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Original Article

Identification of the clinical diagnostic value of miR-10b-5p and the key targets and pathways in glioma, a study based on meta-analysis and bioinformatics

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Abstract: Glioma, a heterogeneous disease, remains incurable despite its apparent uniform pathology. MicroRNA-10b-5p (miRNA-10b-5p) has been studied in several cancers. However, the correlation between miR-10b-5p and glioma remains unknown. Therefore, we intended to investigate the expression of miR-10b-5p in glioma and to uncover the potential molecular regulatory mechanisms of miR-10b-5p in glioma. First, we performed a systematic meta-analysis through searching for published articles, Gene Expression Omnibus (GEO) and The Cancer Genome Atlas (TCGA) databases. Meta-analysis displayed the standard mean difference (SMD) of pooled miR-10b-5p was 2.87 (0.39 to 5.36), suggesting that miR-10b-5p was up-regulated in glioma. Next, a total of 124 possible target genes were collected from two GEO microarrays, TCGA glioblastomas (GBM) samples, and twelve prediction databases. Further GO enrichment analysis showed that the target genes were mainly enriched in axon cargo transport, neuron projection and small GTPase regulator activity. And the KEGG pathway also identified the six significant pathways (MAPK signaling pathway, Oocyte meiosis, Neurotrophin signaling pathway, Axon guidance, Aldosterone-regulated sodium reabsorption, and mTOR signaling pathway) of miR-10b-5p in glioma. Finally, 7 hub genes (PHLPP2, MAKP1, RPS6KA2, PRKCE, YWHAG, EPHA4, and NEDD4L), especially PRKCE and EPHA4, may be the most key potential target genes of miR-10b-5p in glioma. In conclusion, miR-10b-5p was significantly increased in glioma, which might serve as a diagnostic target in glioma. Several pathways especially MAPK signaling pathway, Oocyte meiosis, Axon guidance, and mTOR signaling pathway may be significantly associated with miR-10b in glioma. And the hub genes such as PHLPP2, MAKP1, RPS6KA2, PRKCE, YWHAG, EPHA4, and NEDD4L might be key target genes of miR-10b-5p in glioma. These findings might help the clinical diagnosis of glioma and provide a theoretical basis for the future research on molecular mechanism of glioma.

Keywords: miR-10b-5p, glioma, meta-analysis, GEO, TCGA, bioinformatics

Introduction

Glioma, a prevalent central nervous system tumor with poor prognosis, is classified into 4 grades (I-IV) based on the pathological classification of the tumor by the World Health Organization (WHO) [1]. Glioma can also be classified based on its cellular lineage: diffuse astrocytoma, oligodendroglioma, and glioblastoma (GBM) [2]. A comprehensive treatment strategy based on surgical resection, combined with radiotherapy, chemotherapy, and immunity, and molecular target therapy has been used in recent years. Although treatment technologies have been improved, improvements in patient prognosis are still lacking [3].

MicroRNAs (miRNAs) are endogenous, non-coding, small RNA molecules, commonly consist of 22 nucleotides. They can induce target mRNA degradation and inhibit mRNA translation via targeting complementary regions in the 3'-UTR regions of the mRNA, influencing the level of protein expression [4, 5]. In tumors, miRNAs could function as oncogenes or tumor suppressor genes. Studies have shown that miRNAs are expected to become a new candidate for treatment [6, 7]. The expression of miR-10b-5p was found to be increased in many kinds of cancers, such as breast carcinoma [8, 9]. colorectal cancer [10], gastric cancer [11, 12], hepatocellular carcinoma [13], non-small cell lung cancer [14], pancreatic tumor [15], and glioma

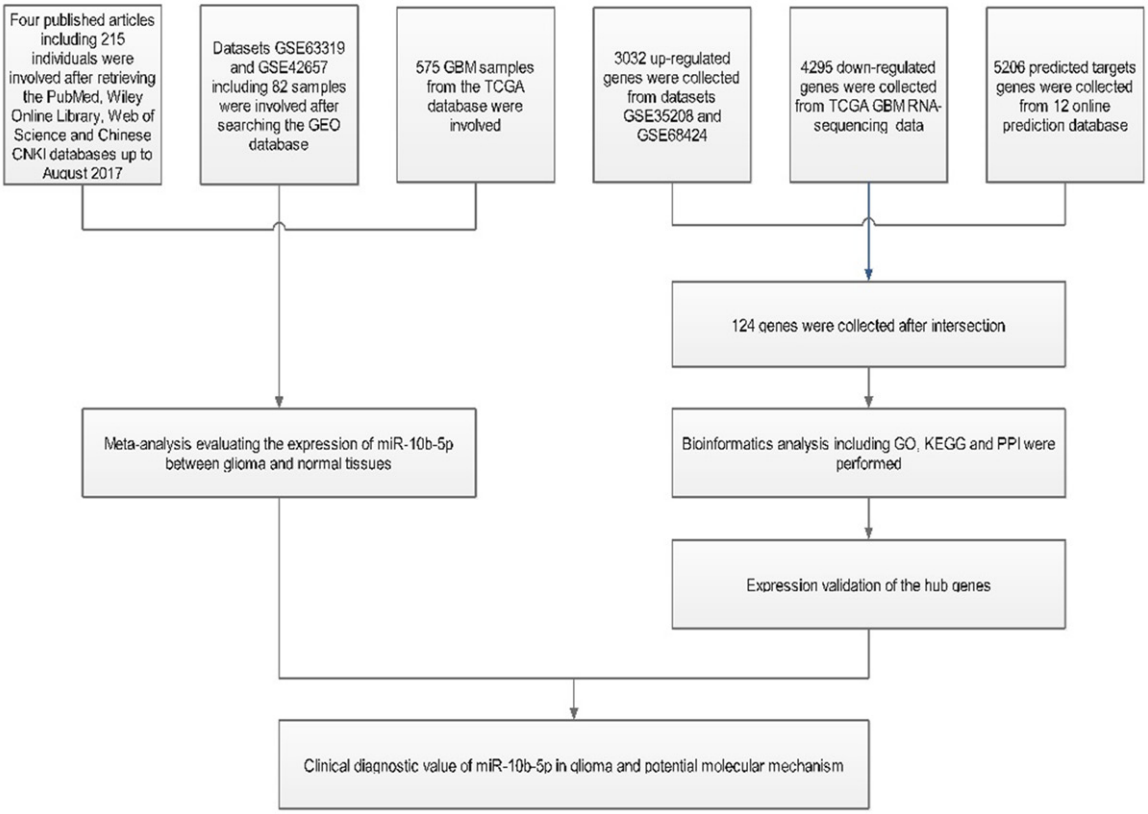

Figure 1. Flow diagram for the whole article design.

[16]. As was reported, the expression of miR-10b-5p was highly associated with advanced grade glioma [17]. However, the association between miR-10b-5p expression and clinicopathological parameters in glioma has not been discussed, and currently, no study has illustrated the regulatory molecular mechanisms of miR-10b-5p in glioma. Therefore, studies aiming to uncover the molecular mechanisms of miR-10b-5p in glioma are needed to be carried out.

Given the above information, in this study, we focused on the diagnostic value and possible underlying molecular mechanisms of miR-10b-5p in glioma. Through microarray data in Gene Expression Omnibus (GEO), GBM RNA-sequencing data in The Cancer Genome Atlas (TCGA) and online prediction databases, we identified the most significant target genes of miR-10b-5p. Then, bioinformatics analysis, including Gene ontology (GO) enrichment, Kyoto Encyclopedia of Genes and Genomes (KEGG) metabolic pathway analysis and Protein-Protein interaction (PPI) network analysis, were adopted to further analyze the significance of the selected target genes. Significantly enriched signal pathways and hub genes are discussed in detail. In summary, this study might offer theoretical evidence for future clinical diagnosis and personal treatment and lay a foundation for future research on glioma.

Materials and methods

Meta-analysis of miR-10b-5p expression in glioma

MiR-10b-5p expression data in glioma were accumulated from published studies and online databases, including the GEO and TCGA databases (**Figure 1**).

Identification of published study

We searched for articles studying the expression of miR-10b-5p in glioma in PubMed, the Wiley Online Library, the Web of Science and Chinese CNKI until August 2017. The inclusion criteria were as follows: (1) both glioma samples and corresponding non-glioma samples were included; (2) the expression profiling data

(Mean ± SD) of miR-10b-5p in glioma and non-glioma samples were available or calculable (3) the subjects involved in the study were homo sapiens.

GEO microarray retrieving

We performed a retrieval in the GEO dataset using the key words: "glioma", "microRNA", "miRNA", "Micro RNA", "Small Temporal RNA", "non-coding RNA", "ncRNA", and "small RNA". In order to screen the differentially expressed genes in glioma, the inclusion criteria were as follows: (1) glioma and corresponding normal brain tissues were analyzed, (2) miR-10b-5p was over-expressed or silenced, (3) mRNA expression in human glioma cells was detected by sequencing. To analyze the expression level of miR-10b-5p in glioma, the eligible microarrays with the following criteria were included: (1) glioma and corresponding normal brain tissues were analyzed, (2) the expression of miR-10b-5p was detected in both glioma and normal brain samples, and (3) the samples were from homo sapiens.

Collection of clinical glioma data from TCGA database

To understand the role of miR-10b-5p in the clinical diagnosis of glioma, the GBM and corresponding normal brain samples in TCGA database were used to collect expression data of miR-10b-5p.

Statistical analysis

The prepared data were further analyzed via the Student's t test, receiver operating characteristic (ROC) curve analysis and meta-analysis using SPSS 24.0 (SPSS, Inc., Chicago, IL), GraphPad Prism 7.0 (GraphPad Software, Inc., La Jolla, CA, USA) and Stata 12.0. Unpaired Student's t test was adopted to compare the expression of miR-10b-5p between glioma and non-glioma tissues. The area under the curve (AUC), sensitivity and specificity were calculated from ROC curve to evaluate the diagnostic capability of miR-10b-5p. In meta-analysis, the standard mean difference (SMD) was used to validate the differential expression of miR-10b-5p. Influence analysis and funnel plot were performed to evaluate heterogeneity and publication bias of included subjects. A p value below 0.05 was considered statistically significant.

Evaluation of common genes via bioinformatics methods

To explore the potential target genes and key pathways of miR-10b-5p in glioma, we collected differentially expressed genes from GEO and TCGA databases. And we also used 12 online prediction databases to gather predicted target genes. Then, we gained the common genes through intersection of the differentially expressed genes and predicted genes. The overlapping genes were further evaluated using bioinformatics methods including GO enrichment, KEGG pathway analysis, and PPI network analysis (**Figure 1**).

Collection of predicted target genes

Twelve prediction databases, including DIANA-microTv4.0, DIANA-microT-CDS, miRanda-rel-2010, miRanda-rel2010, miRDB4.0, miRmap, miRNAMap, PicTar2, PITA, RNA22v2, RNAhybrid2.1, and Targetscan6.2, were used for the prediction of miR-10b-5p target genes. After integrating the results, the frequency of each target gene in the 12 prediction databases was counted. We selected the genes found in at least 4 prediction databases for further study.

Collection of differentially expressed and overlapping genes

Two eligible microarrays (GSE68424 and GSE35208) were downloaded from GEO database. Since miR-10b-5p was silenced in the two eligible microarrays, we identified the up-regulated genes with Log2 (FC) >0. The up-regulated genes from the two eligible microarrays were intersected to select the significantly up-regulated genes. Besides, we downloaded the GBM RNA-sequencing data from the TCGA database and screened the differentially expressed genes. Next, the selected significant up-regulated genes from the microarrays, differentially expressed genes from TCGA GBM samples, and the predicted target genes that were found in at least 4 databases were further intersected to select the key overlapping miR-10b-5p target genes.

GO enrichment and KEGG metabolic pathway analysis

The Database for Annotation, Visualization and Integrated Discovery (DAVID) (https://david.ncifcrf.gov/) online tool was utilized to perform

Table 1. Characteristics of the included subjects in the meta-analysis

Citation (ref.)	Year	Country	Series	Platform	Glioma samples	Non-glioma samples
Sheer D	2012	United Kingdom	GSE42657	GPL8179	57	7
Jha P	2014	India	GSE63319	GPL16384	14	4
Li-hua Son	2011	China	NA	NA	22	6
Yan-ling Kong	2011	China	NA	NA	56	10
Hua D	2012	China	NA	NA	3	3
Ji Y	2015	China	NA	NA	95	20
TCGA	2016	NA	NA	NA	565	10

NA: Not available.

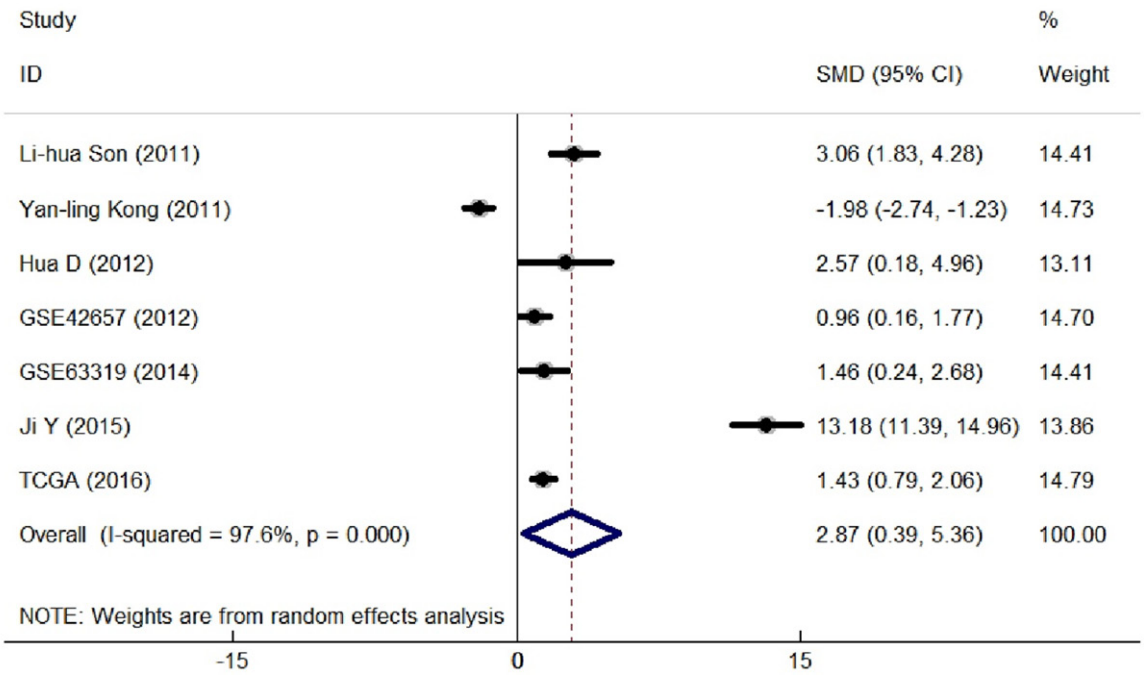

Figure 2. Forest plot for the expression of miR-10b-5p in glioma from 4 published articles, two GEO microarrays, and the TCGA database.

GO enrichment and KEGG pathway analysis. The significant target genes were uploaded into DAVID and the significant GO enriched terms and KEGG pathways were collected. P<0.05 was considered statistically significant.

PPI network construction and module analysis

The significant target genes were uploaded into the STRING database (http://www.string-db.org/) [18, 19] to construct the PPI network. An interaction score >0.4 was used as the threshold for medium interaction confidence. Then, the tabular text output file was downloaded and imported into Cytoscape 3.50. Hub genes were further selected depending on the node degree in the network. Nodes with a degree over 4 were selected as hub genes.

Validation of the hub genes expression

Gene Expression Profiling Interactive Analysis (GEPIA) (http://gepia.cancer-pku.cn/.), an online gene expression analysis tool, provides various gene analysis such as differential expression analysis, correlation analysis, and patient survival analysis based on TCGA and GTEx data [20]. By virtue of this tool, we analyzed the expression of the selected hub genes, hoping to provide more evidence to confirm the relationship between miR-10b-5p and the hub genes.

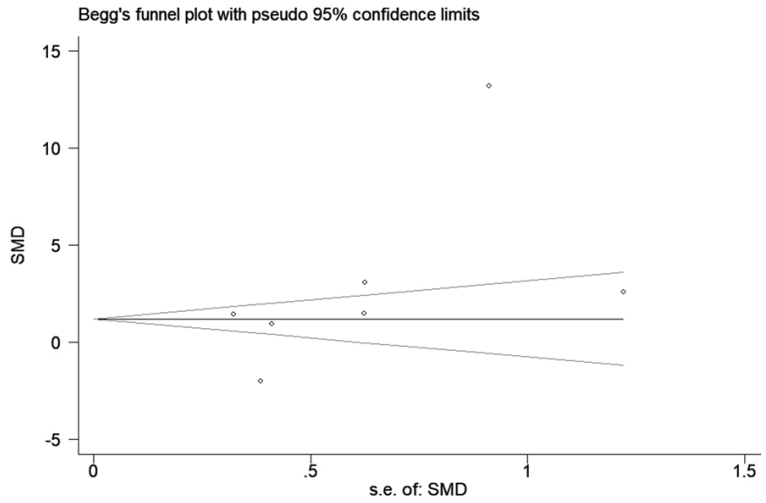

Figure 3. Begg's plot evaluating publication bias among 4 published articles, two GEO microarrays, and the TCGA database.

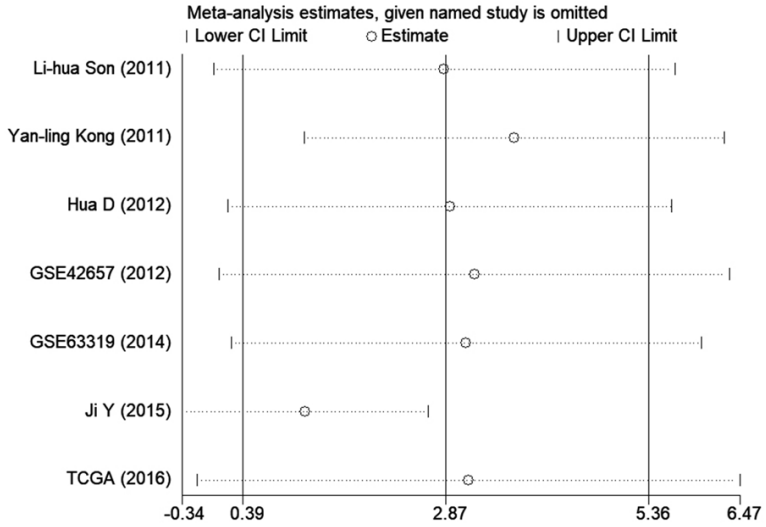

Figure 4. Sensitivity analysis evaluating heterogeneity among 4 published articles, two GEO microarrays, and the TCGA database.

Results

The expression of miR-10b-5p in glioma based on published studies, GEO and the TCGA databases

A total of 4 eligible published studies, which contained a total of 176 glioma samples and 39 non-glioma samples, were included in the study. Combined with the two microarrays (GSE63319 and GSE42657) and the TCGA data, a meta-analysis evaluating the expression of miR-10b-5p performed (**Table 1**). As displayed in **Figure 2**, the pooled SMD was 2.87 (0.39 to 5.36, p=0.023), which suggested the high expression of miR-10b-5p. A significant heterogeneity (I^2= 97.6%, P=0.000) was observed in the forest plot. A Begg's funnel plot was generated to evaluate publication bias (**Figure 3**). The funnel plot was considered symmetric, although there were three subjects out of the funnel. A *p* value of 0.133 also further suggested that there was no publication bias among these studies. A sensitivity analysis was performed to uncover the source of the heterogeneity. As we can observe in **Figure 4**, two published studies, namely Yan-ling Kong's and Ji Y's were suggested to be the source of the heterogeneity. Therefore, we removed these two studies, and the heterogeneity almost disappeared (I^2=54.0%, p=0.069, **Figure 5**). The pooled SMD was 1.69 (0.98 to 2.39, p= 0.000). Taken together, the pooled results of the included studies may help illustrate the high expression of miR-10b-5p in glioma.

We included two eligible microarrays (GSE63319 and GSE42657) that detected the expression of miR-10b-5p in glioma and normal brain tissues. Statistical analysis revealed that the expression of miR-10b-5p was significantly increased in both microarrays. In GSE63319, the expression of miR-10b-5p in glioma versus normal brain tissues was 7.38 ± 0.7684 versus 3.553 ± 0.4633 (p=0.020, **Figure 6A**). The ROC curve also showed prominent diagnostic value of miR-10b-5p. The AUC was 0.929 with a sensitivity of 0.857 and a specificity of 0.75 (p=0.011, **Figure 6B**). In GSE42657, the expression of miR-10b-5p in glioma was 11.21 ± 0.2494, while in the control groups it was 9.475 ± 0.2767 (P=0.019, **Figure 6C**). In addition, the ROC curve analysis

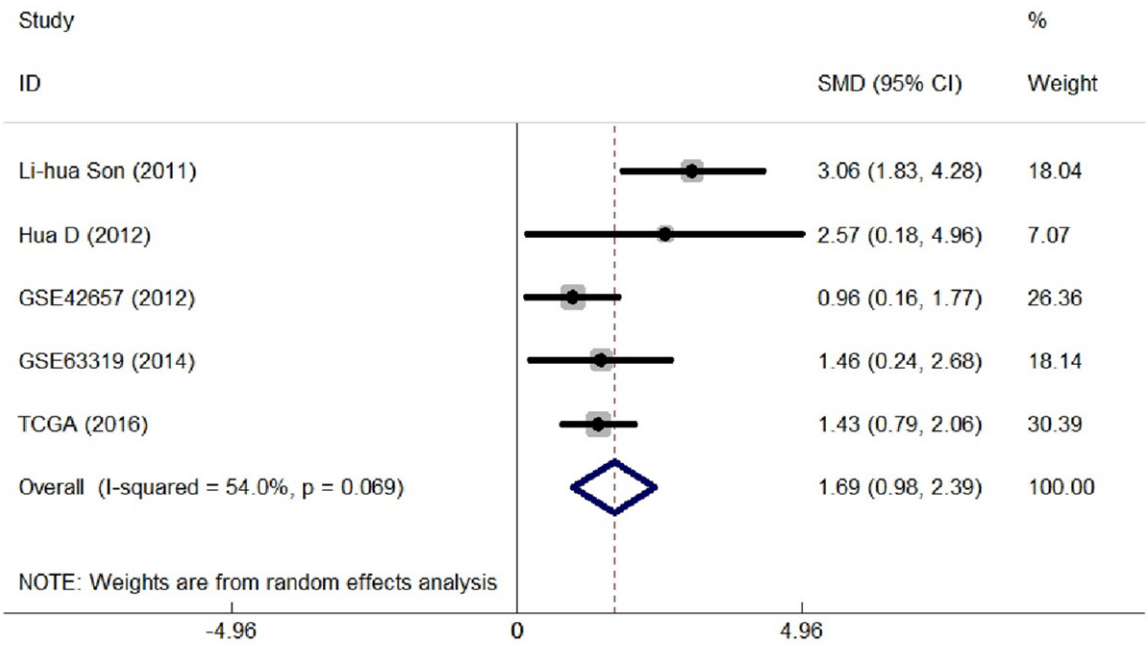

Figure 5. Forest plot for the expression of miR-10b-5p in glioma from 2 published articles, two GEO microarrays, and the TCGA database.

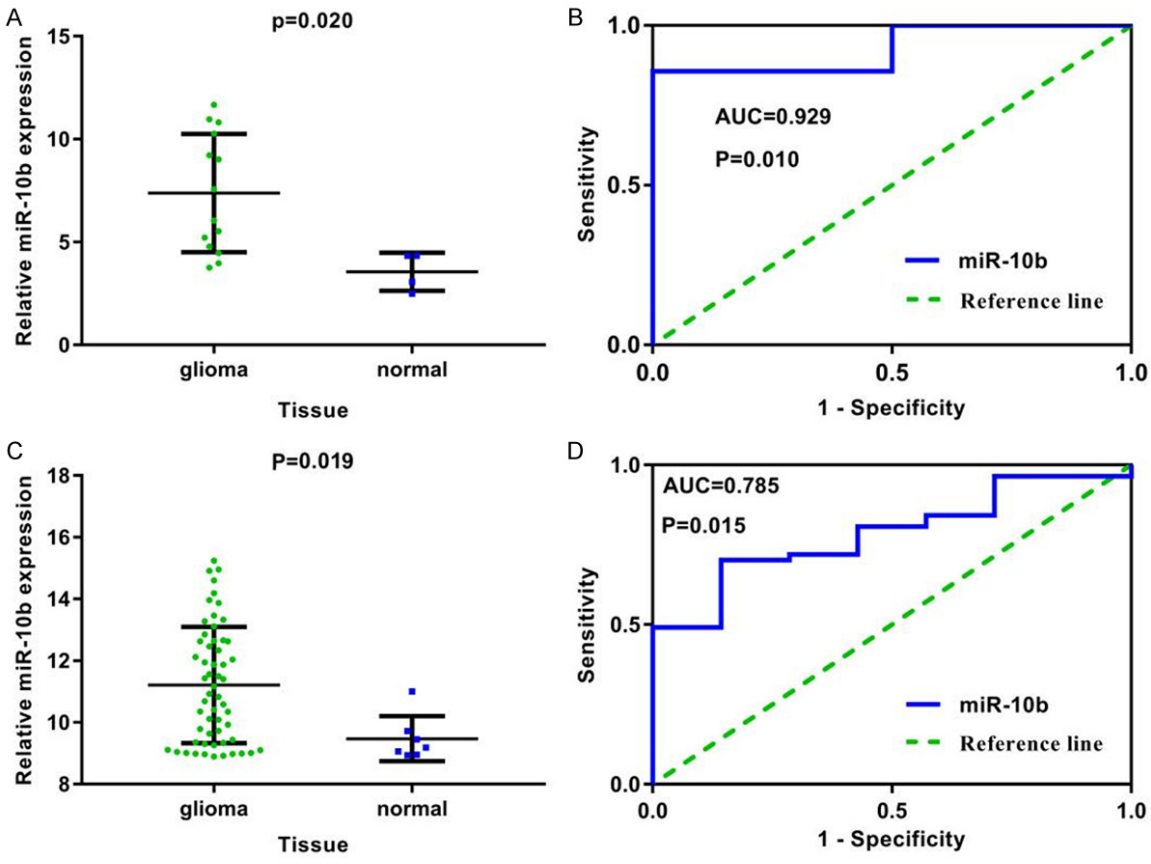

Figure 6. Diagnostic value of miR-10b-5p in gliomas based on GEO microarrays. A. Scatter plot of miR-10b-5p expression in glioma and controls in GSE63319. B. Diagnostic ROC curve analysis for glioma in GSE63319. C. Scatter plot of miR-10b-5p expression in glioma and controls in GSE42657. D. Diagnostic ROC curve analysis for glioma in GSE42657.

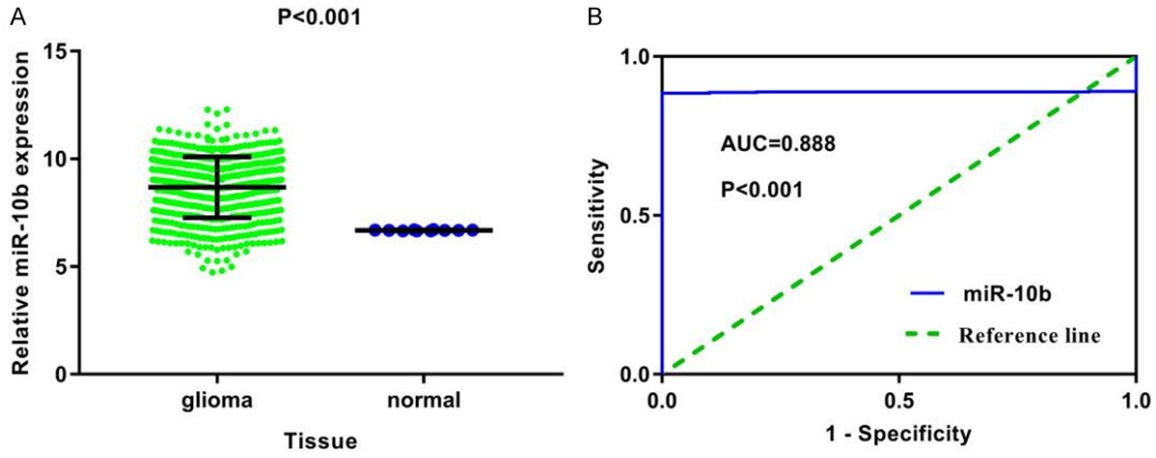

Figure 7. Diagnostic value of miR-10b-5p in glioma based on the TCGA database. A. Scatter plot of miR-10b-5p expression in glioma and controls in the TCGA database. B. Diagnostic ROC curve analysis for glioma in the TCGA database.

revealed that the AUC was 0.785, with a sensitivity of 0.702 and specificity of 0.857 (P=0.015, **Figure 6D**)

Regarding the TCGA data, the expression of miR-10b-5p in glioma was 8.68 ± 1.414, which was significantly higher than the control group at 6.68 ± 0.021 (P<0.001, **Figure 7A**). The ROC curve further verified the diagnostic significance of miR-10b-5p in glioma. The calculated AUC was 0.888, with a sensitivity of 0.885 and specificity of 0.9 (**Figure 7B**).

Differentially expressed and overlapping genes

Gene expression data were extracted from two eligible microarrays: GSE35208 and GSE68424, which silenced miR-10b-5p in glioma and GBM cell lines, respectively. We screened 11,293 and 5,142 up-regulated genes in GSE35208 and GSE68424, respectively. After intersecting the up-regulated genes in the two microarrays, a total of 3129 up-regulated genes were finally collected. Furthermore, since the expression of miR-10b-5p was up-regulated in our meta-analysis, we collected 4295 down-regulated genes from the TCGA GBM samples. Additionally, through 12 online prediction databases, 5206 predictive target genes were collected. The up-regulated genes from the microarray, down-regulated genes from the TCGA GBM samples, and the predicted target genes were further intersected. Finally, a total of 124 common genes were selected (Supplementary 1).

GO enrichment and KEGG metabolic pathway analysis

Through DAVID analysis tool, the 124 target genes were analyzed using GO enrichment and KEGG metabolic pathway analysis. As shown in **Table 2**, significant GO and KEGG enriched terms were listed. GO enrichment was divided into three categories, namely biological processes (BP), cellular components (CC) and molecular functions (MF). In the BP category, we can observe that the 124 target genes mainly enriched in axon cargo transport, microtubule-based transport, regulation of small GTPase mediated signal transduction (**Figure 8**). In the CC category, neuron projection, plasma membrane and plasma membrane part remained the top three enriched terms (**Figure 9**). Regarding the MF category, the three ranked terms were small GTPase regulator activity, GTPase regulator activity and nucleoside-triphosphatase regulator activity (**Figure 9**). KEGG metabolic pathway analysis showed the pathways the 124 target genes mainly grouped in the following 6 pathways: MAPK signaling pathway, Oocyte meiosis, Neurotrophin signaling pathway, Axon guidance, Aldosterone-regulated sodium reabsorption, and mTOR signaling pathway (**Figure 9**).

PPI network construction

The PPI network was able to help identify the most important target genes, namely hub genes, according to the degree of each gene in

Table 2. Significant GO terms and KEGG pathways of the overlapping genes

Category	Term	Count	*P* Value	Benjamini	FDR
GOTERM_BP_FAT	GO: 0008088~axon cargo transport	5	6.21E-06	0.004855777	0.009505
GOTERM_BP_FAT	GO: 0010970~microtubule-based transport	5	4.55E-05	0.017659146	0.069559
GOTERM_BP_FAT	GO: 0051056~regulation of small GTPase mediated signal transduction	9	0.000363	0.090546171	0.55446
GOTERM_BP_FAT	GO: 0030705~cytoskeleton-dependent intracellular transport	5	0.000459	0.085973706	0.699705
GOTERM_BP_FAT	GO: 0006796~phosphate metabolic process	16	0.002783	0.35399211	4.176414
GOTERM_BP_FAT	GO: 0006793~phosphorus metabolic process	16	0.002783	0.35399211	4.176414
GOTERM_BP_FAT	GO: 0046578~regulation of Ras protein signal transduction	7	0.003415	0.360482582	5.102867
GOTERM_BP_FAT	GO:0043244~regulation of protein complex disassembly	4	0.005317	0.449562967	7.836851
GOTERM_BP_FAT	GO: 0032318~regulation of Ras GTPase activity	5	0.005921	0.441236399	8.691193
GOTERM_BP_FAT	GO: 0007018~microtubule-based movement	5	0.00791	0.499317359	11.44771
GOTERM_BP_FAT	GO: 0051130~positive regulation of cellular component organization	6	0.00854	0.489521516	12.3047
GOTERM_BP_FAT	GO: 0043087~regulation of GTPase activity	5	0.010585	0.531599517	15.03325
GOTERM_BP_FAT	GO: 0046907~intracellular transport	11	0.015676	0c.643796085	21.48522
GOTERM_BP_FAT	GO: 0048812~neuron projection morphogenesis	6	0.016365	0.630303735	22.32234
GOTERM_BP_FAT	GO: 0006816~calcium ion transport	5	0.017143	0.620284717	23.25817
GOTERM_BP_FAT	GO: 0006468~protein amino acid phosphorylation	11	0.017246	0.597168219	23.38076
GOTERM_BP_FAT	GO: 0006811~ion transport	12	0.017274	0.574208138	23.41416
GOTERM_BP_FAT	GO: 0016310~phosphorylation	12	0.02263	0.652024695	29.56125
GOTERM_BP_FAT	GO: 0048858~cell projection morphogenesis	6	0.027937	0.708908902	35.19446
GOTERM_BP_FAT	GO: 0007017~microtubule-based process	6	0.031476	0.732781774	38.71444
GOTERM_BP_FAT	GO: 0043242~negative regulation of protein complex disassembly	3	0.032868	0.730203881	40.04921
GOTERM_BP_FAT	GO: 0031175~neuron projection development	6	0.032873	0.712889222	40.05373
GOTERM_BP_FAT	GO: 0032990~cell part morphogenesis	6	0.032873	0.712889222	40.05373
GOTERM_BP_FAT	GO: 0015674~di-, tri-valent inorganic cation transport	5	0.034202	0.710660433	41.30215
GOTERM_BP_FAT	GO: 0032012~regulation of ARF protein signal transduction	3	0.037416	0.727433879	44.22282
GOTERM_BP_FAT	GO: 0000902~cell morphogenesis	7	0.037548	0.713551081	44.33987
GOTERM_BP_FAT	GO: 0008089~anterograde axon cargo transport	2	0.040981	0.730785365	47.30296
GOTERM_BP_FAT	GO: 0030001~metal ion transport	8	0.042943	0.733801983	48.92916
GOTERM_BP_FAT	GO: 0007409~axonogenesis	5	0.045446	0.740903316	50.9366
GOTERM_CC_FAT	GO: 0043005~neuron projection	9	0.001112	0.177858603	1.356195
GOTERM_CC_FAT	GO: 0005886~plasma membrane	37	0.001377	0.114190422	1.676614
GOTERM_CC_FAT	GO: 0044459~plasma membrane part	24	0.005142	0.26097792	6.129915
GOTERM_CC_FAT	GO: 0005874~microtubule	7	0.0065	0.249434417	7.690394
GOTERM_CC_FAT	GO: 0005891~voltage-gated calcium channel complex	3	0.007846	0.242161856	9.214068
GOTERM_CC_FAT	GO: 0042995~cell projection	11	0.009576	0.245909168	11.13686
GOTERM_CC_FAT	GO: 0019898~extrinsic to membrane	9	0.010311	0.22940785	11.94308
GOTERM_CC_FAT	GO: 0034704~calcium channel complex	3	0.01169	0.227948106	13.43725
GOTERM_CC_FAT	GO: 0009898~internal side of plasma membrane	7	0.01261	0.219773052	14.42086
GOTERM_CC_FAT	GO: 0030424~axon	5	0.016142	0.249055007	18.10257
GOTERM_CC_FAT	GO: 0005856~cytoskeleton	16	0.018607	0.259569048	20.58531
GOTERM_CC_FAT	GO: 0005875~microtubule associated complex	4	0.024383	0.303747624	26.13364
GOTERM_CC_FAT	GO: 0044430~cytoskeletal part	12	0.029133	0.329865486	30.42817
GOTERM_CC_FAT	GO: 0005829~cytosol	15	0.029179	0.310835482	30.4682
GOTERM_CC_FAT	GO: 0005626~insoluble fraction	11	0.030837	0.307550752	31.912
GOTERM_CC_FAT	GO: 0034703~cation channel complex	4	0.046787	0.409679981	44.45658
GOTERM_MF_FAT	GO: 0005083~small GTPase regulator activity	8	0.002396	0.422692921	3.026781
GOTERM_MF_FAT	GO: 0030695~GTPase regulator activity	9	0.005622	0.475632909	6.968552
GOTERM_MF_FAT	GO: 0060589~nucleoside-triphosphatase regulator activity	9	0.006398	0.387349878	7.89429
GOTERM_MF_FAT	GO: 0003777~microtubule motor activity	4	0.014888	0.576302915	17.4833
GOTERM_MF_FAT	GO: 0005096~GTPase activator activity	6	0.016058	0.523567383	18.73037
GOTERM_MF_FAT	GO: 0004672~protein kinase activity	10	0.01995	0.536586377	22.75419
GOTERM_MF_FAT	GO: 0005099~Ras GTPase activator activity	4	0.023133	0.534975585	25.90693
GOTERM_MF_FAT	GO: 0004721~phosphoprotein phosphatase activity	5	0.024797	0.512640574	27.50754
GOTERM_MF_FAT	GO: 0004674~protein serine/threonine kinase activity	8	0.025131	0.476708057	27.82527
GOTERM_MF_FAT	GO: 0016791~phosphatase activity	6	0.025829	0.450779765	28.48451

GOTERM_MF_FAT	GO: 0004722~protein serine/threonine phosphatase activity	3	0.036483	0.538704754	37.88266
KEGG_PATHWAY	hsa04010: MAPK signaling pathway	8	0.000585	0.038451993	0.598438
KEGG_PATHWAY	hsa04114: Oocyte meiosis	5	0.003245	0.103157728	3.278365
KEGG_PATHWAY	hsa04722: Neurotrophin signaling pathway	5	0.00499	0.105703171	5.001102
KEGG_PATHWAY	hsa04360: Axon guidance	5	0.005742	0.091944282	5.73476
KEGG_PATHWAY	hsa04960: Aldosterone-regulated sodium reabsorption	3	0.022446	0.262287349	20.7715
KEGG_PATHWAY	hsa04150: mTOR signaling pathway	3	0.034926	0.32765302	30.55374

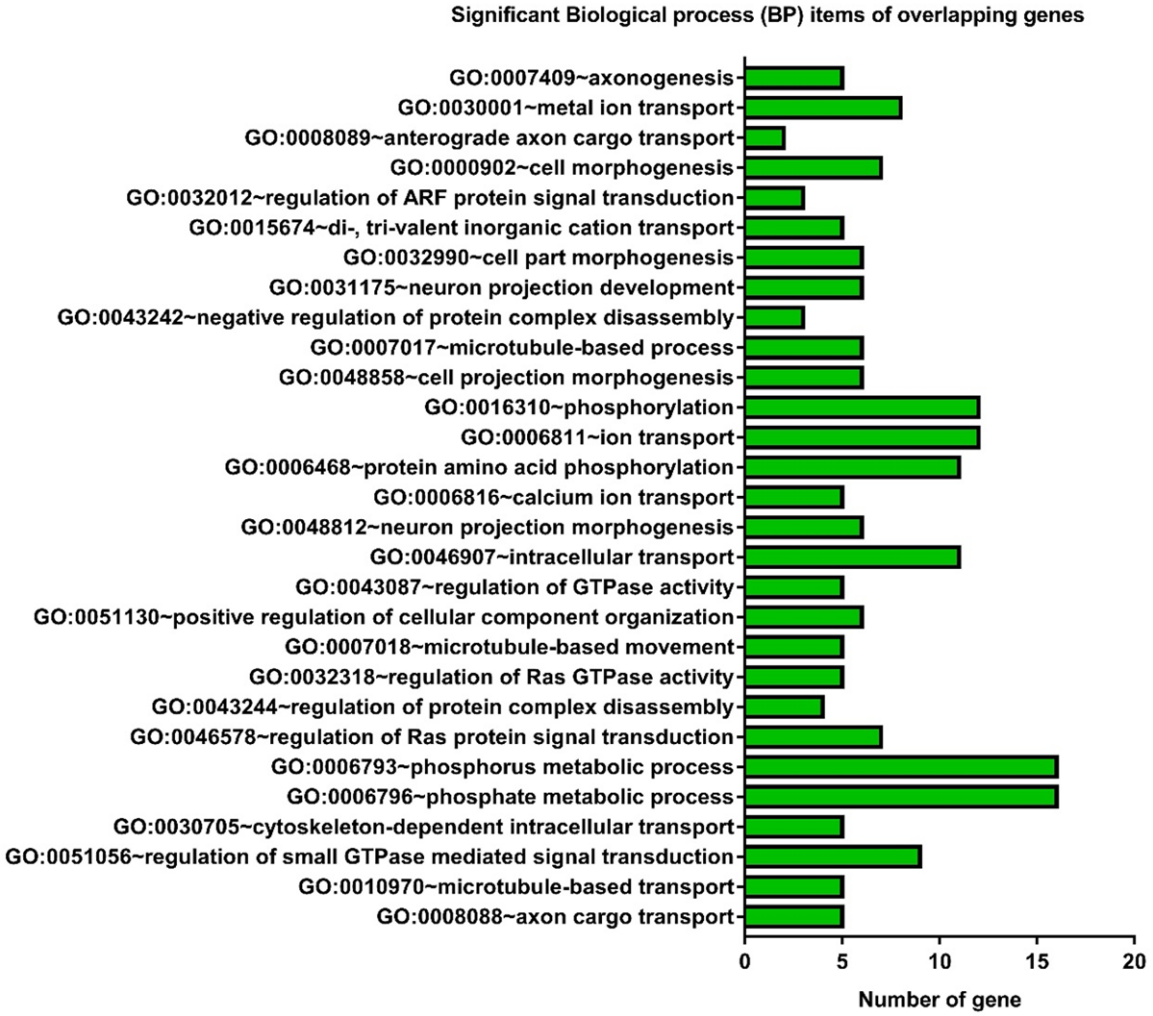

Figure 8. Significant biological process (BP) terms of the overlapping genes.

the network. Therefore, the PPI network was constructed through the STRING database (**Figure 10**), and a total of 7 hub genes (PHLPP2, MAPK1, RPS6KA2, PRKCE, YWHAG, EPHA4 and NEDD4L) were identified through Cytoscape 3.50 due to their relatively high degree (degree >4) (**Figure 11**).

Validation of the hub genes expression

Through GEPIA online gene expression analysis tool, 163 GBM and 207 normal brain samples were used to perform gene expression analysis. We found that 2 hub genes (PRKCE and EPHA4) were significantly down-regulated (**Figure 12**) However, for the rest of the hub genes (PHLPP2, MAPK1, RPS6KA2, YWHAG, and NEDD4L), no statistical expression difference was found (**Figure 13**).

Discussion

It is worth noticing that the expression of miR-10b-5p in glioma was significantly up-regulated

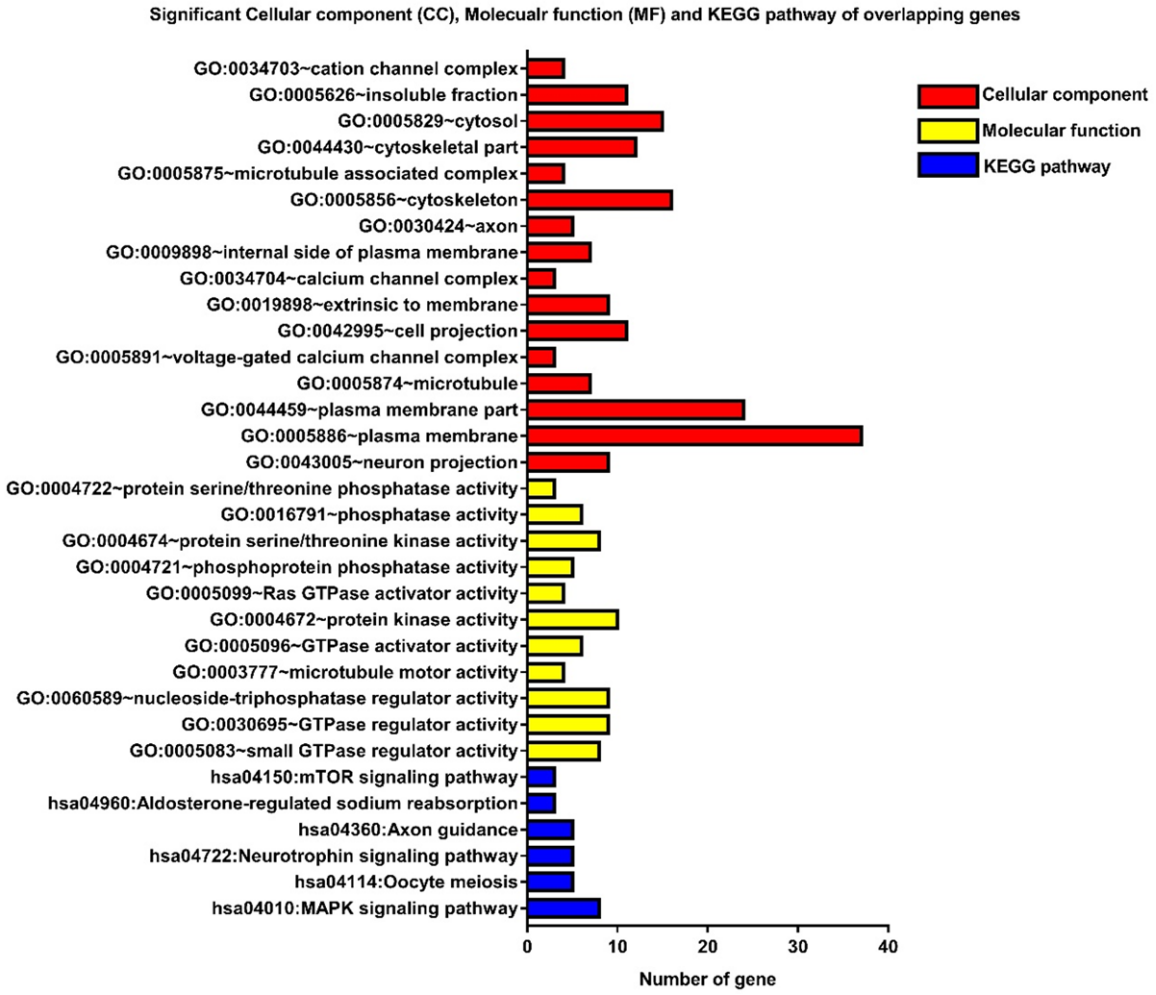

Figure 9. Significant cellular component (CC), molecular function (MF) terms and KEGG pathways of the overlapping genes.

compared with the corresponding normal brain tissues according to the data from the GEO microarrays and TCGA database. Moreover, the ROC curve analysis also revealed that the expression of miR-10b-5p showed satisfactory diagnostic sensitivity and specificity, which made it more able to help in clinical glioma diagnosis.

GO enrichment analysis helped identify the enriched GO terms of the target genes. axon cargo transport, neuron projection, and small GTPase regulator activity were the most enriched in BP, CC and MF, respectively. In the KEGG pathway analysis, six significant pathways were found: MAPK signaling pathway, Oocyte meiosis, Neurotrophin signaling pathway, Axon guidance, Aldosterone-regulated sodium reabsorption and mTOR signaling pathway. Among which, MAPK signaling pathway, Oocyte meiosis, Axon guidance and mTOR signaling pathway have been reported to be correlated with glioma. For example, MAPK/ERK signaling pathway blocking has been found by Li B to be implicated in the inhibition of glioma [21]. Sun S found that beta polypeptide (P4HB) could promote the growth of glioma in vivo through MAPK signaling pathway [22]. Regarding Oocyte meiosis, it was also found by Zhou C to be a significant pathway in glioma [23]. As for Axon guidance, Kunapuli P found that canonical axon guidance pathway was the most affected pathway after re-expression of LGI1 in glioma cells [24]. Plenty of studies have reported that AKT/mTOR signaling pathway participates in the regulation of glioma cell proliferation, migration, and invasion [25-29]. Those previous researches further support our find-

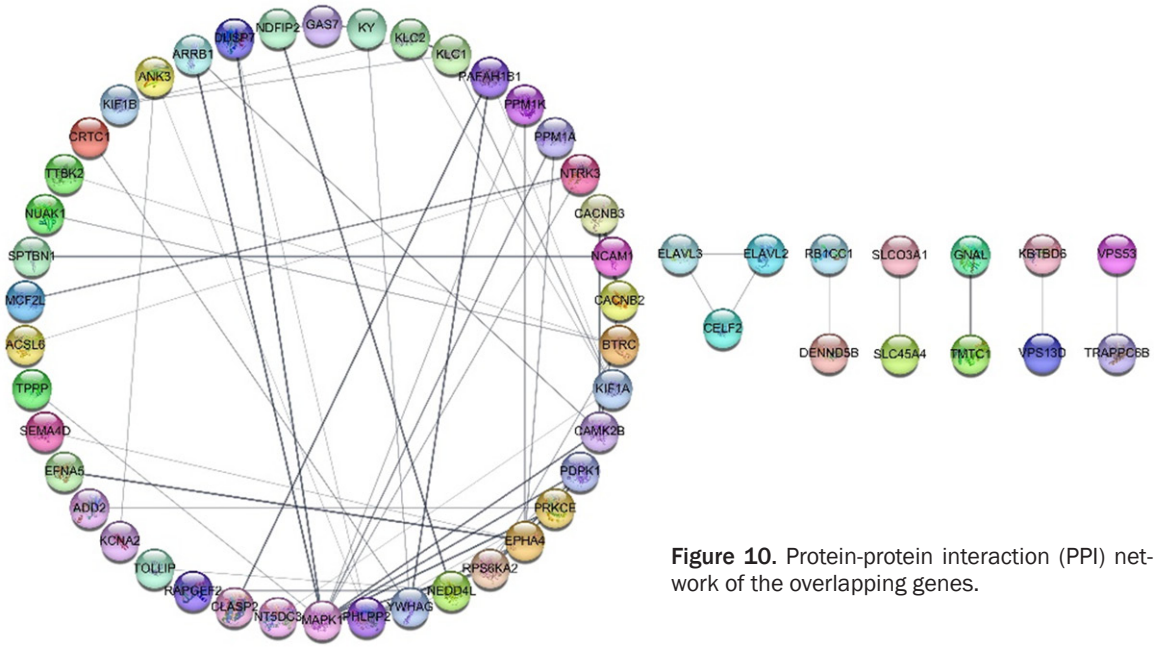

Figure 10. Protein-protein interaction (PPI) network of the overlapping genes.

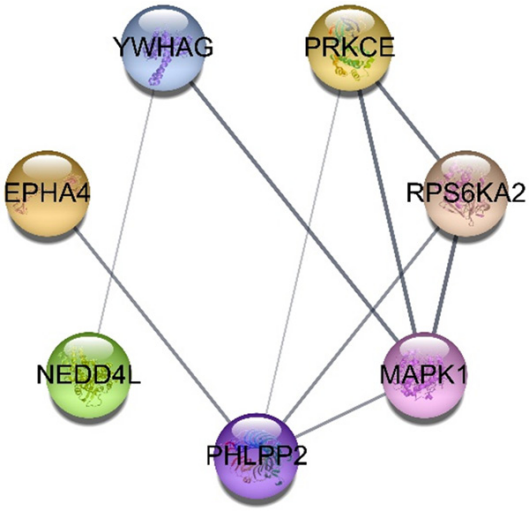

Figure 11. Hub genes selected from protein-protein interaction (PPI) network (degree >4).

ings. MAPK signaling pathway, Oocyte meiosis, Axon guidance, and mTOR signaling pathway did exert important function in glioma. For Neurotrophin signaling pathway and Aldosterone-regulated sodium reabsorption, which have not been reported to be associated with glioma, it is still worthy to focus on exploring their functions in the development of glioma in the future.

Beyond that, the PPI network analysis helped verify that the seven hub genes (PHLPP2, MAKP1, RPS6KA2, PRKCE, YWHAG, EPHA4, and NEDD4L) might be valuable in glioma. Through gene expression analysis, we identified hub genes PRKCE and EPHA4 were significantly down-regulated in GBM samples. Since miR-10b-5p was significantly increased in glioma according to our systematic meta-analysis, the down-regulated hub genes EPHA4 and NEDD4L owned a great chance to act as crucial target genes of miR-10b-5p. As regards the rest five hub genes (PHLPP2, MAPK1, RPS6KA2, YWHAG, and NEDD4L), although no statistical expression difference was observed, there was a trend that the expression of PHLPP2, RPS6KA2, YWHAG and NEDD4L was down-regulated in GBM samples. It still indicated their possibility of acting as key target genes of miR-10b-5p in glioma. Here, we focused on EPHA4 and NEDD4L for detailed discussion.

PRKCE (protein kinase C epsilon) encodes serine- and threonine-specific protein kinase participating in different cellular functions including neuron channel activation, apoptosis, heat shock response, as well as insulin exocytosis. Over the last few years, PRKCE has been discovered to be implicated in different cancer events. Wang H found that PRKCE/MDR1 axis was involved in gallbladder cancer medicine therapy sensitivity [30]. And PRKCE was also found to be a target of miR-146a in papillary thyroid [31]. Besides, PRKCE was an essential

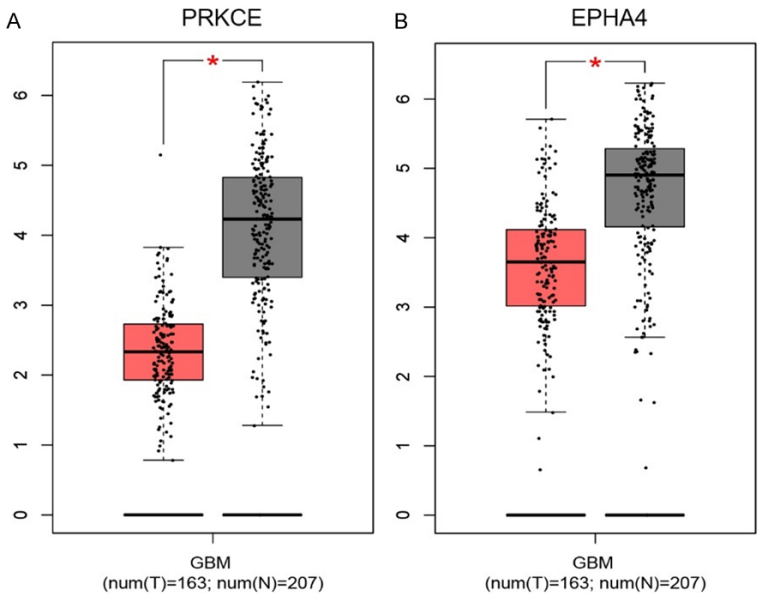

Figure 12. The expression box plot of PRKCE and EPHA4. A. The expression of PRKCE was down-regulated in glioma. B. The expression of EPHA4 was down-regulated in glioma.

factor for the formation of bone metastasis by prostate cancer cells [32]. In Caino MC's study, PRKCE was revealed to be indispensable in NSCLC cell survival, which made it an attractive therapeutic target for NSCLC [33]. Similarly, Huang B discovered that overexpression of PRKCE was correlated with an aggressive phenotype of clear cell renal cell carcinoma, which also made it a promising therapeutic target. In glioma, knockdown of PRKCE was found to suppress growth, induce apoptosis and reduce invasiveness of human glioma cells [34]. However, up to now, no study has illuminated the exact interaction between miR-10b-5p and glioma. Nevertheless, due to PRKCE was influenced after the miR-10b-5p silence in the GEO datasets we included, and the expression of PRKCE was negatively correlated with miR-10b-5p, we could speculate that PRKCE was a promising target of miR-10b-5p in glioma. The interaction between miR-10b-5p and PRKCE might help explain parts of the potential molecular mechanisms of glioma.

EPHA4 (EPH receptor A4), belongs to the ephrin receptor subfamily of the protein-tyrosine kinase family. EPH and EPH-related receptors are known to participate in mediating developmental events, particularly in the nervous system. Recently, EPHA4 was reported to play a crucial role in various cancers. For instance, EPHA4 was found to promote cell proliferation and drug resistance in multiple myeloma [35]. In lung adenocarcinoma, EPHA4 exerted inhibition function in the migration and invasion [36]. And Sun Y found that decreased expression of EPHA4 was correlated with the advanced TNM stage, lymph node metastasis, and worse prognosis of breast cancer [37]. Besides, EPHA4 was also reported as a prognostic factor gastric cancer [38]. As regards glioma, Fukai J revealed EPHA4 was able to promote cell proliferation and migration via EphA4-FGFR1 signaling pathway in the human glioma U251 cell line [39]. However, there was no report about the correlation between EPHA4 and miR-10b-5p. Still, being similar to PRKCE, the expression of EPHA4 was negatively correlated with miR-10b-5p according to the validation through GBM samples. As a result, EPHA4 was expected to be a promising target of miR-10b-5p in glioma, which might help illuminate parts of the molecular interaction mechanisms of glioma.

Nevertheless, limitations existed in this study. All analyses in this paper were performed computationally, thus need further validation through experimentation. However, the information we produced still provided references for the research on the molecular mechanisms of glioma. Similar investigation methods could also be used to other miRNAs and cancers.

Above all, miR-10b-5p was overexpressed in glioma and may act as a satisfactory diagnostic target. Key targets of miR-10b-5p including PHLPP2, MAKP1, RPS6KA2, PRKCE, YWHAG, EPHA4, and NEDD4L, especially PRKCE and EPHA4 may be of great importance in the regulatory molecular mechanisms of glioma. And pathways such as MAPK signaling pathway, Oocyte meiosis, Axon guidance and mTOR signaling pathway may exert crucial function in the molecular regulation of miR-10b-5p in glioma.

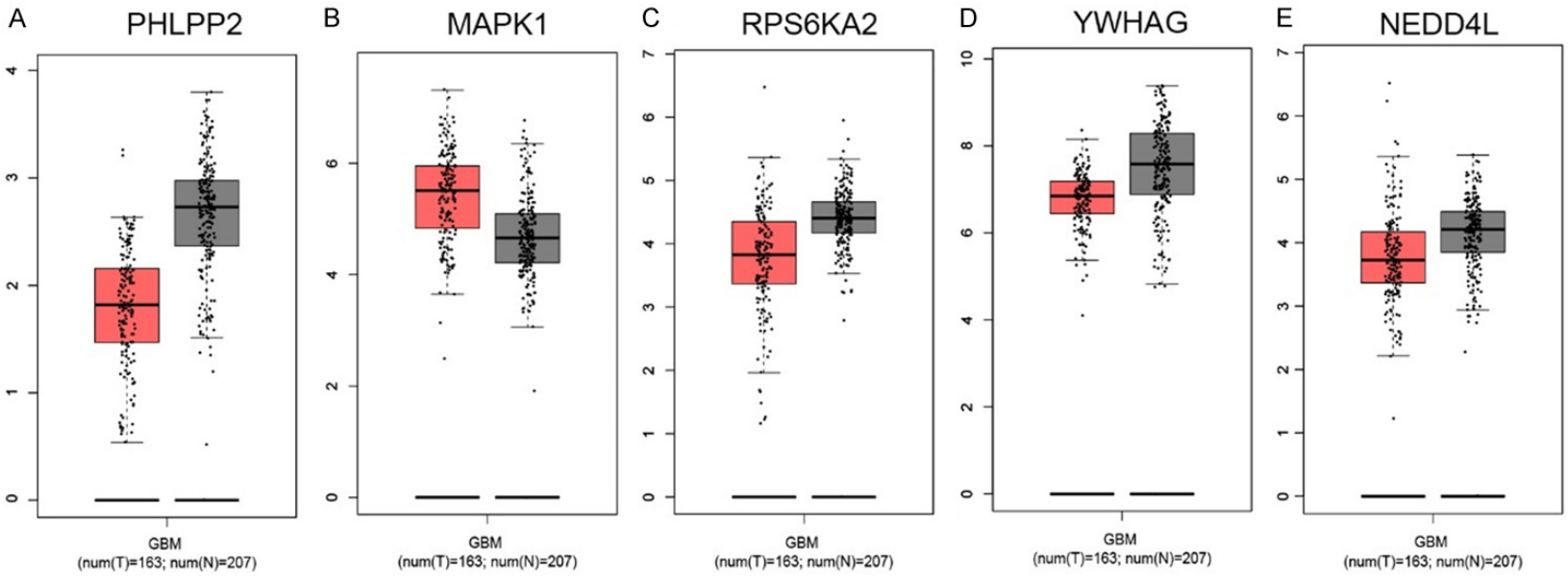

Figure 13. The expression box plot of PHLPP2, MAPK1, RPS6KA2, YWHAG and NEDD4L. A. The expression of PHLPP2 in glioma. B. The expression of MAPK1 in glioma. C. The expression of RPS6KA2 in glioma. D. The expression of YWHAG in glioma. E. The expression of NEDD4L in glioma.

Conclusion

In the current study, up-regulated miR-10b-5p may become a promising diagnostic marker in glioma. Moreover, MAPK signaling pathway, Oocyte meiosis, Axon guidance, mTOR signaling pathway, as well as hub genes PHLPP2, MAKP1, RPS6KA2, PRKCE, YWHAG, EPHA4 and NEDD4L), especially PRKCE and EPHA4 may be the indispensable elements to help illustrate part of the interaction mechanisms between miR-10b-5p and glioma. The current findings might shed light on the future theoretical research and clinical practice.

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Disclosure of conflict of interest

None.

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Supplementary 1. The 124 overlapping genes intersected from up-regulated genes in the microarray, down-regulated genes in the TCGA GBM samples, and the predicted target genes.

CYTH1
SEMA4D
PPM1A
FAXC
CAMK2B
KIAA0930
NFAT5
KIF1B
RPS6KA2
MCF2L
TBC1D24
RB1CC1
TRPM3
KLC2
TPPP
JPH1
PNMA3
YPEL2
CACNB3
PAFAH1B1
RAPH1
STRIP2
NT5DC3
ARRB1
DENND5B
PRKCE
PRDM11
ZNF280B
NRIP3
GAS7
SLC38A1
ACSL6
ANK3
ADD2
ELAVL3
TRAPPC6B
GNAL
NDUFAF4
SLC6A15
TRIM2
MAFK
PIP4K2B
PRRC2B
NUAK1
RALGPS1
NEDD4L
EFNA5
YWHAG
FAM102A
MAPK1
SBF1

CELF2
LRRC8B
ABHD17B
AGAP3
KLF13
TMTC1
PPARGC1B
TMEM63A
DUSP7
LDLRAD4
PDPK1
MEGF9
KY
VPS13D
PGP
ASXL3
RAB3B
DDN
SLC45A4
RUSC2
ZMYND11
PLLP
AGAP1
MGEA5
SPTBN1
DOHH
NCAM1
EPHA4
ELAVL2
DLGAP1
OTUB2
CCSER2
KIAA0513
CACNB2
KIF1A
ZFYVE27
KIAA1462
CRTC1
AAK1
SLC16A7
SLCO3A1
KBTBD6
ZBTB7A
NDFIP2
BCAS4
PDZD8
RASAL2
MMD
PPM1K
FAM126B
GABBR1
VPS53
OSBPL1A
DMRT2

RAPGEF2
SOBP
TOLLIP
SMIM13
BRWD1
PHLPP2
RTN4R
KCNA2
BTRC
TTBK2
INF2
RIMS3
NTRK3
CLASP2
KLC1
SGSM2
FOXP1
ATE1
GPLD1