thermo Fisher Scientific, USA). Reverse transcription of the extracted total RNA into complementary DNA (cDNA) was performed using the Transcriptor First-Stand cDNA Synthesis Kit (Roche). Reverse transcription-quantitative polymerase chain reaction (RT-qPCR) was performed using the FastStart Universal SYBR Green Master (ROX) (Roche, Germany) and quantified using the QuantStudio software (Thermo Fisher Scientific) according to the manufacturer's instructions. Glyceraldehyde 3-phosphate dehydrogenase was used as the endogenous reference gene. The expression level of all mRNAs was determined using the $2^{-\Delta\Delta C_t}$ method. The sequence of all primers used in this study is provided in Table S1. A meaningful analysis between the two groups was performed by an unpaired the t test, and a $P < .05$ was considered statistically significant.

3 | RESULTS

3.1 | Differentially expressed RNAs in GBM

From the TCGA database, we collected data from 174 tissues, including those of 169 GBM and five nontumor brain samples. By setting the cut-off value at $\log FC > 1$ and $\log FC < -1$ with false discovery rate and $P < .05$, we identified 869 upregulated lncRNAs, 774 downregulated lncRNAs, 3327 upregulated mRNAs, and 3008 downregulated mRNAs (Figure S1A,B). From the GEO database, we collected the microarray data of GSE103227, including five GBM and five non-tumoral brain samples, and the microarray data of GSE63319, including 11 GBM and four epileptic tissues. We identified 698 upregulated lncRNAs, 629 downregulated lncRNAs, 1927 upregulated mRNAs, and 1836 downregulated mRNAs in GSE103227 (Figure S1C,D) and 40 upregulated miRNAs and 50 downregulated miRNAs (Figure S1E) in GSE63319 with thresholds set at $\log FC > 1$ and $\log FC < -1$ with $P < .05$. After integrated bioinformatical analyses, we identified a total of 210 DElncRNAs and 2508 DEmRNAs from the TCGA and GEO databases,

including 97 upregulated DElncRNAs (Figure 1A), 113 downregulated DElncRNAs (Figure 1B), 1188 upregulated DEmRNAs (Figure 1C), and 1320 downregulated DEmRNAs (Figure 1D) in the GBM tissues compared with the normal brain tissues.

3.2 | The ceRNA network in GBM

To comprehend the functions of DElncRNAs in GBM, we chose the interaction pairs of lncRNAs-miRNAs from the miRcode databases and the interaction pairs of miRNAs-mRNAs from the miRTarBase, TargetScan, and miRDB databases and constructed an abnormal ceRNA network of lncRNA-miRNA-mRNA. The results revealed that a total of 34 DElncRNAs and 14 DEmiRNAs formed 104 interaction pairs, while a total of 12 DEmiRNAs and 852 DEmRNAs formed 920 interaction pairs. Further, we integrated the candidate mRNAs and the DEmRNAs to obtain the final target DEmRNAs of the DEmiRNAs. Finally, a total of 29 DElncRNAs, 11 DEmiRNAs, and 113 DEmRNAs were employed to construct the ceRNA network using Cytoscape, as shown in Figure 2.

3.3 | The GO and the KEGG pathway enrichment analysis

The GO functional enrichment and the KEGG pathway enrichment analyses were performed on DEmRNAs in the lncRNA-related ceRNA network. Using the ClusterProfile package in R software, GO analysis showed that DEmRNAs were associated with "DNA binding," "histone acetyltransferase binding," "transmembrane receptor protein tyrosine kinase activity," and so on (Figure 3; Table 1). Pathway analysis using the ClusterProfiler package showed that DEmRNAs have a clear relationship with a variety of cancer-associated pathways, such as MAPK signaling pathway, apelin signaling pathway, Ras signaling pathway, forkhead box O signaling pathway, and autophagy-animal (Figure 4; Table 2).

3.4 | Construction of protein-protein interaction networks

To construct a PPI network, we used the online tool STRING database to explore the relationship between DEmRNAs in the ceRNA network. We used Cytoscape

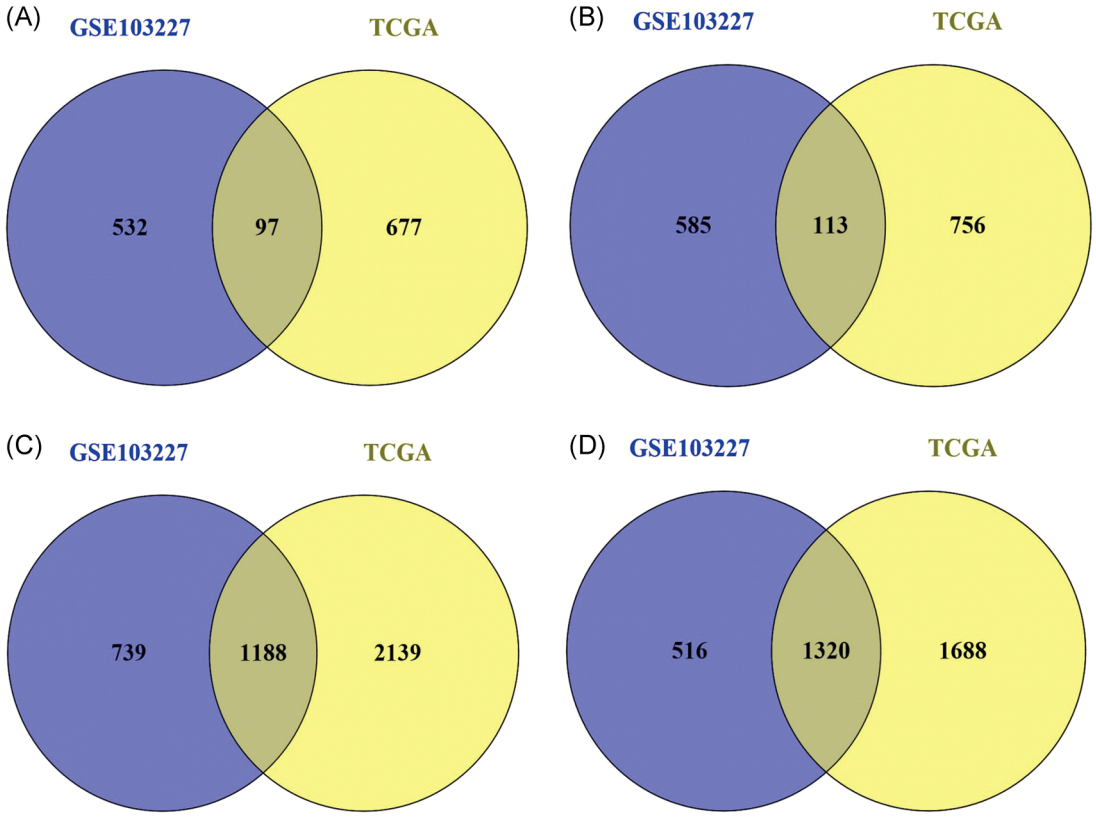

FIGURE 1 Identification of consistent expression of DElncRNAs and DEmRNAs in GSE103227 and TCGA using the online tool Venny for integrated analysis. A, Represents upregulated DElncRNAs. B, Represents downregulated DElncRNAs. C, Represents upregulated DEmRNAs. D, Represents downregulated DEmRNAs. DEmRNA, differentially expressed messenger RNA; DElncRNA, differentially expressed long noncoding RNA; TCGA, The Cancer Genome Atlas

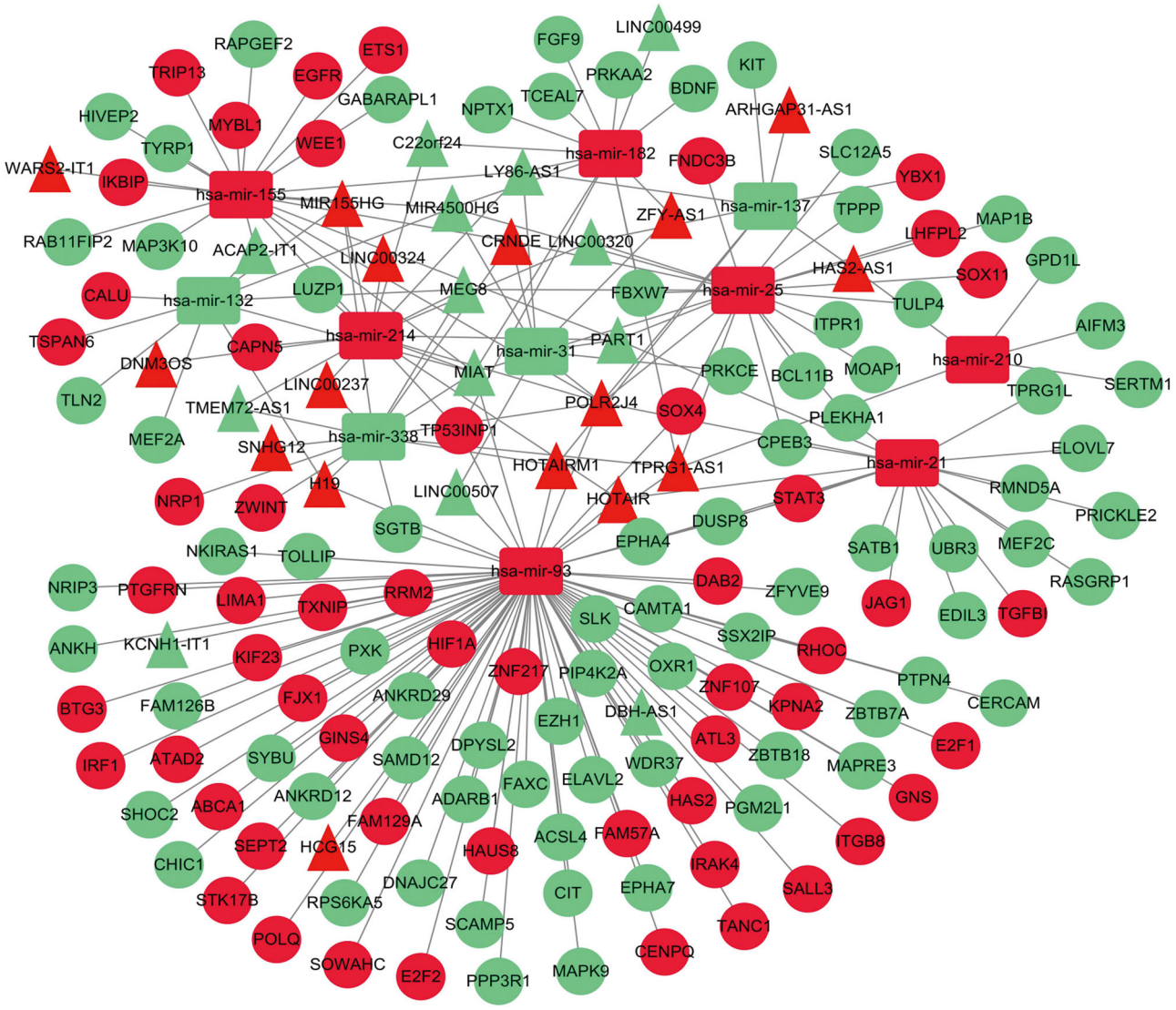

FIGURE 2 The ceRNA network of lncRNA-miRNA-mRNA in GBM. Red nodes represent upregulated DERNAs and green nodes represent downregulated DERNAs. DElncRNAs, DEmiRNAs, and DEmRNAs are represented by triangles, rectangles, and ellipses, respectively. ceRNA, competitive endogenous RNA; DEmRNAs, differentially expressed messenger RNA; GBM, glioblastoma; lncRNA, long noncoding RNA; miRNA. microRNA; mRNA, messenger RNA

software as a tool for PPI network visualization (Figure 5A). Moreover, based on the degree of linkage between DEmRNAs and the hub gene, we identified the top ten DEmRNAs as the hub genes with the highest degree of connectivity, including *EGFR, KIT*, *IRAK4*, *RHOC*, *SHOC2*, *STAT3*, *BDNF*, *EPHA4*, *MAPK9*, and *MEF2A* (Figure 5B).

3.5 | Relevant lncRNAs associated with survival of patients with GBM

To explore the prognostic capability of DElncRNAs in GBM, 29 DElncRNAs in the ceRNA network and the overall survival of GBM patients were analyzed using Kaplan-Meier curve analysis. The results showed that six of 29 DElncRNAs had a significant relationship with overall survival ($P < .05$). These six DElncRNAs were *DBH-AS1*, *MIR155HG*, *DNM3OS*, *CRNDE*, *HOTAIRM1*, and *HOTAIR* and were negatively correlated with overall survival (Figure 6).

3.6 | Experiments verify the six DElncRNAs associated with survival in GBM

To validate the results of the six survival-related DElncRNAs in our constructed ceRNA network as a potential biomarker in GBM, we used RT-qPCR to detect their

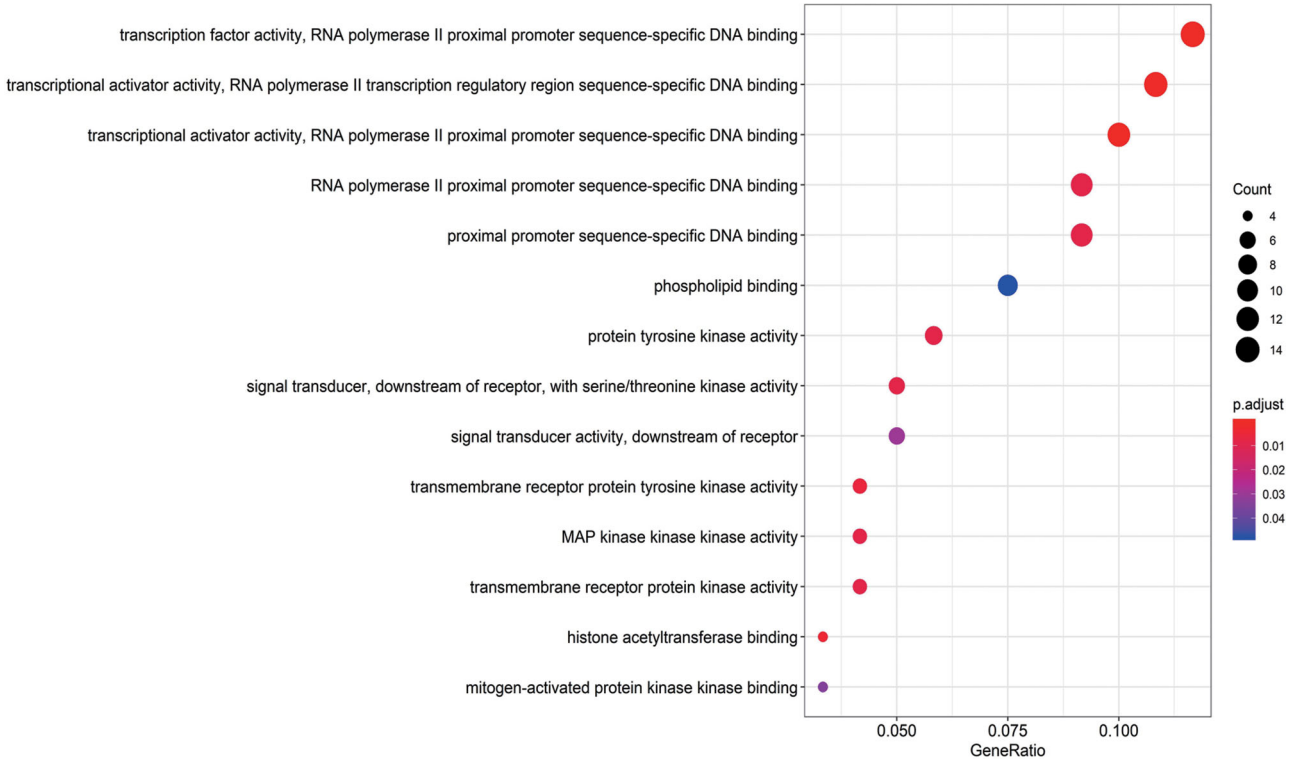

FIGURE 3 GO functional enrichment analysis of DEmRNAs in lncRNA-related ceRNA networks. The main parameters comprised the gene ratio, counts, and adjusted *P* values. ceRNA, competitive endogenous RNA; DEmRNAs, differentially expressed messenger RNA; GO, Gene Ontology; lncRNA, long noncoding RNA

expression levels in 30 glioma tissues and five nontumor brain tissues. We used multi-database joint analysis to show that the expression levels of *MIR155HG*, *DNM3OS*, *CRNDE*, *HOTAIRM1*, and *HOTAIR* were increased in glioma tissues, and our RT-qPCR results were also consistent with the analysis results (Figure 7A-E). Furthermore, the results showed that the expression level of DBH-AS1 was significantly lower in glioma tissues, which was consistent with our analysis (Figure 7F). In addition, we validated the relationship between the expression levels of *DBH-AS1*, *MIR155HG*, *DNM3OS*, *CRNDE*, *HOTAIRM1*, and *HOTAIR* and the overall survival of 30 glioma patients using Kaplan-Meier curve analysis (Figure 8).

3.7 | Identification of four small-molecule compounds for the treatment of GBM based on CMap analysis

We uploaded 54 upregulated DEmRNAs and 79 down-regulated DEmRNAs from the ceRNA network to the CMap website. According to the shear standard, a total of four small-molecule compounds are available that can be potential drugs for the treatment of patients with GBM. A schematic diagram of the 2D and 3D structure of these compounds is shown in Figure 9 and the corresponding data have been provided in Table 3.

4 | DISCUSSION

GBM accounts for a vast majority of malignant brain tumors in the central nervous system and has high mortality and disability rates.[1] Although aggressive treatments, such as maximal resection, adjuvant radiotherapy, and chemotherapy are used for patients with GBM, the survival rate is still difficult to improve,[15] due to the poor understanding of the pathogenesis of GBM and the lack of an effective therapeutic target. Previous studies have reported that differential genes play an important role in the pathology of tumors and may be potential therapeutic targets.[16–18] To understand and identify potential therapeutic targets in GBM, we explored the mechanisms underlying the pathogenesis of GBM in this study. Several recent studies have shown that lncRNAs play an important role in the pathological processes of tumors,[19,20] but few reports have focused on the expression profiles of lncRNAs in GBM.

lncRNAs have complex roles in the regulation of multiple signaling pathways, and the ceRNA network hypothesis explains the complex relationship of

TABLE 1 GO functional annotation enrichment analysis of DEmRNAs in lncRNA-related ceRNA networks in GBM

ID	Description	Adjusted *P* values	Count	Gene names
GO:0001077	Transcriptional activator activity, RNA polymerase II proximal promoter sequence-specific DNA binding	.000155667	12	MEF2C/BCL11B/CAMTA1/MEF2A/STAT3/MYBL1/ETS1/IRF1/HIF1A/YBX1/SOX4/SOX11
GO:0000982	Transcription factor activity, RNA polymerase II proximal promoter sequence-specific DNA binding	.000155667	14	MEF2C/BCL11B/ZBTB7A/CAMTA1/MEF2A/STAT3/MYBL1/ETS1/IRF1/HIF1A/ZNF217/YBX1/SOX4/SOX11
GO:0001228	Transcriptional activator activity, RNA polymerase II transcription regulatory region sequence-specific DNA binding	.000615708	13	MEF2C/BCL11B/CAMTA1/MEF2A/STAT3/MYBL1/ETS1/IRF1/HIF1A/YBX1/SOX4/SOX11/E2F2
GO:0035035	Histone acetyltransferase binding	.003570336	4	ZBTB7A/MEF2A/ETS1/HIF1A
GO:0004714	Transmembrane receptor protein tyrosine kinase activity	.005813928	5	EPHA7/EPHA4/KIT/NRP1/EGFR
GO:0004702	Signal transducer, downstream of receptor, with serine/threonine kinase activity	.008051205	6	MAP3K10/MAPK9/EPHA7/EPHA4/KIT/EGFR
GO:0000978	RNA polymerase II proximal promoter sequence-specific DNA binding	.008051205	11	MEF2C/BCL11B/ZBTB7A/MEF2A/STAT3/MYBL1/ETS1/IRF1/HIF1A/ZNF217/YBX1
GO:0004709	MAPK activity	.008051205	5	MAP3K10/EPHA7/EPHA4/KIT/EGFR
GO:0004713	Protein tyrosine kinase activity	.008051205	7	FGF9/EPHA7/EPHA4/KIT/NRP1/WEE1/EGFR
GO:0019199	Transmembrane receptor protein kinase activity	.008051205	5	EPHA7/EPHA4/KIT/NRP1/EGFR
GO:0000987	Proximal promoter sequence-specific DNA binding	.008051205	11	MEF2C/BCL11B/ZBTB7A/MEF2A/STAT3/MYBL1/ETS1/IRF1/HIF1A/ZNF217/YBX1
GO:0005057	Signal transducer activity, downstream of receptor	.029668514	6	MAP3K10/MAPK9/EPHA7/EPHA4/KIT/EGFR
GO:0031434	MAPK binding	.034182191	4	EPHA7/EPHA4/KIT/EGFR
GO:0005543	Phospholipid binding	.047810486	9	ITPR1/PLEKHA1/RAPGEF2/TULP4/RASGRP1/PXK/ZFYVE9/ABCA1/JAG1

Abbreviations: ceRNA, competitive endogenous RNA; DEmRNAs, differentially expressed messenger RNA; GBM, glioblastoma; GO, Gene Ontology; lncRNA, long noncoding RNA; MAPK, mitogen-activated protein kinase.

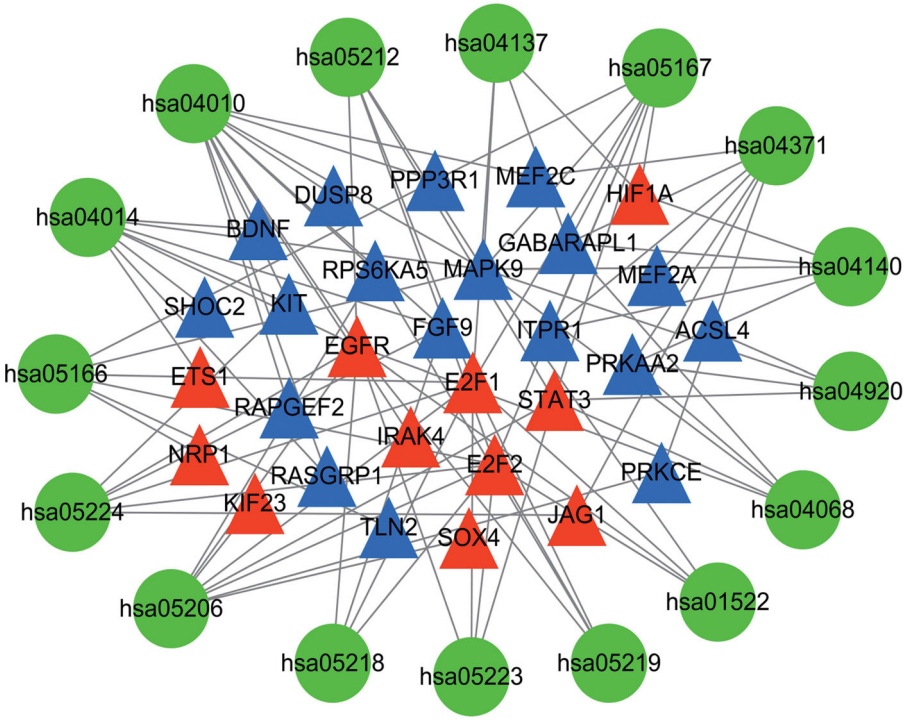

FIGURE 4 KEGG cell signaling pathway analysis of DEmRNAs in lncRNA-related ceRNA networks. Red nodes represent upregulated DEmRNAs, blue nodes represent downregulated DEmRNAs, and green nodes represent the ID of cell signaling pathways. DEmRNAs and the ID of cell signaling pathways are represented by triangles and circles, respectively. ceRNA, competitive endogenous RNA; DEmRNAs, differentially expressed messenger RNA; KEGG, Kyoto Encyclopedia of Genes and Genome; lncRNA, long noncoding RNA

TABLE 2 KEGG cell signaling pathway analysis of DEmRNAs in lncRNA-related ceRNA networks in GBM

ID	Description	Adjusted *P* value	Count	Gene names
hsa04010	MAPK signaling pathway	.000435264	12	BDNF/DUSP8/MEF2C/FGF9/MAPK9/RAPGEF2/PPP3R1/RASGRP1/RPS6KA5/KIT/IRAK4/EGFR
hsa04371	Apelin signaling pathway	.005667834	7	PRKCE/ITPR1/GABARAPL1/MEF2C/PRKAA2/MEF2A/JAG1
hsa05167	Kaposi sarcoma-associated herpesvirus infection	.005667834	8	ITPR1/GABARAPL1/MAPK9/PPP3R1/STAT3/E2F1/HIF1A/E2F2
hsa05219	Bladder cancer	.010469554	4	RPS6KA5/E2F1/EGFR/E2F2
hsa05212	Pancreatic cancer	.010469554	5	MAPK9/STAT3/E2F1/EGFR/E2F2
hsa04014	Ras signaling pathway	.012910378	8	BDNF/FGF9/MAPK9/RASGRP1/KIT/SHOC2/ETS1/EGFR
hsa05224	Breast cancer	.022422348	6	FGF9/KIT/E2F1/JAG1/EGFR/E2F2
hsa01522	Endocrine resistance	.022422348	5	MAPK9/E2F1/JAG1/EGFR/E2F2
hsa05166	Human T-cell leukemia virus 1 infection	.028574019	7	TLN2/MAPK9/PPP3R1/E2F1/ETS1/NRP1/E2F2
hsa04137	Mitophagy-animal	.028574019	4	GABARAPL1/MAPK9/E2F1/HIF1A
hsa05223	Non-small cell lung cancer	.028574019	4	STAT3/E2F1/EGFR/E2F2
hsa04920	Adipocytokine signaling pathway	.030871251	4	MAPK9/PRKAA2/ACSL4/STAT3
hsa05206	MiRNAs in cancer	.030899348	8	PRKCE/RPS6KA5/STAT3/E2F1/SOX4/KIF23/EGFR/E2F2
hsa05218	Melanoma	.03094176	4	FGF9/E2F1/EGFR/E2F2
hsa04140	Autophagy-animal	.038917517	5	ITPR1/GABARAPL1/MAPK9/PRKAA2/HIF1A
hsa04068	FoxO signaling pathway	.041647437	5	GABARAPL1/MAPK9/PRKAA2/STAT3/EGFR

Abbreviations: ceRNA, competitive endogenous RNA; DEmRNAs, differentially expressed messenger RNA; FoxO, forkhead box O; GBM, glioblastoma; KEGG, Kyoto Encyclopedia of Genes and Genome; lncRNA, long noncoding RNA; MAPK, mitogen-activated protein kinase; miRNA, microRNA.

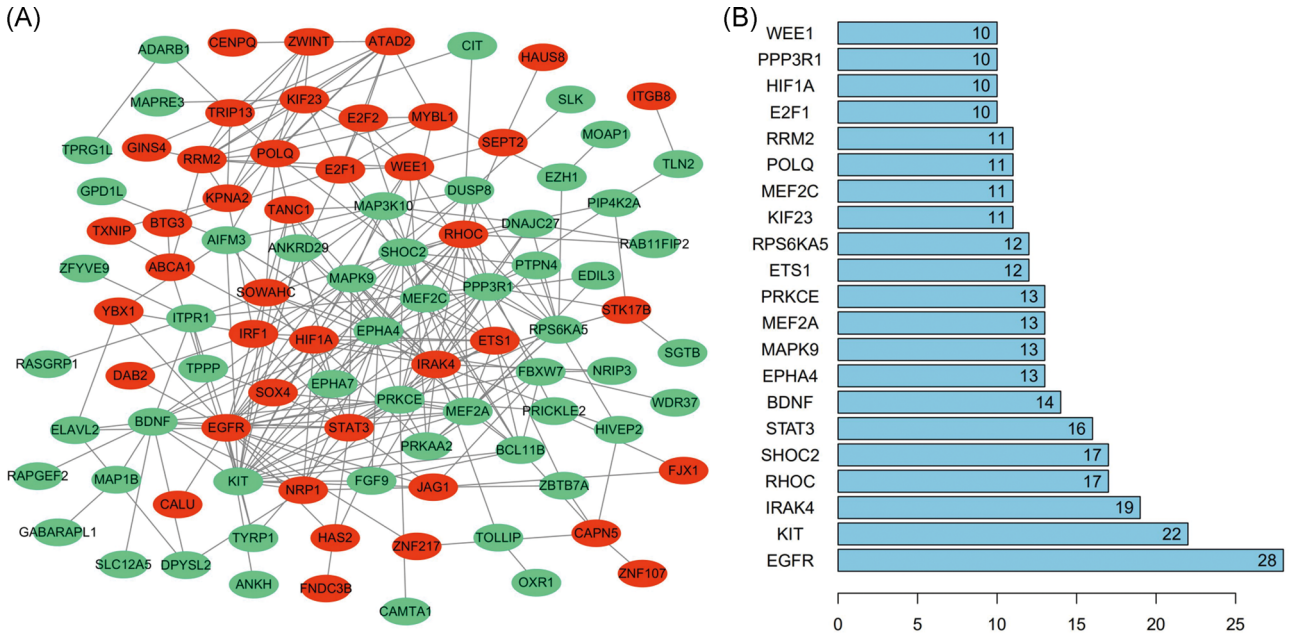

FIGURE 5 PPI network construction of DEmRNAs in ceRNA networks. A, Red nodes represent upregulated DEmRNAs and green nodes represent downregulated DEmRNAs. B, The top ten hub genes with the highest degree of connectivity between DEmRNAs. ceRNA, competitive endogenous RNA; DEmRNA, differentially expressed messenger RNA; PPI, protein-protein interaction

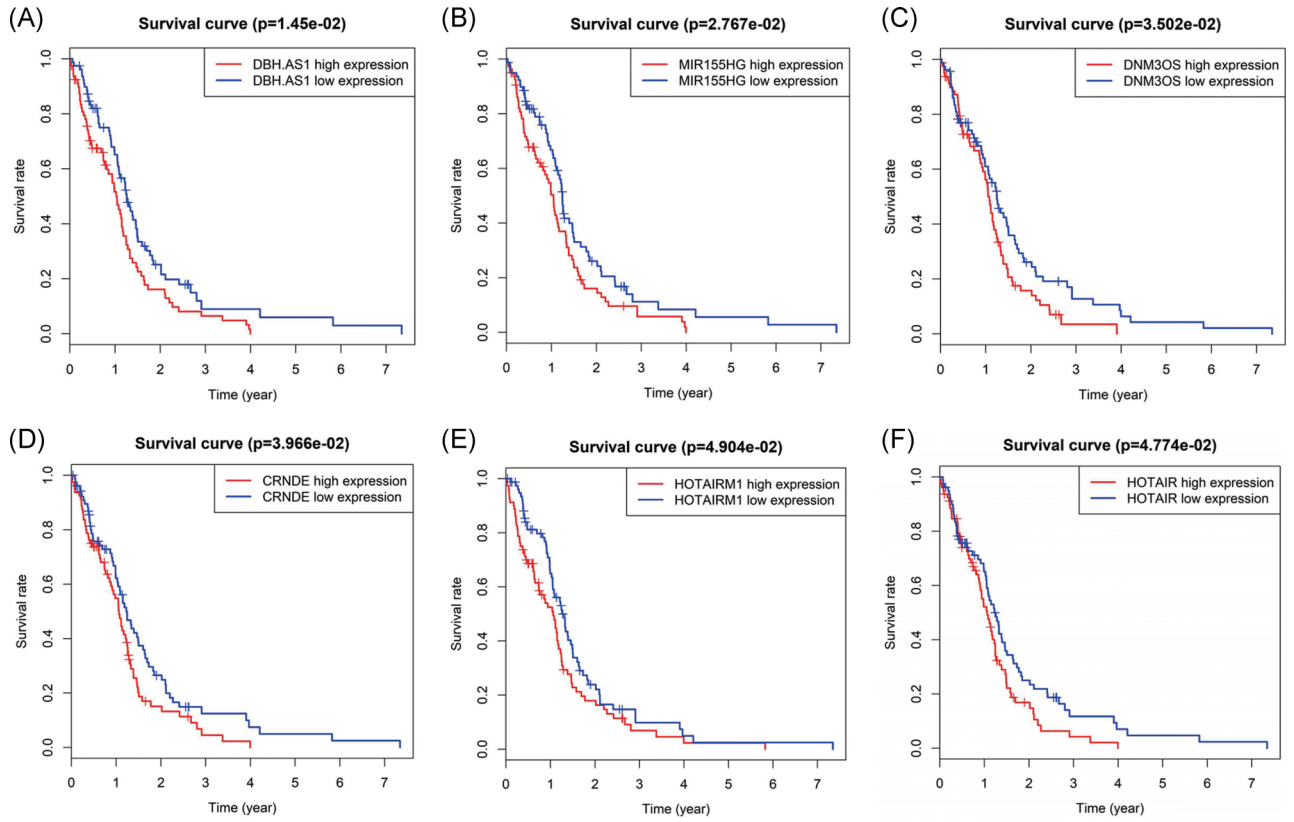

FIGURE 6 The association between overall survival and six DElncRNAs in the ceRNA network using Kaplan-Meier curve analysis. Six DElncRNAs were significantly ($P < .05$) associated with overall survival. These included *DBH-AS1*, *MIR155HG*, *DNM3OS*, *CRNDE*, *HOTAIRM1*, and *HOTAIR*. ceRNA, competitive endogenous RNA; DElncRNA, differentially expressed long noncoding RNA

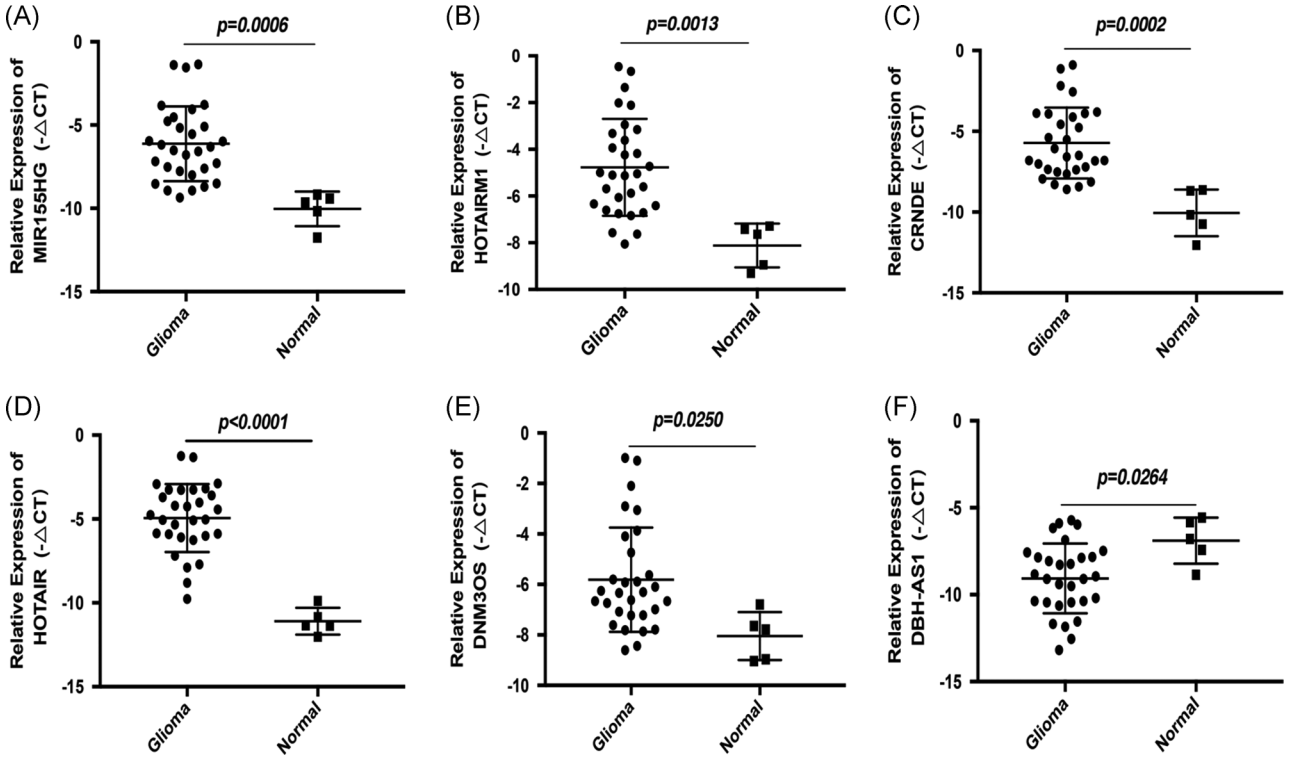

FIGURE 7 The expression levels of six survival-related lncRNAs in the ceRNA network were detected by RT-qPCR. These included *MIR155HG*, *HOTAIRM1*, *CRNDE*, *HOTAIR*, *DNM3OS*, and *DBH-AS1* ($P < .05$). ceRNA, competitive endogenous RNA; lncRNA, long noncoding RNA; RT-qPCR, reverse transcription-quantitative polymerase chain reaction

lncRNAs, miRNAs, and mRNAs. The ceRNA hypothesis proposes a new regulatory mechanism that is mainly dominated by lncRNAs.[7] Several studies have reported the role of lncRNAs in GBM, but few have focused on the lncRNA-related ceRNA network, especially using multi-database integrated bioinformatical analysis.

In the present study, we identified differentially expressed lnRNAs, miRNAs, and mRNAs in GBM and normal brain tissue samples from the TCGA and the GEO databases. We selected the intersecting differentially expressed lncRNAs and mRNAs in the two databases and further used bioinformatics tools to construct a lncRNA-related ceRNA network. Using GO and KEGG analyses, we further analyzed the functions and pathways of the DEmRNAs in the ceRNA network. We analyzed the relevant lncRNAs involved in the ceRNA network and the related clinical information and found that six lncRNAs were associated with overall survival. Finally, we used the drug development tool CMap to analyze the differentially expressed mRNAs in the ceRNA network and obtained four small-molecule compounds that could be potential drugs for the treatment of GBM.

Using GO and KEGG, we analyzed the differentially expressed mRNAs in the ceRNA network. Based on the ceRNA network theory, lncRNAs participate in biological processes through miRNA regulation of mRNA expression. Therefore, the functions and potential pathways of lncRNAs may be similar to those of mRNAs. The results of the GO analysis demonstrated that mRNAs are mainly enriched in several major regions, such as DNA binding, histone acetyltransferase binding, and transmembrane receptor protein tyrosine kinase activity. KEGG results revealed that some enriched pathways have been reported in previous studies regarding cancer. MAPK is a key signaling pathway involved in the proliferation, apoptosis, migration, and infiltration of GBM.[21] The Ras signaling pathway is involved in cell growth, apoptosis, and differentiation in various cancers.[22] For instance, metapristone inhibits proliferation and migration of non-small cell lung cancer by influencing the expression of key genes in the RAS/RAF/MEK/MAPK and PI3K/AKT signaling pathways. According to reports by Farhan et al,[23] the FoxO signaling pathway plays multiple regulatory roles in the pathological processes of cancer, such as apoptosis, cell cycle, regulation of hormones and growth factors, and angiogenesis.

We used the TCGA and GEO databases to obtain data of differentially expressed lncRNAs, miRNAs, and mRNAs for integrated analysis and used these to construct a ceRNA network. In the ceRNA network, we showed the core lncRNAs, miRNAs, and mRNAs after the intersection. Perhaps this network can help us further understand the pathogenesis of GBM at the genetic level.

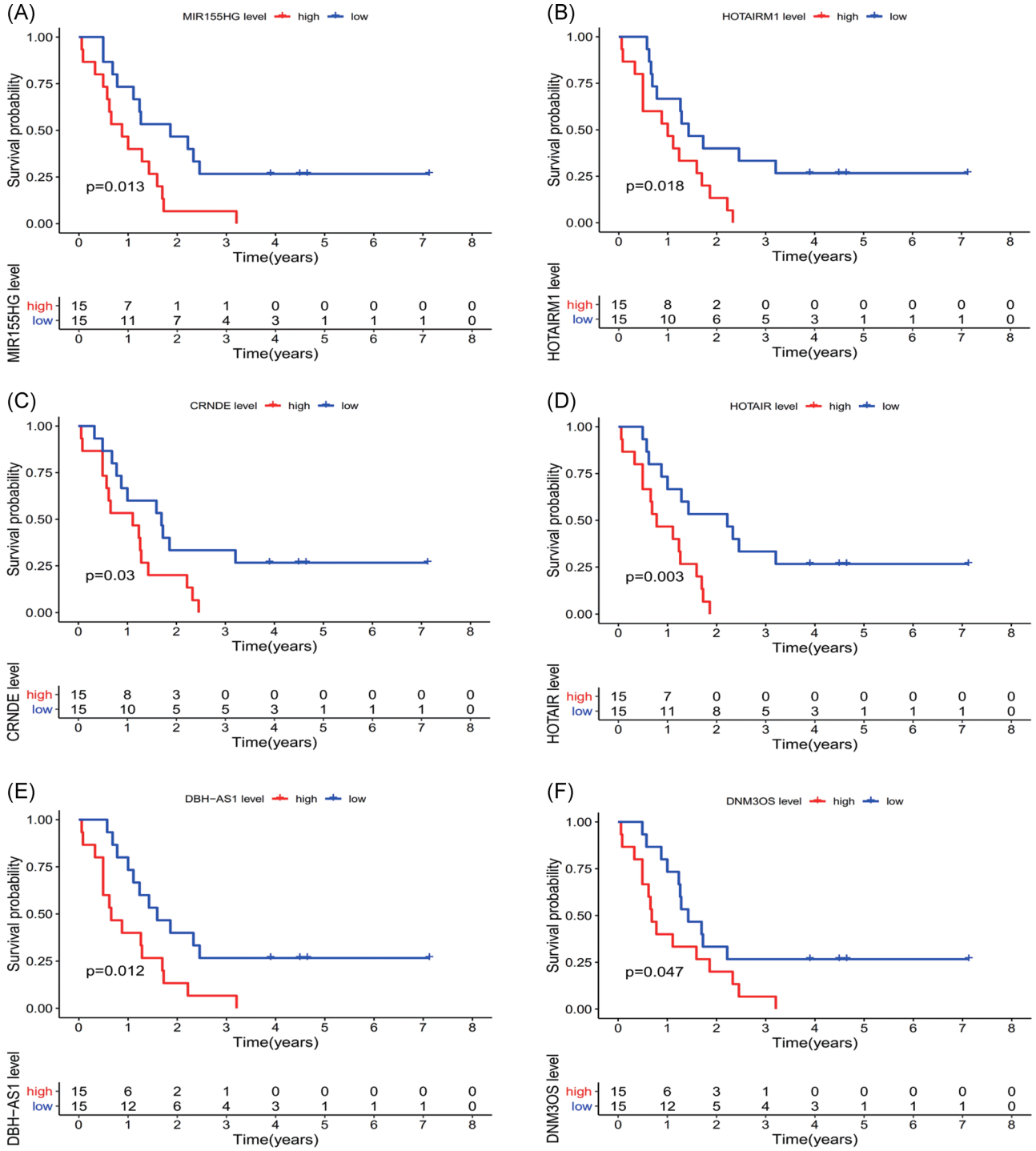

FIGURE 8 The relationship between the expression level of the six experimentally validated DElncRNAs in 30 glioma samples and the overall survival of the patients. These included *MIR155HG*, *HOTAIRM1*, *CRNDE*, *HOTAIR*, *DNM3OS*, and *DBH-AS1* ($P < .05$). DElncRNA, differentially expressed long noncoding RNA

There are some interactions that have been confirmed in previous reports. For example, H19 promotes proliferation, migration, and invasion of breast cancer cells by competitively binding to *miR-93-5p* to regulate the *STAT3* expression.[24] *NSC141562* is a small-molecule compound that can be used to treat GBM as it inhibits the *MIR155HG*/*miR-155* axis.[25] Several lncRNAs in ceRNA networks have been reported to have a regulatory role in GBM, such as *H19*, *Linc00320*, *HOTAIRM1*, *CRNDE*, and *HOTAIR*.[4,26–29] These reports can further confirm the credibility of the analysis in our study.

We used differentially expressed mRNAs in the ceRNA network to construct a PPI network, in which ten key mRNAs, including *EGFR*, *KIT*, *IRAK4*, *RHOC*,

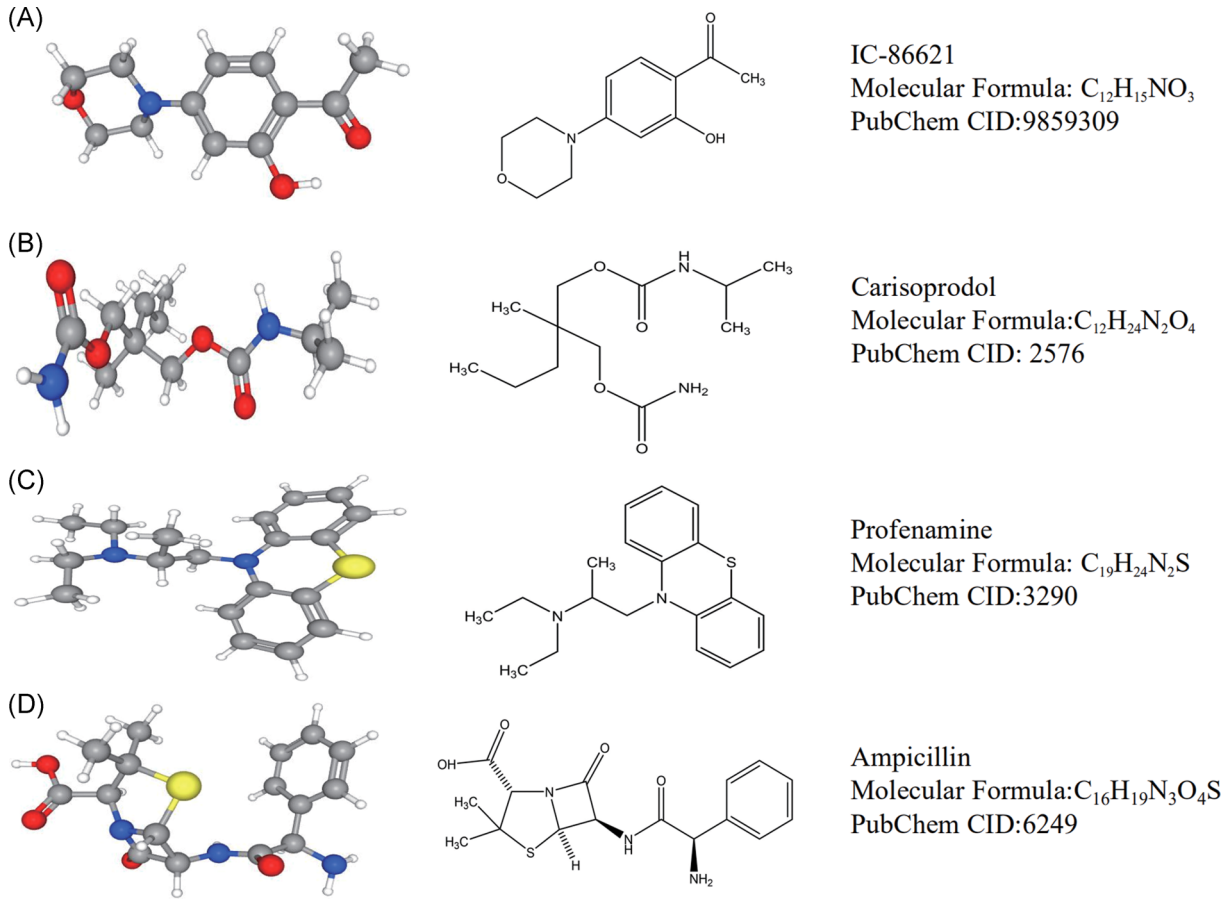

FIGURE 9 2D and 3D structures of the four compounds identified by the connectivity map analysis: (A) IC-86621, (B) carisoprodol, (C) profenamine, and (D) ampicillin

SHOC2, *STAT3*, *BDNF*, *EPHA4*, *MAPK9*, and *MEF2A*, were screened based on the degree of connectivity between individual mRNAs. Most of these mRNAs have been reported to be involved in the pathogenesis of GBM. For example, Sean A. Misek et al[30] reported that activated *EGFR* is an obvious oncogenic factor involved in multiple pathways to promote GBM, such as MAPK and PI3K/Akt pathways. *KIT* is a receptor for stem cell factors, whose activation state is involved in the development and progression of glioma cells.[31] Kumar et al[32] pointed out that *IRAK4* plays an important role in regulating the sensitivity of temozolomide in the treatment of glioma. The data downloaded from the TCGA database contains patients' basic clinical information. We analyzed the overall survival of patients with respect to lncRNAs and mRNAs in the ceRNA network. Six of 29 lncRNAs were associated with the patient's overall survival. These were *DBH-AS1*, *MIR155HG*, *DNM3OS*, *CRNDE*, *HOTAIRM1*, and *HOTAIR*. Therefore, the results of this study further demonstrated that DElncRNAs involved in the constructed ceRNA network have a significant relationship with the prognosis of patients.

TABLE 3 Four small-molecule compounds identified as potential drugs for GBM treatment in CMap analysis

Cmap name	Enrichment	*P* value
IC-86621	−0.823	.00189
Carisoprodol	−0.818	.00203
Profenamine	−0.789	.00404
Ampicillin	−0.739	.00911

Abbreviations: CMap, connectivity map; GBM, glioblastoma.

Finally, CMap is an online tool used for drug discovery. Using this tool, we obtained four small-molecule compounds that may be potential drugs for the treatment of GBM. Previous reports have indicated the potential therapeutic effects of these small-molecule compounds in cancer. For example, the DNA-dependent protein kinase (DNA-PK) is involved in a variety of pathways in repairing and stabilizing the double-strand breaks of DNA. Its inhibitor IC-86621 has a potential role in the treatment of cancer as it hinders the repair of DNA double-strand breaks.[33] Ampicillin sodium salt has always been a standard antibacterial drug, but it has a significant antiproliferative effect on a variety of cancer cells. Some traditional medicines have been newly

discovered to have therapeutic effects, and it is possible to understand their pharmacological effects in a faster manner, which would facilitate their early application in clinical settings. These drugs include atorvastatin, which is regarded as a synthetic lipid-lowering agent and has been found to have a significant therapeutic effect on a chronic subdural hematoma in recent years.[34]

5 | CONCLUSION

In conclusion, we constructed a ceRNA network using differential lncRNAs, miRNAs, and mRNAs involved in GBM obtained from the TCGA and GEO databases and studied the relevant clinical information. To the best of our knowledge, a large sample analysis of lncRNA expression profiles based on two databases is relatively rare. Our results provide a way to uncover potential ceRNA networks in GBM, which can help us better understand the pathogenesis of GBM at the genetic level and identify potential drugs for the treatment of GBM.

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CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

AUTHOR CONTRIBUTIONS

ZL and XW conceived and designed the experiments, performed the experiments, analyzed the data, contributed reagents, materials, and analysis tools. GY and CZ contributed reagents, materials, analysis tools, authored or reviewed drafts of the paper. RZ and JY performed the experiments, prepared figures or tables. YZ and JH performed the experiments, prepared figures, or tables, performed the statistical analyses. BO analyzed the data, prepared figures, or tables. SZ contributed reagents/materials/analysis tools, authored or reviewed drafts of the paper, approved the final draft.

DATA AVAILABILITY STATEMENT

The author confirms that data from the public database for this study have explained the source of the data in detail in the manuscript and provided a link address.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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