

# Circular RNA hsa\_circ\_0005114-miR-142-3p/miR-590-5p-adenomatous polyposis coli protein axis as a potential target for treatment of glioma

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Received September 12, 2019; Accepted October 22, 2020

DOI: 10.3892/ol.2020.12320

**Abstract.** Glioma is the most common type of brain tumor and is associated with a high mortality rate. Despite recent advances in treatment options, the overall prognosis in patients with glioma remains poor. Studies have suggested that circular (circ)RNAs serve important roles in the development and progression of glioma and may have potential as therapeutic targets. However, the expression profiles of circRNAs and their functions in glioma have rarely been studied. The present study aimed to screen differentially expressed circRNAs (DECs) between glioma and normal brain tissues using sequencing data collected from the Gene Expression Omnibus database (GSE86202 and GSE92322 datasets) and explain their mechanisms based on the competing endogenous (ce)RNA regulatory hypothesis. In total, 424 commonly downregulated DECs (with the Gene\_symbol annotated in the circBase database) in these two datasets were identified. Using the CircInteractome and Starbase databases, 18 micro (mi)RNAs (miRs) were predicted to interact with DECs, while 22 glioma-related genes obtained from the Comparative Toxicogenomics Database were predicted to be regulated by 15 miRNAs via the miRwalk 2.0 database. A ceRNA network was established based on 115 DECs, 15 miRNAs and 22 mRNAs. LinkedOmics online analysis using The Cancer Genome Atlas (TCGA) data showed that hsa-miR-142-3p/hsa-miR-590-5p and their target gene adenomatous polyposis coli protein (APC) were all significantly associated with overall survival rate and their prognosis trend was opposite, revealing that high expression levels of hsa-miR-142-3p/hsa-miR-590-5 were associated with a poor overall survival rate, while high

APC expression with a good overall survival rate. UALCAN analysis using TCGA data of glioblastoma multiforme and the GSE25632 and GSE103229 microarray datasets showed that hsa-miR-142-3p/hsa-miR-590-5p was upregulated and APC was downregulated. Thus, hsa-miR-142-3p/hsa-miR-590-5p-APC-related circ/ceRNA axes may be important in glioma, and hsa\_circ\_0005114 interacted with both of these miRNAs. Functional analysis showed that hsa\_circ\_0005114 was involved in insulin secretion, while APC was associated with the Wnt signaling pathway. In conclusion, hsa\_circ\_0005114-miR-142-3p/miR-590-5p-APC ceRNA axes may be potential targets for the treatment of glioma.

## Introduction

Glioma is the most common malignant primary brain tumor, with an incidence of 4.7-7.3/100,000 individuals (1-4) and a mortality rate of 1.2-4.0/100,000 individuals (5). Despite advances in treatment options (such as radical surgery and chemo-radiotherapy), the prognosis of most patients remains poor due to high recurrence rates and invasiveness, with the 5-year survival rate of only 3-6% for grade IV (1,4). Therefore, it is important to further resolve the mechanisms underlying glioma progression to identify novel targets and develop new therapeutic approaches.

Circular (circ)RNAs are a newly identified class of non-coding (nc)RNAs with a covalent loop structure lacking the 5'-end cap and 3'-end poly A tail, which prevents degradation by RNA exonucleases and confers strong stability in specific cells, such as tumor cells (6-8). Therefore, abnormal expression of circRNAs may be a potential mechanism for the development and progression of cancer, including glioma. This hypothesis has been demonstrated in several studies. For example, Zhou *et al* (9) demonstrated that hsa\_circ\_0008344 was significantly upregulated in glioblastoma tissues compared with the adjacent normal brain tissue. Zhou *et al* also reported that knockdown of hsa\_circ\_0008344 suppressed glioblastoma cell proliferation, colony formation, migration and invasion, but facilitated apoptosis. Wang *et al* (10) found that hsa\_circ\_0001649 expression was decreased in glioma specimens and cell lines. In addition, downregulated hsa\_circ\_0001649 is significantly associated with advanced grade and poor prognosis for patients with glioma. The functions and mechanism of

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**Key words:** glioma, circular RNAs, competitive endogenous RNA, prognosis

circRNAs remain unclear; however, accumulating evidence indicates circRNAs have micro (mi)RNA (miR) response elements (MREs) (6-8), while MREs are widely known to be located in the 3'-untranslated region (3'-UTR) of the target mRNAs and miRNAs inhibit mRNA translation by binding to MREs. Thus, circRNAs may directly bind to miRNAs, impacting the interaction between miRNAs and mRNAs and contributing to the progression of glioma. This is known as the competitive endogenous RNA (ceRNA) hypothesis (6-8). This mechanism of circRNA function has been reported in several studies. For example, circ-pituitary homeobox (PITX)1 is upregulated in cancerous tissues and four cell lines of glioma. Dual-luciferase reporter and rescue assays indicated that circ-PITX1 may exert its oncogenic functions by sponging miR-518a-5p, leading to the release of the miR-518a-5p-mediated repression of interleukin 17 receptor D (IL17RD) (11). Similarly, hsa\_circ\_0034642 levels are also increased in glioma tissues and cells. Mechanistically, hsa\_circ\_0034642 sponges miR-1205 to regulate basic leucine zipper ATF-like transcription factor (BATF)3 levels to facilitate cell proliferation, migration and invasion (12). Circ-epididymis-specific  $\alpha$ -mannosidase (MAN2B2) regulates S100 calcium-binding protein A8 (S100A8) expression by inhibiting miR-1205 and increasing S100A8 expression rescues the tumor suppressor effects of knockout of circ-MAN2B2 (13). Upregulated hsa\_circ\_0074362 plays a role in glioma progression by regulating the miR-1236-3p/homeobox B7 pathway (14), and downregulated hsa\_circ\_0001946 may act as a ceRNA, inhibiting glioblastoma progression by modulating miR-671-5p and cerebellar degeneration-related protein 1 (15). Overall, these findings imply that circRNA/ceRNA axes may be potential targets for the treatment of glioma; however, few studies have investigated this.

The present study aimed to further screen crucial circRNA/ceRNA axes for glioma by using the circRNA sequencing data of Yuan *et al* (16) and Zhu *et al* (17). Only the common differentially expressed circRNAs (DECs) in these two datasets were used to construct the ceRNA network based on the interactions between circRNAs-miRNAs and miRNAs-mRNAs. The expression and prognostic ability of these hub miRNAs and mRNAs were also validated using The Cancer Genome Atlas (TCGA) database and microarray data, which indirectly illustrated the possible proto- or antioncogenic roles of circRNAs. The current study may provide some novel therapeutic targets for glioma.

## Materials and methods

**Data collection.** In total, two circRNA datasets of glioblastoma with the accession numbers GSE86202 (16) and GSE92322 (17) were downloaded from the Gene Expression Omnibus (GEO) database (<http://www.ncbi.nlm.nih.gov/geo/>). Three glioma and paired normal brain tissues were used for RNA sequencing on Illumina HiSeq 2500 (*Homo sapiens*, GPL16791) in GSE86202 dataset (16), while 10 samples (five glioma and paired normal brain tissues) were included for circRNA sequencing on Illumina HiSeq 2000 (*Homo sapiens*, GPL11154) in GSE92322 dataset (17).

**Screening of DECs.** The normalized data of each dataset was downloaded from the GEO database. The gene symbol and

circID of circRNAs were annotated using the circBase database (<http://www.circbase.org>) (18) according to the chromosome location and information of the positive-negative chain. The DECs were identified using edgeR package (<http://www.bioconductor.org/packages/release/bioc/html/edgeR.html>) (19).  $\log_2FC$  (fold-change)  $>1$  and  $P < 0.05$  were defined as the significance threshold values. The heatmap of DECs was created using pheatmap package (version 1.0.8; <https://cran.r-project.org/web/packages/pheatmap/>) of R language (version 3.4.1; <http://www.r-project.org/>) (20).

**Construction of circRNA-miRNA-mRNA ceRNA network.** The miRNAs that interacted with common DECs in two datasets were predicted using the CircInteractome (<https://circinteractome.nia.nih.gov>) (21) and StarBase (<http://starbase.sysu.edu.cn/starbase2/>) (22) databases. Only the shared miRNAs predicted in these two databases were used for further analysis. The target genes of these miRNAs were subsequently predicted using the miRwalk 2.0 database (23), which included 12 prediction programs (miRWalk, MicroT4, miRanda, miRBridge, miRDB, miRMap, miRNAmap, PICTAR2, PITA, RNA22, RNAhybrid and TargetsCan). Only the target genes predicted by at least six algorithms were retained, which were then compared with established glioma-related genes collected from the Comparative Toxicogenomics database (CTD) (24) to obtain glioma-associated miRNA-mRNA interaction pairs. The circRNA-miRNA-mRNA-ceRNA network was then established based on these interaction relationships between DECs-miRNAs and miRNAs-mRNAs using Cytoscape software (version 3.6.1; [www.cytoscape.org/](http://www.cytoscape.org/)) (25).

**Functional enrichment analysis for DECs and target genes in the ceRNA network.** Enrichment analyses for Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways and Gene Ontology (GO) [including biological process (BP), cellular component (CC) and molecular function (MF)] terms were conducted using the ClusterProfiler tool (26) to predict the possible function of DECs and target genes in the ceRNA network.  $P < 0.05$  adjusted for multiple testing according to Benjamini and Hochberg (27) was regarded as the cut-off value.

**Prognosis and expression validation for miRNAs and target genes in the ceRNA network.** The LinkedOmics online database (<http://www.linkedomics.org/>) (28) was searched to explore the associations between miRNAs/mRNAs and overall survival (OS) rate for glioma based on a Cox regression, after which Kaplan-Meier survival curves were automatically provided. TCGA-glioblastoma multi-forme-low grade glioma (GBMLGG) miRNAseq/RNA-seq and clinical data were selected for miRNAs/mRNAs analysis. The UALCAN web-portal (<http://ualcan.path.uab.edu/index.html>) (29) was used for validation of the expression of target genes of miRNAs between unmatched glioblastoma multi-forme and normal control tissues using TCGA data, in which the statistical significance was estimated using unpaired Student's t-tests. GSE103229 (including five glioblastoma and five normal control tissues) and GSE25632 (including 82 glioblastoma and five normal controls) (30) microarray datasets in the GEO database were used to determine the expression

Table I. circRNAs identified for glioma.

circID	Gene_symbol	GSE92322		GSE86202	
		logFC	P-value	logFC	P-value
hsa_circ_0001368	KLHL24	-7.03946	1.62x10 <sup>-7</sup>	-11.743	3.62x10 <sup>-3</sup>
hsa_circ_0098551	YAF2	-6.11209	1.81x10 <sup>-4</sup>	-9.32971	1.72x10 <sup>-2</sup>
hsa_circ_0100496	DGKH	-5.23572	6.47x10 <sup>-3</sup>	-8.82079	2.37x10 <sup>-2</sup>
hsa_circ_0001367	KLHL24	-5.88609	5.50x10 <sup>-4</sup>	-10.9425	6.07x10 <sup>-3</sup>
hsa_circ_0064615	SLC4A7	-5.87691	3.79x10 <sup>-4</sup>	-10.562	7.75x10 <sup>-3</sup>
hsa_circ_0060425	PTPRT	-5.01633	1.54x10 <sup>-2</sup>	-7.7421	4.77x10 <sup>-2</sup>
hsa_circ_0001936	BRWD3	-5.02396	1.54x10 <sup>-2</sup>	-8.73538	2.51x10 <sup>-2</sup>
hsa_circ_0104727	SH3GL3	-5.67125	7.75x10 <sup>-4</sup>	-11.395	4.53x10 <sup>-3</sup>
hsa_circ_0005114	RIMS2	-8.09913	3.48x10 <sup>-11</sup>	-6.44629	3.90x10 <sup>-2</sup>
hsa_circ_0104726	SH3GL3	-4.80592	1.18x10 <sup>-2</sup>	-10.2547	9.46x10 <sup>-3</sup>
hsa_circ_0000120	MAN1A2	-4.33712	3.20x10 <sup>-2</sup>	-11.2985	4.83x10 <sup>-3</sup>
hsa_circ_0069718	DCUN1D4	-5.42038	2.74x10 <sup>-3</sup>	-10.1942	9.83x10 <sup>-3</sup>
hsa_circ_0130887	PEX3	-5.26036	6.47x10 <sup>-3</sup>	-10.2911	9.24x10 <sup>-3</sup>
hsa_circ_0004516	C12orf24	-4.78292	1.18x10 <sup>-2</sup>	-9.66325	1.38x10 <sup>-2</sup>
hsa_circ_0117841	SLC4A10	-5.46976	2.74x10 <sup>-3</sup>	-10.8846	6.30x10 <sup>-3</sup>
hsa_circ_0001369	KLHL24	-5.94584	2.61x10 <sup>-4</sup>	-10.6354	7.40x10 <sup>-3</sup>

Only the top 16 most significantly downregulated circRNAs that may act as a sponge of miR-142-3p/miR-590-5p are listed. FC, fold change; circ, circular.

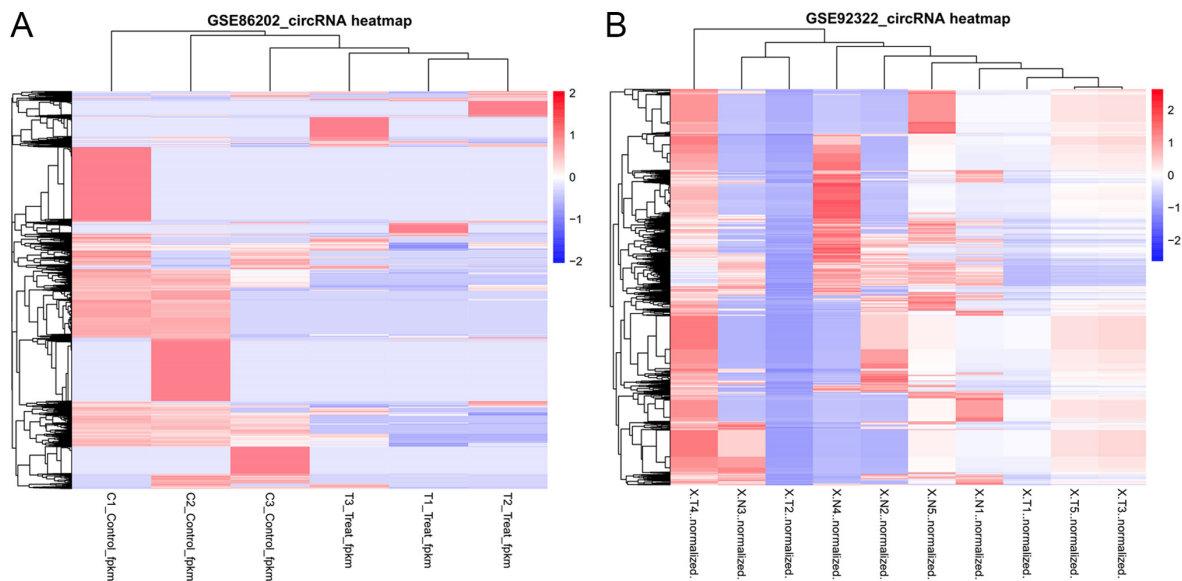


Figure 1. Heat map analysis of differentially expressed circRNAs in the (A) GSE86202 and (B) GSE92322 datasets. circ, circular.

of crucial miRNAs with GEO2R, an interactive web-based statistical tool (<https://www.ncbi.nlm.nih.gov/geo/geo2r/>).  $P < 0.05$  was considered to indicate a statistically significant difference.

## Results

**Identification of DECs.** In the GSE86202 dataset, 23,463/37,085 circRNAs were annotated to the circID in the circBase database and 6,131 had corresponding Gene\_symbols. In the

GSE92322 dataset, 12,863/59,386 circRNAs were annotated to the circID in the circBase database and 4,676 of them had corresponding Gene\_symbol.

Using the edgeR package, a total of 22 upregulated (20 had circID and Gene\_symbol) and 2,068 (1,874 had circID and 1,332 had Gene\_symbol) DECs were identified between glioma and paired normal brain tissues in the GSE86202 dataset. Meanwhile, 29 upregulated (zero had circID and Gene\_symbol) and 1,205 (1,196 had circID and 884 had Gene\_symbol) DECs were identified in the GSE92322 dataset (Table I). The heat maps

Table II. Kyoto Encyclopedia of Genes and Genomes pathways enriched for differentially expressed circRNAs.

ID	Description	Adjusted P-value	Gene ID
hsa04723	Retrograde endocannabinoid signaling	1.65x10 <sup>-2</sup>	CACNA1A/CACNA1C/GABRB2/GABRG3/GNAQ/NAPEPLD/NDUFA10/NDUFS1/PRKACB/PRKCB/RIMS1
hsa04012	ErbB signaling pathway	1.65x10 <sup>-2</sup>	AKT2/AKT3/PAK1/PAK3/PIK3CA/PRKCB/PTK2/SOS2
hsa04911	Insulin secretion	1.65x10 <sup>-2</sup>	CACNA1C/GNAQ/KCNMA1/PCLO/PRKACB/PRKCB/RIMS2/RYR2
hsa04020	Calcium signaling pathway	1.65x10 <sup>-2</sup>	RYR2/CACNA1A/CACNA1C/CACNA1E/CAMK4/GNAQ/PDE1C/PHKB/PRKACB/PRKCB/RYR2/SLC8A1
hsa04725	Cholinergic synapse	1.65x10 <sup>-2</sup>	AKT2/AKT3/CACNA1A/CACNA1C/CAMK4/GNAQ/PIK3CA/PRKACB/PRKCB
hsa05211	Renal cell carcinoma	1.65x10 <sup>-2</sup>	AKT2/AKT3/CREBBP/PAK1/PAK3/PIK3CA/SOS2
hsa05205	Proteoglycans in cancer	2.22x10 <sup>-2</sup>	AKT2/AKT3/ANK2/ANK3/ARHGEF12/PAK1/PIK3CA/PRKACB/PRKCB/PTK2/SOS2/TIAM1
hsa05231	Choline metabolism in cancer	2.27x10 <sup>-2</sup>	AKT2/AKT3/DGKB/DGKH/DGKI/PIK3CA/PRKCB/SOS2
hsa04660	T cell receptor signaling pathway	2.30x10 <sup>-2</sup>	AKT2/AKT3/DGKI/4CACNA1C/PAK1/PAK3/PIK3CA/SOS2
hsa04810	Regulation of actin cytoskeleton	2.58x10 <sup>-2</sup>	APC/ARHGEF12/ARHGEF7/DOCK1/MYH10/PAK1/PAK3/PIK3CA/PIKFYVE/PTK2/SOS2/TIAM1
hsa04024	cAMP signaling pathway	3.38x10 <sup>-2</sup>	AKT2/AKT3/RYR2/CACNA1C/CAMK4/CREBBP/PAK1/PIK3CA/PRKACB/RYR2/TIAM1
hsa04720	Long-term potentiation	3.38x10 <sup>-2</sup>	CACNA1C/CAMK4/CREBBP/GNAQ/PRKACB/PRKCB
hsa04919	Thyroid hormone signaling pathway	3.38x10 <sup>-2</sup>	AKT2/AKT3/CREBBP/MED13L/PIK3CA/PRKACB/PRKCB/THRB
hsa05032	Morphine addiction	3.38x10 <sup>-2</sup>	CACNA1A/GABRB2/GABRG3/PDE1C/PDE8A/PRKACB/PRKCB
hsa04014	Ras signaling pathway	3.38x10 <sup>-2</sup>	AKT2/AKT3/NTRK2/PAK1/PAK3/PIK3CA/PRKACB/PRKCB/RAPGEF5/RASA2/SOS2/TIAM1
hsa04961	Endocrine and other factor-regulated calcium reabsorption	3.38x10 <sup>-2</sup>	RYR2/GNAQ/PRKACB/PRKCB/SLC8A1
hsa00280	Valine, leucine and isoleucine degradation	3.48x10 <sup>-2</sup>	HADHB/HMGCLL1/HMGCS1/MCCC1/PCCA
hsa00510	N-Glycan biosynthesis	3.48x10 <sup>-2</sup>	FUT8/MAN1A2/MAN2A1/ST6GAL2/TUSC3
hsa04662	B cell receptor signaling pathway	3.48x10 <sup>-2</sup>	AKT2/AKT3/4CACNA1C/PIK3CA/PRKCB/SOS2
hsa05170	Human immunodeficiency virus 1 infection	3.94x10 <sup>-2</sup>	AKT2/AKT3/CUL5/GNAQ/4CACNA1C/PAK1/PAK3/PDIA3/PIK3CA/PRKCB/PTK2
hsa04070	Phosphatidylinositol signaling system	3.94x10 <sup>-2</sup>	DGKB/DGKH/DGKI/PIK3C3/PIK3CA/PIKFYVE/PRKCB
hsa05214	Glioma	3.95x10 <sup>-2</sup>	AKT2/AKT3/CAMK4/PIK3CA/PRKCB/SOS2
hsa04728	Dopaminergic synapse	4.67x10 <sup>-2</sup>	AKT2/AKT3/CACNA1A/CACNA1C/CLOCK/GNAQ/PRKACB/PRKCB

APC, adenomatous polyposis coli protein.

of these DECs are displayed in Fig. 1. These findings suggested that these DECs may be effective to classify the samples into two groups.

*Functional enrichment analysis for DECs.* A comparison between the two aforementioned datasets reported that 424 downregulated DECs with Gene\_symbol were shared. The functions of DECs were enriched according to their corresponding Gene\_symbols. The results showed that

23 KEGG pathways were enriched (Table II and Fig. 2A), mainly including ‘retrograde endocannabinoid signaling’, ‘ErbB signaling pathway’ and ‘insulin secretion’ for hsa\_circ\_0005114. Meanwhile, KEGG pathways enriched for hsa\_circ\_0100496 were ‘calcium signaling pathway’, ‘cholinergic synapse’ and ‘choline metabolism in cancer’, and ‘N-Glycan biosynthesis’ was enriched for hsa\_circ\_0000120. Furthermore, 143 GO terms (including 87 BPs, 38 CCs and 18 MFs; Table III and Fig. 2B) were also enriched, including

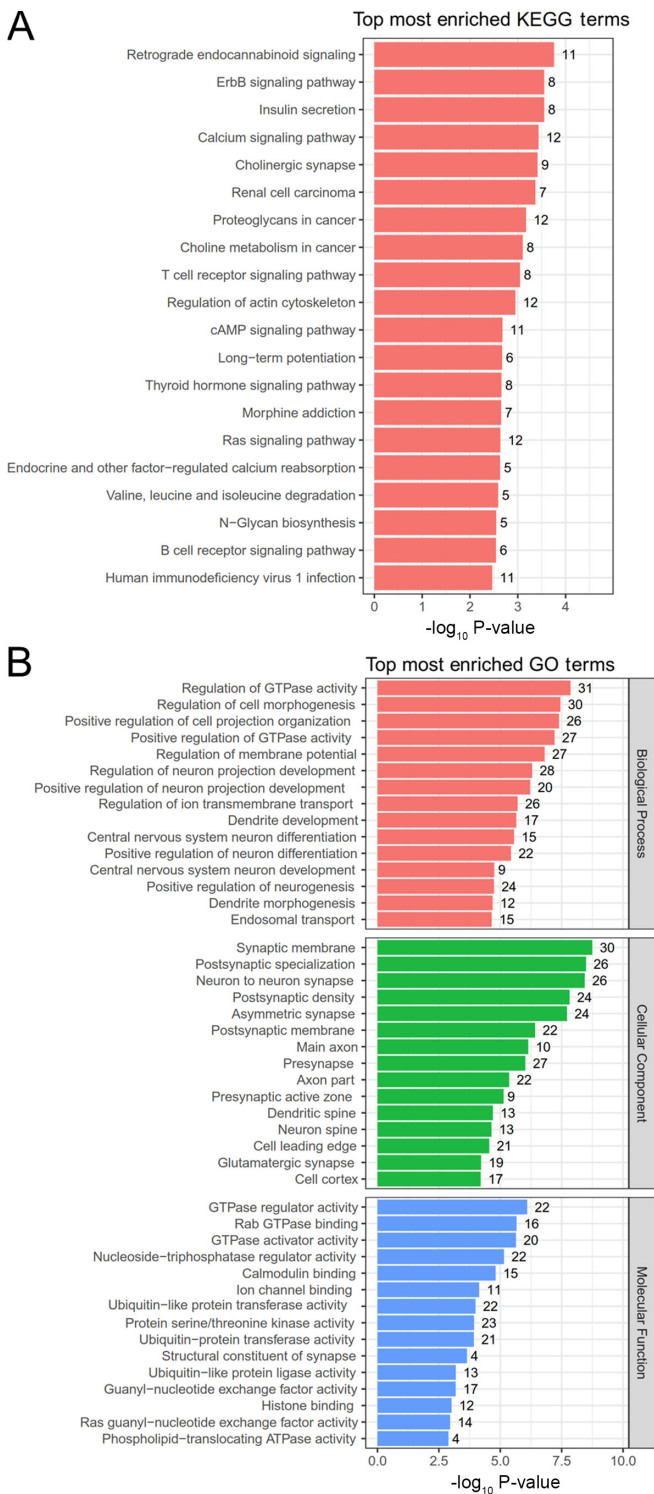


Figure 2. Function enrichment analysis for differentially expressed circular RNAs. (A) Top 20 KEGG pathways and (B) top 15 biological process, cellular component and molecular function GO terms. KEGG, Kyoto Encyclopedia of Genes and Genomes; GO, Gene Ontology.

'regulation of cell morphogenesis' (hsa\_circ\_0005114 and hsa\_circ\_0001936), 'regulation of neuron projection development' (hsa\_circ\_0005114), 'central nervous system neuron differentiation' (hsa\_circ\_0117841), 'positive regulation of neuron differentiation' (hsa\_circ\_0005114, hsa\_circ\_0104727) and 'cell-cell junction' (hsa\_circ\_0001368). These findings suggested that hsa\_circ\_0005114 may be crucial for the

development of glioma since it was enriched in KEGG pathways and GO terms.

*Construction of the ceRNA network and functional enrichment.* After uploading the circID (197 common in the two datasets) to the CircInteractome database, 313 miRNAs were predicted to interact with DECs. Using Gene\_symbol, 276 miRNAs were predicted to interact with DECs using Starbase. Among them, 18 miRNAs were shared, including hsa-miR-139-5p, -142-3p, -184, -217, -324-5p, -326, -330-5p, -338-3p, -346, -375, -384, -421, -496, -543, -590-5p, -599, -615-3p and -875-5p). Subsequently, the target genes were predicted for these 18 miRNAs using the miRwalk 2.0 database, which were then compared with 65 pre-established glioma-related genes collected from the CTD database. In total, 22 genes were shared, including BRD4, SUZ12, VEGFA, NOTCH3, NOTCH2, RUNX3, CTNND2, EGFR, CDK6, PML, BHLHE40, EGF, NOTCH1, TNFSF10, LZTR1, RUNX1, MDM4, MET, IL1B, JAG1, APC and RECK. Then, the ceRNA network was established based on 115 DECs, 15 miRNAs and the aforementioned 22 mRNAs (Fig. 3).

Function analysis for the target genes of miRNAs in this ceRNA network showed that 44 KEGG pathways (Table IV and Fig. 4A) and 736 GO terms (including 713 BPs, three CCs and 20 MFs) (Table V and Fig. 4B) were enriched, such as 'regulation of actin cytoskeleton' (Table IV), various cancer pathways (including 'miRNAs in cancer', 'breast cancer', 'endometrial cancer', 'gastric cancer', 'hepatocellular carcinoma' and 'colorectal cancer'; Fig. 4A), 'Wnt signaling pathway', 'cell cycle arrest', 'cell fate commitment' (Fig. 4B) and cell junction assembly (Table V), in all of which the APC gene was included. These findings suggested that the circRNAs-miRNAs that regulated APC may be crucial for the development of glioma.

*Prognosis and expression analysis for miRNAs and target genes.* LinkedOmics online analysis using TCGA data showed that 19 mRNAs and nine miRNAs were significantly associated with OS (Table VI and Fig. 5). These prognosis-related miRNAs and mRNAs constituted 20 interaction relationships in the ceRNA network. However, the prognosis trend between hsa-miR-217/hsa-miR-324-5p/hsa-miR-590-5p/hsa-miR-599/hsa-miR-875-5p and their target genes were consistent, with high expression levels of both miRNAs and their target genes indicating a poor prognosis, which is not in accordance with the negative regulatory mechanisms of miRNAs on mRNAs. Thus, only hsa-miR-139-5p-NOTCH1/RUNX1/CDK6/TNFSF10/VEGFA and hsa-miR-142-3p-APC-related ceRNA axes may be important. Furthermore, as all the circRNAs in the ceRNA network were downregulated, its interactive miRNAs should be upregulated according to the ceRNA theory (6-8); however, similarly to circRNAs, high expression levels of hsa-miR-139-5p were also associated with an excellent overall survival rate, indicating that hsa-miR-139-5p was also downregulated in glioma. Thus, hsa-miR-139-5p-related interaction relationships were excluded from further analyses and only hsa-miR-142-3p-APC-related ceRNA axes (in which high hsa-miR-142-3p expression was associated with poor OS, while high APC expression was associated with a good prognosis, as shown in Fig. 5) were crucial.

Table III. Significant GO terms enriched for differentially expressed circRNAs.

GO term	ID	Description	Adjusted P-value	Gene ID
BP	GO:0022604	Regulation of cell morphogenesis	4.70x10 <sup>-5</sup>	ARHGAP44/ARHGEF7/BRWD1/BRWD3/CAPRIN1/CORO1C/CUX1/DLG1/DOCK1/FMNL2/HECW1/HECW2/MKLN1/MYH10/NTRK2/PAK1/PAK3/PARP6/PHIP/PSEN1/PTK2/RHOBTB3/RIMS1/RIMS2/ROBO1/ROBO2/SYT1/TIAM1/TNIK/ZMYM4
BP	GO:0031346	Positive regulation of cell projection organization	4.70x10 <sup>-5</sup>	APC/ARHGEF7/ATP8A2/AUTS2/CAPRIN1/CBFA2T2/CCDC88A/CORO1C/CPEB3/CUX1/HTT/KDM1A/LRRC7/MAGI2/NTRK2/PAK1/PAK3/PARP6/PSEN1/RIMS1/RIMS2/ROBO1/ROBO2/SYT1/TENM3/TIAM1
BP	GO:0042391	Regulation of membrane potential	1.09x10 <sup>-4</sup>	AKAP6/AKT2/ANK2/ANK3/APP/CACNA1A/CACNA1C/CACNA1E/DGKI/DLG1/FGF14/GABRB2/GABRG3/GCLM/GNAQ/KCNH1/KCNK2/KCNMA1/NALCN/NDUFS1/NTRK2/PSEN1/RIMS1/RIMS2/RYR2/SLC8A1/SLMAP
BP	GO:0010975	Regulation of neuron projection development	2.93x10 <sup>-4</sup>	ARHGAP44/ATP8A2/CAMSAP2/CAPRIN1/CBFA2T2/CCDC88A/CPEB3/CUX1/FBXO7/HECW1/HECW2/KDM1A/LRRC7/MAGI2/NTRK2/PAK1/PAK3/PARP6/PSEN1/PTK2/RIMS1/RIMS2/ROBO1/ROBO2/SYT1/TENM3/TIAM1/TNIK
BP	GO:0034765	Regulation of ion transmembrane transport	8.62x10 <sup>-4</sup>	AKAP6/AKT2/ANK2/ANK3/APP/ATG5/CACNA1A/CACNA1C/CACNA1E/CRBN/DLG1/FGF14/HECW1/HECW2/HTT/KCNH1/KCNMA1/KLHL24/NALCN/PKD2/PSEN1/RYR2/SLC8A1/SLMAP/STK39/UBQLN1
BP	GO:0021953	Central nervous system neuron differentiation	9.64x10 <sup>-4</sup>	ADARB1/AGTPBP1/DCLK1/DCLK2/GIGYF2/HERC1/LRP6/NFIB/NTRK2/PSEN1/PTK2/ROBO1/ROBO2/SATB2/SLC4A10
BP	GO:0045666	Positive regulation of neuron differentiation	1.17x10 <sup>-4</sup>	ATP8A2/CAPRIN1/CBFA2T2/CPEB3/CUX1/KDM1A/LRRC7/MAGI2/NTRK2/PAK1/PAK3/PARP6/PSEN1/RIMS1/RIMS2/ROBO1/ROBO2/SH3GL3/SYT1/TCF4/TENM3/TIAM1
BP	GO:0021954	Central nervous system neuron development	4.84x10 <sup>-3</sup>	ADARB1/DCLK1/DCLK2/NFIB/NTRK2/PTK2/ROBO1/ROBO2/SLC4A10
BP	GO:0050769	Positive regulation of neurogenesis	4.84x10 <sup>-3</sup>	APP/ATP8A2/CAPRIN1/CBFA2T2/CPEB3/CUX1/KDM1A/LRRC7/MAGI2/MAN2A1/NTRK2/PAK1/PAK3/PARP6/PSEN1/RIMS1/RIMS2/ROBO1/ROBO2/SH3GL3/SYT1/TCF4/TENM3/TIAM1
BP	GO:0097479	Synaptic vesicle localization	5.75x10 <sup>-3</sup>	AP3B2/CADPS2/DGKI/ERC2/MAGI2/PCLO/PRKCB/PSEN1/RIMS1/RIMS2/STXBP5L/SYN3/SYT1
CC	GO:0097060	Synaptic membrane	5.96x10 <sup>-7</sup>	ANK2/ANK3/ANKS1B/ARHGAP32/ATP2B1/CACNA1C/CADPS2/CNKS2/CPEB3/DENND1A/DGKI/DLG1/DLG2/ERC1/ERC2/GABRB2/GABRG3/KCNH1/KCNMA1/LRRC7/PICALM/PSD3/PSEN1/RIMS1/RIMS2/SLC8A1/STRN/SYNE1/SYT1/TIAM1
CC	GO:0099572	Postsynaptic specialization	5.96x10 <sup>-7</sup>	ANKS1B/ARHGAP32/ARHGAP44/CACNA1C/CNKS2/CPEB3/DCLK1/DGKI/DLG1/DLG2/GABRB2/KCNH1/LRRC7/MAGI2/MIB1/NTRK2/PAK3/PCLO/PSD3/SEPT11/SH3GL3/STRN/SYN3/TANC2/TIAM1/TNIK

Table III. Continued.

GO term	ID	Description	Adjusted P-value	Gene ID
CC	GO:0098984	Neuron to neuron synapse	5.96x10 <sup>-7</sup>	ANKS1B/ARHGAP32/ARHGAP44/CACNA1C/ CNKSR2/CPEB3/DCLK1/DGKI/DLG1/DLG2/KCNH1/ LRRC7/MAGI2/MIB1/NTRK2/PAK3/PCLO/PSD3/ SEPT11/SH3GL3/STRN/SYN3/SYT1/TANC2/TIAM1/ TNIK
CC	GO:0014069	Postsynaptic density	1.84x10 <sup>-6</sup>	ANKS1B/ARHGAP32/ARHGAP44/CACNA1C/ CNKSR2/CPEB3/DCLK1/DGKI/DLG1/DLG2/KCNH1/ LRRC7/MAGI2/MIB1/NTRK2/PAK3/PCLO/PSD3/ SH3GL3/STRN/SYN3/TANC2/TIAM1/TNIK
CC	GO:0098793	Presynapse	5.86x10 <sup>-5</sup>	APP/ARHGAP44/ATP2B1/CADPS2/DENND1A/DGKI/ ERC1/ERC2/HTT/ICA1/KCNH1/KCNK2/MCTP1/ NTRK2/PCLO/PICALM/PRKCB/PSEN1/RIMS1/ RIMS2/RPH3A/SH3GL3/STX12/SYN3/SYT1/TNIK/ WDR7
CC	GO:0048786	Presynaptic active zone	3.58x10 <sup>-4</sup>	APP/ARHGAP44/ATP2B1/DGKI/ERC1/ERC2/PCLO/ RIMS1/RIMS2
CC	GO:0098978	Glutamatergic synapse	2.05x10 <sup>-3</sup>	ARHGAP44/ATP2B1/CADPS2/CAMK4/CNKSR2/ DGKB/DGKI/DLG1/DLG2/ERC2/MYH10/PAK3/ SEPT11/SH3GL3/SYN3/SYT1/TANC2/TIAM1/TNIK
CC	GO:0005938	Cell cortex	2.05x10 <sup>-3</sup>	AKT2/ARHGAP32/ARHGEF7/ERC2/EXOC6B/ FMN2/MKLN1/MYH10/PCLO/PKD2/PSEN1/PTK2/ RHOBTB3/RIC8B/RIMS1/RIMS2/SEPT11
CC	GO:0043025	Neuronal cell body	2.28x10 <sup>-3</sup>	AMFR/ARHGEF7/ATP2B1/CACNA1A/CACNA1C/ CNKSR2/DENND1A/DGKI/GIGYF2/KCNH1/KCNK2/ KLHL24/LMTK2/LRP6/MYH10/PDE1C/PICALM/ PSEN1/SLC1A4/SLC4A10/STRN/TIAM1/TMEM50A
CC	GO:0005911	Cell-cell junction	8.24x10 <sup>-3</sup>	AKAP6/ANK2/ANK3/APC/APP/ASH1L/DLG1/ KLHL24/LRRC7/MAGI2/NFASC/PAK1/PIKFYVE/ PKD2/SDCCAG8/SLC8A1/SPECC1L/STRN/TIAM1/ TJP1
MF	GO:0017137	Rab GTPase binding	4.74x10 <sup>-4</sup>	ACAP2/CLEC16A/DENND1A/DENND1B/DENND5B/ ERC1/GAPVD1/PICALM/RHOBTB3/RIMS1/RIMS2/ RPH3A/STXBP5L/TBC1D12/TBC1D1/TBCK
MF	GO:0044325	Ion channel binding	7.48x10 <sup>-3</sup>	AKAP6/ANK2/ANK3/DLG1/HTT/KCNH1/PKD2/ RIMS1/RIMS2/RYR2/SLC8A1
MF	GO:0004143	Diacylglycerol kinase activity	4.94x10 <sup>-2</sup>	DGKB/DGKH/DGKI

Only terms enriched by significantly differentially expressed circRNAs that may act as a sponge of miR-142-3p/miR-590-5p are listed. The genes enriched in each term were corresponding to differentially expressed circRNAs. GO, Gene Ontology; BP, biological process; CC, cellular component; MF, molecular function; circ, circular; APC, adenomatous polyposis coli protein.

Accordingly, hsa\_circ\_0000120/ 0001367/ 0001368/ 0001369/ 0001936/ 0004516/ 0005114/ 0060425/0064615/ 0130887-hsa-miR-142-3p-APC ceRNA axes may be potential targets for the development of glioma. Additionally, UALCAN analysis using TCGA data of glioblastoma multiforme demonstrated that APC was significantly downregulated in glioma compared with in normal tissues (P=0.0000822); while GSE25632 microarray analysis showed the expression of hsa-miR-142-3p was significantly higher in glioma compared with that in normal tissues (log<sub>2</sub>FC=1.21, P=0.00284), further

confirming the creditability of these crucial ceRNA axes for glioma (data not shown).

In addition, both of GSE25632 (log<sub>2</sub>FC=5.75, P=0.0226) and GSE103229 (log<sub>2</sub>FC=1.65, P=0.0208) microarray analyses revealed that hsa-miR-590-5p was upregulated in glioma compared with that in normal tissues (data not shown). In fact, the interaction between hsa-miR-590-5p and APC was also predicted by RNA22 and RNAhybrid using miRwalk2.0 analysis (data not shown). Therefore, circRNAs interacting with hsa-miR-590-5p were also important, including

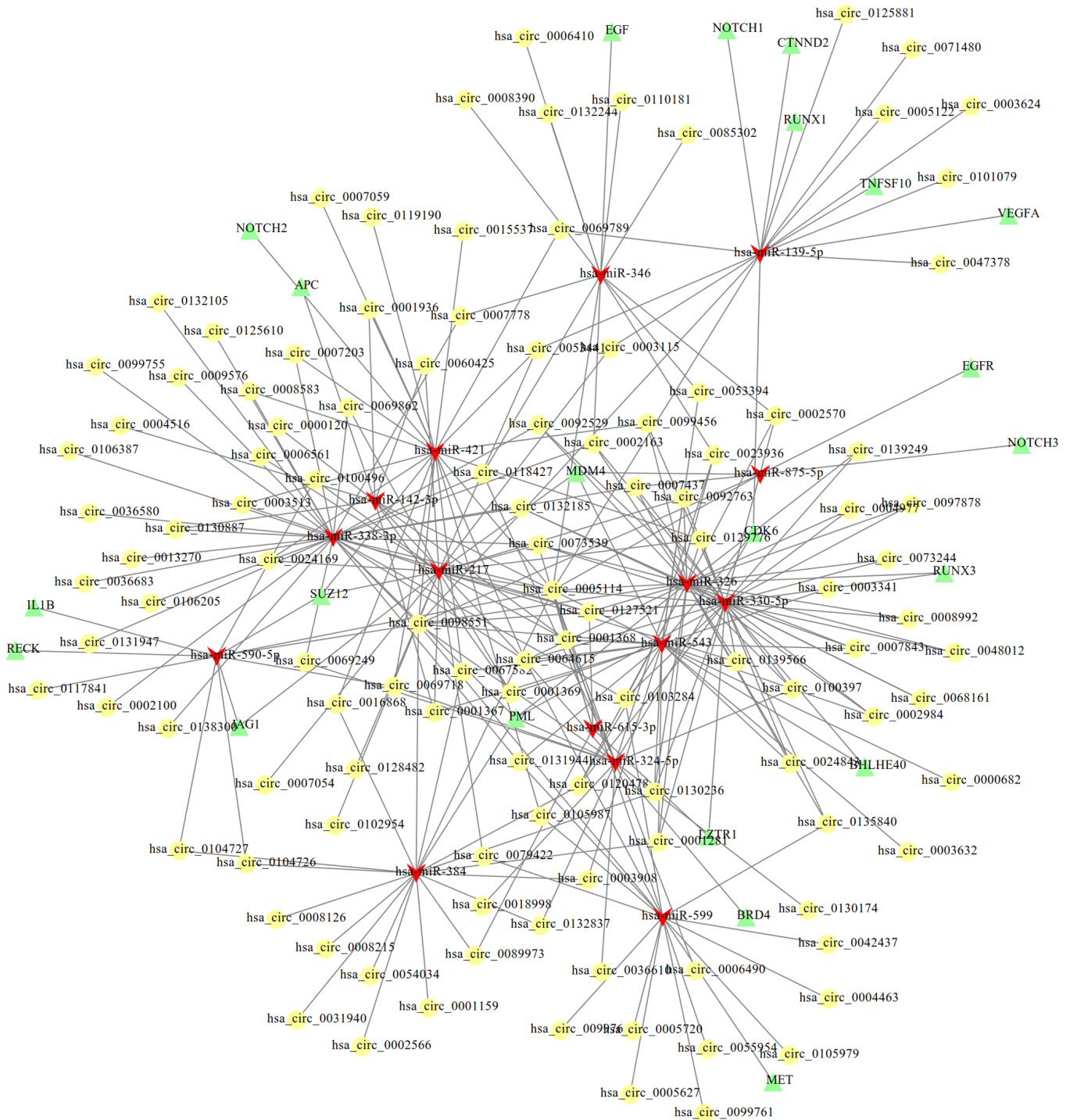


Figure 3. Competing endogenous RNA interaction network of circRNA-miRNA-mRNA. Yellow circles represent circRNAs, green triangles represent mRNAs and red inverted arrowheads represent miRNAs.

hsa\_circ\_0005114, 0069718, 0098551, 0100496, 0104726, 0104727 and 0117841 (Fig. 3), among which hsa\_circ\_0005114 interacted with hsa-miR-142-3p. Therefore, the hsa\_circ\_0005114-miR-142-3p/miR-590-5p-APC ceRNA axis may represent a potential mechanism for the development of glioma.

**Discussion**

In the present study, 16 crucial downregulated circRNAs were identified that may sponge miR-142-3p/miR-590-5p to suppress

glioma progression via regulating the APC gene. Most of them, to the best of our knowledge, were newly reported circRNAs associated with cancer, except for hsa\_circ\_0001368 that has a tumor-suppression role in gastric cancer by regulating the miR-6506-5p/forkhead box (FOX) protein O3 axis (31). However, hsa\_circ\_0005114 may be particularly important as it was a circRNA interacting with both miR-142-3p and miR-590-5p.

The present analysis did not identify the roles of hsa\_circ\_0005114 in cancer; however, hsa\_circ\_0005114 is derived



Table IV. Kyoto Encyclopedia of Genes and Genomes pathways enriched for genes in the competing endogenous RNA network.

ID	Description	Adjusted P-value	Gene ID
hsa05206	microRNAs in cancer	3.02x10 <sup>-8</sup>	APC/CDK6/EGFR/MDM4/MET/NOTCH1/NOTCH2/NOTCH3/RECK/VEGFA
hsa05224	Breast cancer	3.50x10 <sup>-8</sup>	APC/CDK6/EGF/EGFR/JAG1/NOTCH1/NOTCH2/NOTCH3
hsa05165	Human papillomavirus infection	6.64x10 <sup>-7</sup>	APC/CDK6/EGF/EGFR/JAG1/NOTCH1/NOTCH2/NOTCH3/VEGFA
hsa04658	Th1 and Th2 cell differentiation	4.91x10 <sup>-5</sup>	JAG1/NOTCH1/NOTCH2/NOTCH3/RUNX3
hsa01522	Endocrine resistance	5.38x10 <sup>-5</sup>	EGFR/JAG1/NOTCH1/NOTCH2/NOTCH3
hsa04330	Notch signaling pathway	7.36x10 <sup>-5</sup>	JAG1/NOTCH1/NOTCH2/NOTCH3
hsa05218	Melanoma	3.22x10 <sup>-4</sup>	CDK6/EGF/EGFR/MET
hsa05212	Pancreatic cancer	3.31x10 <sup>-4</sup>	CDK6/EGF/EGFR/VEGFA
hsa01521	EGFR tyrosine kinase inhibitor resistance	3.62x10 <sup>-4</sup>	EGF/EGFR/MET/VEGFA
hsa05219	Bladder cancer	1.22x10 <sup>-3</sup>	EGF/EGFR/VEGFA
hsa05213	Endometrial cancer	3.13x10 <sup>-3</sup>	APC/EGF/EGFR
hsa05226	Gastric cancer	3.21x10 <sup>-3</sup>	APC/EGF/EGFR/MET
hsa05223	Non-small cell lung cancer	3.81x10 <sup>-3</sup>	CDK6/EGF/EGFR
hsa04010	MAPK signaling pathway	3.81x10 <sup>-3</sup>	EGF/EGFR/RUNX3/MET/VEGFA
hsa05225	Hepatocellular carcinoma	4.05x10 <sup>-3</sup>	APC/CDK6/EGFR/MET
hsa05214	Glioma	4.59x10 <sup>-3</sup>	CDK6/EGF/EGFR
hsa05210	Colorectal cancer	6.37x10 <sup>-3</sup>	APC/EGF/EGFR
hsa04510	Focal adhesion	6.37x10 <sup>-3</sup>	EGF/EGFR/MET/VEGFA
hsa04151	PI3K-Akt signaling pathway	6.44x10 <sup>-3</sup>	CDK6/EGF/EGFR/MET/VEGFA
hsa04015	Rap1 signaling pathway	6.52x10 <sup>-3</sup>	EGF/EGFR/MET/VEGFA
hsa04066	HIF-1 signaling pathway	8.06x10 <sup>-3</sup>	EGF/EGFR/VEGFA
hsa05163	Human cytomegalovirus infection	8.22x10 <sup>-3</sup>	CDK6/EGFR/RUNX3/VEGFA
hsa04014	Ras signaling pathway	8.80x10 <sup>-3</sup>	EGF/EGFR/MET/VEGFA
hsa04919	Thyroid hormone signaling pathway	1.08x10 <sup>-2</sup>	NOTCH1/NOTCH2/NOTCH3
hsa05020	Prion diseases	1.29x10 <sup>-2</sup>	RUNX3/NOTCH1
hsa04068	FoxO signaling pathway	1.38x10 <sup>-2</sup>	EGF/EGFR/TNFSF10
hsa05162	Measles	1.38x10 <sup>-2</sup>	CDK6/RUNX3/TNFSF10
hsa04934	Cushing syndrome	2.02x10 <sup>-2</sup>	APC/CDK6/EGFR
hsa05160	Hepatitis C	2.02x10 <sup>-2</sup>	CDK6/EGF/EGFR
hsa05144	Malaria	2.08E-02	RUNX3/MET
hsa05164	Influenza A	2.48x10 <sup>-2</sup>	RUNX3/PML/TNFSF10
hsa05202	Transcriptional misregulation in cancer	3.00x10 <sup>-2</sup>	MET/PML/RUNX1
hsa05230	Central carbon metabolism in cancer	3.27x10 <sup>-2</sup>	EGFR/MET
hsa05221	Acute myeloid leukemia	3.27x10 <sup>-2</sup>	PML/RUNX1
hsa05120	Epithelial cell signaling in Helicobacter pylori infection	3.27x10 <sup>-2</sup>	EGFR/MET
hsa05205	Proteoglycans in cancer	3.27x10 <sup>-2</sup>	EGFR/MET/VEGFA
hsa05211	Renal cell carcinoma	3.27x10 <sup>-2</sup>	MET/VEGFA
hsa04115	p53 signaling pathway	3.36x10 <sup>-2</sup>	CDK6/MDM4
hsa04520	Adherens junction	3.36x10 <sup>-2</sup>	EGFR/MET
hsa04810	Regulation of actin cytoskeleton	3.51x10 <sup>-2</sup>	APC/EGF/EGFR
hsa05220	Chronic myeloid leukemia	3.55x10 <sup>-2</sup>	CDK6/RUNX1
hsa04012	ErbB signaling pathway	4.28x10 <sup>-2</sup>	EGF/EGFR
hsa04540	Gap junction	4.47x10 <sup>-2</sup>	EGF/EGFR
hsa05323	Rheumatoid arthritis	4.55x10 <sup>-2</sup>	RUNX3/VEGFA

APC, adenomatous polyposis coli protein.

Table V. Significant GO terms enriched for genes in the competing endogenous RNA network.

GO term	ID	Description	Adjusted P-value	Gene ID
BP	GO:0016055	Wnt signaling pathway	2.09x10 <sup>-5</sup>	APC/CTNND2/EGF/EGFR/MET/NOTCH1/RECK/RUNX1
BP	GO:0198738	Cell-cell signaling by wnt	2.09x10 <sup>-5</sup>	APC/CTNND2/EGF/EGFR/MET/NOTCH1/RECK/RUNX1
BP	GO:0030111	Regulation of Wnt signaling pathway	2.17x10 <sup>-5</sup>	APC/CTNND2/EGF/EGFR/NOTCH1/RECK/RUNX1
BP	GO:0060828	Regulation of canonical Wnt signaling pathway	7.32x10 <sup>-5</sup>	APC/CTNND2/EGF/EGFR/NOTCH1/RECK
BP	GO:0007050	Cell cycle arrest	7.32x10 <sup>-5</sup>	APC/CDK6/MDM4/NOTCH1/NOTCH2/PML
BP	GO:0045165	Cell fate commitment	9.34x10 <sup>-5</sup>	APC/JAG1/NOTCH1/NOTCH2/NOTCH3/PML
BP	GO:0060070	Canonical Wnt signaling pathway	1.31x10 <sup>-4</sup>	APC/CTNND2/EGF/EGFR/NOTCH1/RECK
BP	GO:0042176	Regulation of protein catabolic process	3.36x10 <sup>-4</sup>	APC/EGF/EGFR/IL1B/MDM4/PML
BP	GO:0048871	Multicellular organismal homeostasis	7.99x10 <sup>-4</sup>	APC/EGFR/IL1B/MET/NOTCH1/VEGFA
BP	GO:0033044	Regulation of chromosome organization	1.45x10 <sup>-3</sup>	APC/BRD4/IL1B/PML/VEGFA
BP	GO:0034329	Cell junction assembly	3.62x10 <sup>-3</sup>	APC/CTNND2/RUNX1/VEGFA
BP	GO:1901652	Response to peptide	3.98x10 <sup>-3</sup>	APC/IL1B/JAG1/NOTCH1/TNFSF10
BP	GO:0071900	Regulation of protein serine/threonine kinase activity	4.29x10 <sup>-3</sup>	APC/EGF/EGFR/IL1B/VEGFA
BP	GO:0045930	Negative regulation of mitotic cell cycle	1.45x10 <sup>-3</sup>	APC/EGFR/MDM4/PML
BP	GO:0034330	Cell junction organization	4.78x10 <sup>-3</sup>	APC/CTNND2/RUNX1/VEGFA
BP	GO:0007043	Cell-cell junction assembly	5.19x10 <sup>-3</sup>	APC/CTNND2/RUNX1
BP	GO:0010948	Negative regulation of cell cycle process	5.30x10 <sup>-3</sup>	APC/MDM4/PML/SUZ12
BP	GO:0106106	Cold-induced thermogenesis	6.58x10 <sup>-3</sup>	APC/NOTCH1/VEGFA
BP	GO:0045216	Cell-cell junction organization	7.56x10 <sup>-3</sup>	APC/CTNND2/RUNX1
MF	GO:0008013	Beta-catenin binding	3.47x10 <sup>-2</sup>	APC/CTNND2
MF	GO:0031625	Ubiquitin protein ligase binding	3.47x10 <sup>-2</sup>	APC/EGFR/PML
MF	GO:0044389	Ubiquitin-like protein ligase binding	3.78x10 <sup>-2</sup>	APC/EGFR/PML

Only terms enriched by adenomatous polyposis coli protein are listed. GO, Gene Ontology; BP, biological process; MF, molecular function; APC, adenomatous polyposis coli protein.

from the regulating synaptic membrane exocytosis (RIMS)2 gene. Thus, the function of hsa\_circ\_0005114 may be similar to that of RIMS2. Notably, Mukasa *et al.* (32) observed that RIMS2 expression is significantly higher in normal brain tissues compared with glioblastoma. In addition, it was reported that RIMS2 mediates the cAMP-guanidine nucleotide exchange factor II pathway to promote incretin-potentiated insulin secretion (33,34). The downregulation of RIMS2 may lead to the lower levels of insulin and the development of diabetes and obesity, as while 11.1% of patients with glioma (35) and 30% of those with brain tumor are diagnosed with obesity and insulin resistance/impaired glucose tolerance (36). Therefore, hsa\_circ\_0005114 may be involved in glioma by influencing the insulin secretion pathway, which was also predicted in the present function enrichment analysis. However, how hsa\_circ\_0005114 influences insulin secretion needs further investigation. A recent study proposed that high insulin index is positively associated with a high risk of glioma development (37). However, another study reported that decreased insulin receptor expression impairs cellular

functions and represses orthotopic glioblastoma (38). These conflicting reports may be attributed to the dual roles of insulin (39).

In addition to insulin secretion mechanisms, the present study predicted that hsa\_circ\_0005114 may exert tumor suppressor functions by upregulating miR-142-3p and miR-590-5p, which subsequently inhibited the expression of APC. Studies have demonstrated that miR-590-5p is upregulated in liver (40), colorectal (41), gastric (42) and cervical cancer (43). miR-590-5p promotes cancer cell proliferation, invasion and therapy resistance by targeting FOXO1 (34), TGF- $\beta$  R2 (44), matrix metalloproteinases (41), reversion-inducing cysteine-rich protein with Kazal motifs (36) and cell adhesion molecule L1-like (43). Furthermore, a previous study also showed that miR-590-3p is upregulated in human glioma tissues (especially high grade) and radioresistant human glioblastoma cells (45). The use of anti-miR-590-3p suppresses cell viability, decreases colony formation capacity, increases the apoptotic rate and enhances the radiosensitivity. A luciferase reporter assay demonstrated that leucine-rich

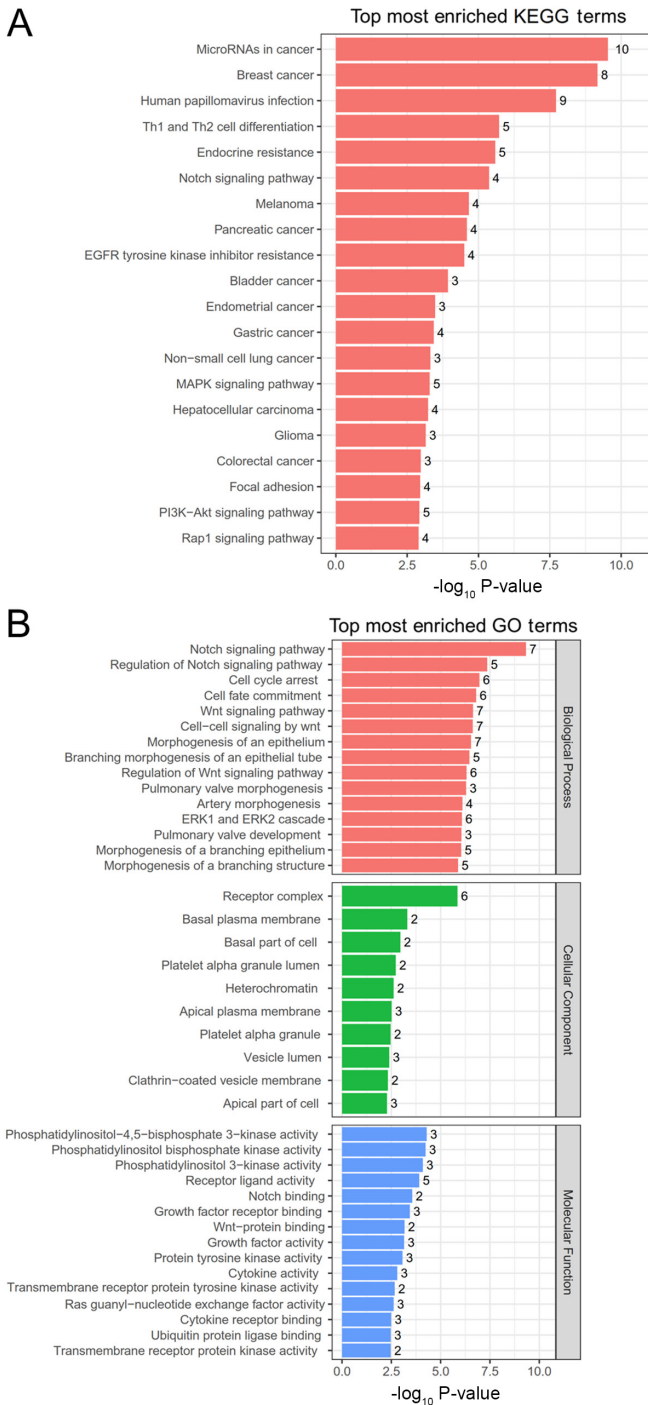


Figure 4. Function enrichment analysis for target genes of miRNAs. (A) Top 20 KEGG pathways and (B) top 15 biological process, cellular component and molecular function GO terms. KEGG, Kyoto Encyclopedia of Genes and Genomes; GO, Gene Ontology.

repeats and immunoglobulin-like domains protein 1 is the direct target of miR-590-3p. Similarly, other studies using quantitative PCR also detected that miR-142-3p expression is significantly upregulated in renal cell carcinoma (46) and nasopharyngeal carcinoma (47) compared with adjacent tissues. Downregulation of miR-142-3p significantly suppresses cell proliferation, migration and cell cycle progression, promotes apoptosis *in vitro* (46) and blocks tumor growth in a mouse model via upregulating its targeted genes, including suppressor of cytokine signaling 6 (47) and Ras-related C3 botulinum

Table VI. Overall survival-related mRNAs and miRNAs.

A, mRNA			
RNA	Cox regression test	P-value	FDR (BH)
SUZ12	7.823x10 <sup>-1</sup>	4.984x10 <sup>-05</sup>	2.991x10 <sup>-4</sup>
VEGFA	4.428x10 <sup>-1</sup>	1.000x10 <sup>-50</sup>	1.000x10 <sup>-49</sup>
NOTCH3	5.14x10 <sup>-1</sup>	2.21x10 <sup>-11</sup>	6.64x10 <sup>-11</sup>
RUNX3	5.539x10 <sup>-1</sup>	1.000x10 <sup>-38</sup>	1.000x10 <sup>-37</sup>
CTNND2	-6.151x10 <sup>-1</sup>	1.000x10 <sup>-26</sup>	1.000x10 <sup>-25</sup>
EGFR	1.815x10 <sup>-1</sup>	2.405x10 <sup>-6</sup>	1.443x10 <sup>-5</sup>
CDK6	4.738x10 <sup>-1</sup>	1.000x10 <sup>-17</sup>	1.000x10 <sup>-16</sup>
PML	8.536x10 <sup>-1</sup>	3.663x10 <sup>-12</sup>	2.198x10 <sup>-11</sup>
BHLHE40	4.879x10 <sup>-1</sup>	3.331x10 <sup>-15</sup>	1.998x10 <sup>-14</sup>
EGF	3.093x10 <sup>-1</sup>	2.355x10 <sup>-9</sup>	7.065x10 <sup>-9</sup>
NOTCH1	5.332x10 <sup>-1</sup>	1.000x10 <sup>-52</sup>	1.000x10 <sup>-52</sup>
TNFSF10	3.983x10 <sup>-1</sup>	2.207x10 <sup>-13</sup>	6.621x10 <sup>-13</sup>
RUNX1	5.332x10 <sup>-1</sup>	1.000x10 <sup>-52</sup>	1.000x10 <sup>-52</sup>
MDM4	3.306x10 <sup>-1</sup>	1.131x10 <sup>-7</sup>	6.784x10 <sup>-7</sup>
MET	2.378x10 <sup>-1</sup>	1.000x10 <sup>-16</sup>	1.000x10 <sup>-16</sup>
IL1B	1.159x10 <sup>-1</sup>	1.164x10 <sup>-4</sup>	2.329x10 <sup>-4</sup>
JAG1	6.596x10 <sup>-1</sup>	1.000x10 <sup>-33</sup>	1.000x10 <sup>-32</sup>
APC	-7.618x10 <sup>-1</sup>	1.000x10 <sup>-36</sup>	1.000x10 <sup>-35</sup>
RECK	4.158x10 <sup>-1</sup>	6.612x10 <sup>-3</sup>	1.984x10 <sup>-2</sup>

B, miRNA			
RNA	Cox regression test	P-value	FDR (BH)
hsa-miR-139-5p	-1.909x10 <sup>-1</sup>	3.418x10 <sup>-3</sup>	1.025x10 <sup>-2</sup>
hsa-miR-142-3p	4.080x10 <sup>-1</sup>	6.370x10 <sup>-9</sup>	3.822x10 <sup>-8</sup>
hsa-miR-217	2.006x10 <sup>-1</sup>	2.422x10 <sup>-6</sup>	1.453x10 <sup>-5</sup>
hsa-miR-324-5p	2.797x10 <sup>-1</sup>	6.083x10 <sup>-3</sup>	3.650x10 <sup>-2</sup>
hsa-miR-346	-6.408x10 <sup>-1</sup>	2.317x10 <sup>-9</sup>	1.390x10 <sup>-8</sup>
hsa-miR-590-5p	5.611x10 <sup>-1</sup>	2.461x10 <sup>-7</sup>	7.384x10 <sup>-7</sup>
hsa-miR-599	4.453x10 <sup>-1</sup>	9.471x10 <sup>-7</sup>	2.841x10 <sup>-6</sup>
hsa-miR-615-3p	9.771x10 <sup>-1</sup>	1.000x10 <sup>-8</sup>	1.000x10 <sup>-7</sup>
hsa-miR-875-5p	1.369x10 <sup>-1</sup>	5.275x10 <sup>-4</sup>	1.361x10 <sup>-3</sup>

Only mRNAs and miRNAs significantly associated with overall survival P≤0.05 are shown. FDR, False Discovery Rate; miRNA, micro RNA; APC, adenomatous polyposis coli protein; hsa, *Homo sapiens*; BH, Benjamini and Hochberg.

toxin substrate 1 in colorectal cancer (48). High expression of miR-142-3p is correlated with histological differentiation and a poor prognosis for patients with esophageal squamous cell carcinoma (49). In line with the aforementioned findings, the present study reported that miR-590-5p and miR-142-3p were upregulated in glioblastoma tissues compared with normal controls and were associated with a worse prognosis. However, the targeted genes of these two miRNAs were not completely validated in cancer. It was predicted that APC, a regulator of the WNT signaling pathway (50), may be a direct target for

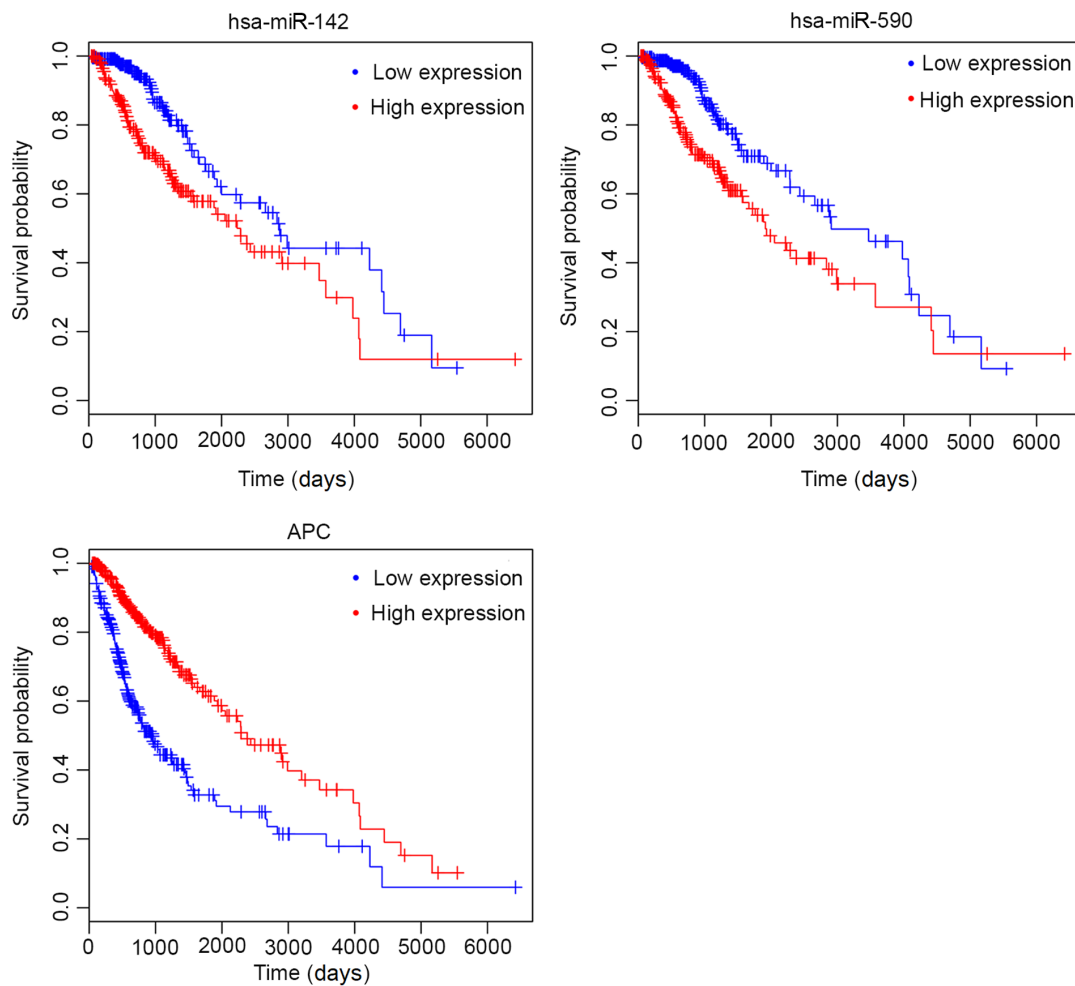


Figure 5. Association with overall survival of miRNAs and mRNA. Cox regression test was used as the statistical method to calculate the association. hsa, *Homo sapiens*.

both miR-590-5p and miR-142-3p. Their negative regulatory relationship was indirectly investigated by Naseri *et al* (51) who showed that delivery of anti-miR-142-3p by exosomes to breast cancer cells leads to a significant increase in APC mRNA levels. In addition, Wu *et al* (52) used dual-luciferase and western blot analysis in mesenchymal stem cells to demonstrate that miR-590-3p binds to the 3'UTR of APC mRNA. Notably, APC encodes a protein that acts as an antagonist of the Wnt signaling pathway (53), which is often activated in cancer (54). Thus, APC may be downregulated during carcinogenesis, which has been confirmed in various cancer types, including glioma (55-57). For example, Cole *et al* (55) reported that APC expression is decreased in pancreatic ductal adenocarcinoma tissues, and that APC siRNA treatment promotes cell proliferation and migration. Wang *et al* (56) reported that the expression of APC mRNA is significantly decreased in ovarian tumor cells and tissues compared with in normal ovarian cells and tissues. In addition, overexpression of APC induces increased apoptosis of ovarian tumor cells by decreasing the ATP binding cassette subfamily B member 1 (also known as multidrug resistance gene 1)/chemokine (C-X-C motif) ligand 1 signaling pathway. Zhang *et al* (57) demonstrated that APC expression is downregulated in colorectal cancer tissues, which is associated with the expression of miR-494.

Overexpression of miR-494 promotes colorectal cancer cell proliferation by inhibiting APC and consequently inducing Wnt/ $\beta$ -catenin signaling. Western blot assays of Li *et al* (58) also suggested that miR-106a-5p reduces APC protein levels and upregulates target proteins of the Wnt/ $\beta$ -catenin pathway, resulting in the invasion of glioblastoma cells (58). In accordance with these studies, the present study reported that APC expression was decreased in glioblastoma tissues compared with normal controls and high expression of APC was associated with a good prognosis. Functional enrichment analysis showed APC was involved in Wnt signaling pathway, cell fate commitment, and cell junction.

Overall, the present study suggested that hsa\_circ\_0005114-miR-142-3p/miR-590-5p-APC ceRNA axes may be mechanisms for the development and progression of glioma. These axes may also have potential as novel targets for the treatment of glioma. However, additional *in vitro* and *in vivo* experiments, such as gene interaction experiments involving knockout/overexpression of hsa\_circ\_0005114-miR-142-3p/miR-590-5p-APC and their influence on tumor cells (using cell proliferation, apoptosis, invasion and migration assays) and tumor growth, are required to validate these conclusions in the future, and the lack of these experiments is a limitation of the present study.

## Acknowledgements

Not applicable.

## Funding

The present study was funded by grants from The Natural Science Foundation of China (grant no. 81400404) and The Science and Technology of Jilin Province (grant no. 20200201470JC).

## Availability of data and materials

The GSE86202, GSE92322, GSE103229 and GSE25632 datasets were downloaded from the Gene Expression Omnibus repository (<http://www.ncbi.nlm.nih.gov/geo/>). LinkedOmics and UALCAN database used the miRNA and mRNA sequencing data of The Cancer Genome Atlas (<https://gdc-portal.nci.nih.gov/>) for analysis.

## Authors' contributions

BW, LW and JWZ conceived and designed the study. BW collected and analyzed the data. LW and JWZ contributed to the interpretation of data. BW and LW drafted the manuscript. JWZ revised the manuscript. All authors read and approved the final manuscript.

## Ethics approval and consent to participate

Not applicable.

## Patient consent for publication

Not applicable.

## Competing interests

The authors declare that they have no competing interests.

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