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Circ-0001801 contributes to cell proliferation, migration, invasion and epithelial to mesenchymal transition (EMT) in glioblastoma by regulating miR-628-5p/HMGB3 axis

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Abstract. – OBJECTIVE: Glioblastoma (GBM) is a fast-growing type of central nervous system tumor with high invasiveness and recurrence. Circular RNA (circRNA) circ-0001801 (also named as circPCMTD1) was recognized as a novel biomarker in cancers. However, the pathological mechanism of circ-0001801 in GBM is still largely obscured. To the best of our knowledge, it is the first time to reveal the involvement of circ-0001801/miR-628-5p/HMGB3 axis in the progression of GBM cells.

PATIENTS AND METHODS: Expression of circ-0001801, microRNA (miR)-628-5p and high mobility group box 3 (HMGB3) in GBM tumors and cells was detected by quantitative real time-polymerase chain reaction (qRT-PCR). Stability of circ-0001801 was determined by RNase R. 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide (MTT) was performed to analyze cell viability. Cell migration and invasion were assessed using transwell assay. The interaction between miR-628-5p and circ-0001801 or HMGB3 was confirmed by Dual-Luciferase reporter system. Protein expression of HMGB3, N-cadherin, E-cadherin and Vimentin was detected by Western blot.

RESULTS: The up-regulation of circ-0001801 and HMGB3 and the down-regulation of miR-628-5p were both observed in GBM tumors and cells compared with the normal ones. Depletion of circ-0001801 reduced GBM cell proliferation, migration, invasion and EMT. In addition, we discovered that circ-0001801 was a sponge of miR-628-5p and HMGB3 was a target of miR-628-5p. Furthermore, miR-628-5p inhibitor abolished circ-0001801 silencing-mediated inhibition of cell progression in GBM. Similarly, HMGB3 restored circ-0001801 silencing-mediated repression on GBM cell progression. We also noticed that circ-0001801 could improve HMGB3 expression by sponging miR-628-5p in GBM.

CONCLUSIONS: Overexpression of circ-0001801 accelerates cell proliferation, migration, invasion and EMT in GBM by absorbing miR-628-5p and facilitating HMGB3 expression, representing promising targeted therapy for GBM.

Key Words:
Circ-0001801, MiR-628-5p, HMGB3, Progression, GBM.

Abbreviations

GBM: Glioblastoma; BBB: blood-brain barrier; HMGB3: high mobility group box 3.

Introduction

Glioblastoma (GBM) is a malignant brain cancer characterized by aggressive growth, rich vasculature, distant metastasis and poor prognosis[1,2]. Advanced treatments have improved therapeutic outcomes. However, GBM vascularization and blood-brain barrier (BBB) could block the penetration of anti-tumor agents, reducing the treatment efficiency[3-5]. Therefore, investigating new biomarkers implicated in the occurrence and development of GBM is in great needed.

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Circular RNAs (circRNAs) are non-coding RNAs widely presented in the cytoplasm of eukaryotic cells[6]. They are capable of altering transcription, transporting RNAs as well as binding to proteins. Numerous studies have elucidated that differential expression of circRNAs was closely linked with tumorigenesis of many cancers[7-9]. For example, hsa_circRNA_101996 expedited the malignancy of cervical cancer by accelerating cell growth and invasion through targeting miR-8075 and improving TPX2 expression[10]. Similarly, hsa_circRNA_100290 functioned as competing endogenous RNA (ceRNA) to bind to miR-378a and facilitate cell proliferation through glycolysis process in oral squamous cell carcinoma [11]. Overexpression of hsa_circRNA_103809 was also reported to contribute to cell growth, migration and invasion in hepatocellular carcinoma by interacting with miR-377-3p through FGFR1/ERK pathway[12]. Specifically, circPCMTD1 (circ-0001801) contributed to cell survival, migration and invasion in glioma by sponging miR-224-5p[13]. Therefore, it is essential to investigate the molecular mechanism of circ-0001801 for GBM progression.

MicroRNAs (miRNAs) are single-stranded non-coding RNAs comprising of 16-25 nucleotides[14]. Generally, miRNAs are involved in many pathological processes, including cell metabolism, growth, migration, infiltration, differentiation, inflammation, invasion and autophagy [15-17]. They could negatively regulate gene expression by interacting with the messenger RNAs (mRNAs) and leading to protein translation blockage[18]. Thus, dysregulation of specific miRNA was associated with the pathogenesis of many diseases. For example, up-regulation of miR-628-5p facilitated the development of cardiac allograft vasculopathy after orthotopic heart transplantation[19]. By comparison, miR-628-5p acted as tumor suppressor to reduce glioma cell cycle and proliferation by interacting with DDX59[20]. Therefore, we assumed that circ-0001801 might exert its function by acting as a sponge of miR-628-5p in glioma.

We attempted to elucidate the biological mechanism of circ-0001801 in GBM cell development. The levels of circ-0001801 and miR-628-5p in GBM tissues and cells were detected to disclose the roles of them. Loss-of-function experiment was employed to reveal the effects of circ-0001801 on GBM cell progression. Rescue experiment was conducted to further evaluate the function of circ-0001801, miR-628-5p and high mobility group box 3 (HMGB3) in GBM. Hence, our study revealed that circ-0001801 promotes cell proliferation, migration, invasion and EMT in GBM by improving HMGB3 level through absorbing miR-628-5p for the first time.

Patients and Methods

Tissue Samples

GBM patients (n=50) were recruited from The First Affiliated Hospital of Sun Yat-sen University. All the patients have not received preoperative treatment. GBM tumors and the adjacent normal tissues were collected by surgery and immediately stored at -80°C. All the participants signed informed consents and the protocols were approved by the Ethics Committee of The First Affiliated Hospital of Sun Yat-sen University.

Cell Transfection

LN229, T98 cells were purchased from American Type Culture Collection (ATCC, Manassas, VA, USA), U251 and BT325 cells were obtained from Beijing Neurosurgical Institute, Tiantan Hospital (Beijing, China) and normal human astrocytes (NHA) cells were purchased from Lonza (Alpharetta, GA, USA). The cells were incubated in complete Dulbecco's Modified Eagle's Medium (DMEM, Gibco, Rockville, MD, USA). Small interfering RNA (siRNA) targeting circ-0001801 (si-circ-0001801#1 and si-circ-0001801#2), control (si-con), pcDNA and pcDNA-HMGB3 were synthesized by Genepharma (Shanghai, China). MiR-628-5p, miR-628-5p inhibitor (anti-miR-628-5p), control (miR-con) and control inhibitor (anti-miR-con) were purchased from RiboBio (Guangzhou, China). Cell transfection was conducted using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA).

Quantitative Real Time-Polymerase Chain Reaction (qRT-PCR)

RNA extraction from GBM tissues and the adjacent normal tissues was performed by TRIzol reagent (Invitrogen, Carlsbad, CA, USA). RNA extraction from the nuclear and cytoplasm of GBM cells was conducted using Nuclear and Cytoplasmic Protein Extraction Kit (Yasen, Shanghai, China). The cDNA for circ-0001801, miR-628-5p and HMGB3 was synthesized by All-in-One™ Synthesis Kit (FulenGen, Guangzhou, China). After that, qRT-PCR was performed using SYBR green (Applied Biosystems,

Foster City, CA, USA). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and U6 were exploited as internal references. The primers for circ-0001801, miR-628-5p and HMGB3 were listed: circ-0001801, (Forward, 5'-CTGAAGTTATGGAAGCATTG-3'; Reverse, 5'-ATGGCTTCCAATATTGCACTTG-3'); miR-628-5p, (Forward, 5'-GCTGACATATTTACTAGAG-3'; Reverse, 5'-GAACATGTCTGCGTATCTC-3'); HMGB3 (Forward, 5'-GACCAGCTAAGGGAGGCAA-3'; Reverse, 5'-ACAGGAAGAATCCAGACGGT-3'); GAPDH, (Forward, 5'-AGGTCGGTGTGAACGGATTTG-3'; Reverse, 5'-GGGGTCGTTGATGGCAACA-3'); U6, (Forward, 5'-ACCCTGAGAAATACCCTCACAT-3'; Reverse, 5'-GACGACTGAGCCCCTGATG-3').

Examination of the Stability of Circ-0001801

The stability of circ-0001801 was evaluated using RNase R (Geneseed Biotech, Guangzhou, China). In brief, T98 and U251 cells were plated on 24-well plates overnight. Then, T98 and U251 cells were treated with RNase R (100 μg/mL). Finally, the cells were collected and subjected to qRT-PCR.

3-(4,5-Dimethyl-2-Thiazolyl)-2,5-Diphenyl-2-H-Tetrazolium Bromide (MTT) Assay

Transfected T98 and U251 cells were inoculated in 96-well plates. After proliferating for 24 h, 48 h and 72 h, the cells were added with 10 μL MTT (Beyotime, Shanghai, China) for 4 h. Then, the cells were added with 100 μL dimethyl sulfoxide (DMSO, Sigma-Aldrich, St. Louis, MO, USA) for 2 h to terminate the reaction. The optical density (OD) value at 490 nm was measured by a spectrophotometer.

Transwell Assay

Transwell was exploited to determine cell migration and invasion. For migration assay, transfected T98 and U251 cells were plated on the upper chamber of the transwell (Corning, Corning, NY, USA). After migrating for 48 h, the cells at the lower chamber were stained with 0.1% crystal violet. For invasion assay, transfected T98 and U251 cells were plated on the upper chamber of transwell pre-treated with Matrigel (Sigma-Aldrich). The following procedure was the same as the migration assay. Lastly, the migrated and invaded T98 and U251 cells were analyzed by a microscope.

Western Blot

Protein HMGB3, N-cadherin, E-cadherin, Vimentin and GAPDH were isolated from transfected T98 and U251 cells. The isolated proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), transferred to polyvinylidene difluoride (PVDF) membranes (Millipore, Billerica, MA, USA) and blocked by 5% nonfat milk. Next, the membranes were incubated with primary antibodies against HMGB3, N-cadherin, E-cadherin, Vimentin and GAPDH (Abcam, Cambridge, MA, USA) and horseradish peroxidase (HRP)-conjugated secondary antibody (Sangon, Shanghai, China).

Dual-Luciferase Reporter Assay

The interaction between miR-628-5p and circ-0001801 or HMGB3 was proved by Dual-Luciferase reporter assay. In brief, wild type circ-0001801 (WT circ-0001801), HMGB3 (WT-HMGB3), mutant type circ-0001801 (MUT circ-0001801) and HMGB3 (MUT-HMGB3) luciferase vectors were constructed. Next, those vectors were co-transfected into T98 and U251 cells with miR-628-5p or miR-con. Finally, luciferase activities were measured using a luminometer.

Statistical Analysis

All the data were presented as means ± standard deviation (SD). Statistical analysis was performed by GraphPad Prism 7 (San Diego, CA, USA). The two-tailed Student's *t*-test was used to analyze the differences between groups. The correlation between miR-628-5p and circ-0001801 or HMGB3 was analyzed by Pearson's correlation coefficient. *p*-value less than 0.05 ($p<0.05$) was considered statistically significant.

Results

Overexpression of Circ-0001801 in GBM

The interrelation between circ-0001801 and GBM cell progression was initially evaluated by qRT-PCR. Apparently, circ-0001801 expression was up-regulated in GBM tumors and cells (LN229, T98, U251, BT325) compared with the normal counterparts (Figure 1A-B). Furthermore, we observed that circ-0001801 was mainly expressed in cell cytoplasm, revealing that circ-0001801 might exert its function in the cytoplasm of GBM cells (Figure 1C-D). To analyze the stability of circ-0001801, T98 and U251 cells

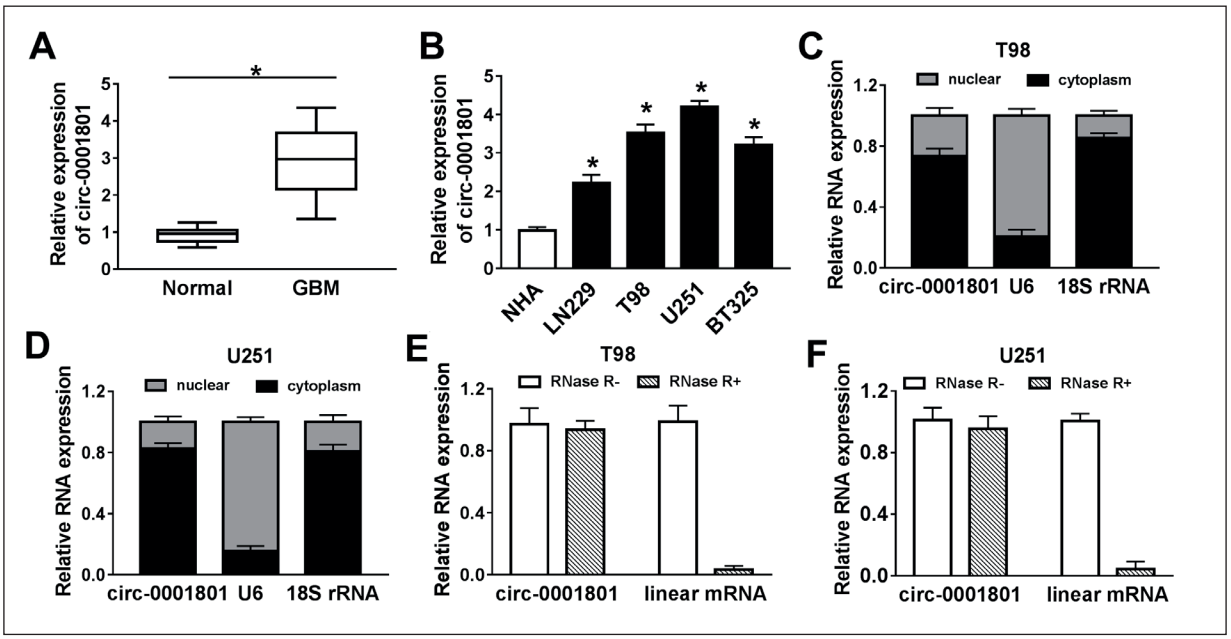

Figure 1. Circ-0001801 was up-regulated in GBM. **A-B,** Circ-0001801 expression in GBM tumors and cells (LN229, T98, U251, BT325) compared with normal tissues and cells (NHA) was measured by qRT-PCR. **C-D,** The expression of circ-0001801, U6 and 18S rRNA in the nuclear and cytoplasm of GBM cells was measured by qRT-PCR. **E-F,** The expression of circ-0001801 and linear mRNA in GBM cells treated with RNase R was measured by qRT-PCR. *$p<0.05$.

were treated with RNase R. The result exhibited that the linear mRNA was degraded by RNase R whereas circ-0001801 remained unchanged after RNase R treatment, indicating circ-0001801 was resistant to RNase R (Figure 1E-F). These findings clarified that circ-0001801 was closely associated with GBM cell development.

Elimination of Circ-0001801 Repressed Cell Proliferation, Migration, Invasion and EMT in GBM

The influence of circ-0001801 on GBM cell progression was evaluated in T98 and U251 cells transfected with si-con, si-circ-0001801#1 or si-circ-0001801#2. Declined expression of circ-0001801 in GBM cells transfected with si-circ-0001801#1 and si-circ-0001801#2 indicated the transfection efficiency was high (Figure 2A). T98 and U251 cells transfected with si-circ-0001801#1 were employed for the subsequent studies owing to the relatively high transfection efficiency. In addition, cell growth was restricted by circ-0001801 silencing (Figure 2B-C). Meanwhile, the depletion of circ-0001801 reduced GBM cell migration and invasion (Figure 2D-E). To investigate the regulatory effect of circ-0001801 on cell EMT, we analyzed the expression of EMT markers. As illustrated in Figure 2F-I, the expression of E-cadherin was increased while N-cadherin and Vimentin were decreased after circ-0001801 knockdown. Taken together, circ-0001801 elimination suppressed cell progression in GBM.

Circ-0001801 Was a Sponge of MiR-628-5p

By searching from starBase v2.0, we noticed that there were potential binding sites between circ-0001801 and miR-628-5p (Figure 3A). To validate the prediction, the Dual-Luciferase reporter system was constructed by co-transfecting with circ-0001801 WT or MUT and miR-628-5p or miR-con in T98 and U251 cells. As displayed in Figure 3B-C, luciferase activity of circ-0001801 WT was suppressed by miR-628-5p. However, luciferase activity of circ-0001801 MUT transfection cells remained unchanged. These findings confirmed the interaction between circ-0001801 and miR-628-5p. Specifically, miR-628-5p expression was elevated by circ-0001801#1 silencing, revealing that circ-0001801#1 could regulate miR-628-5p expression (Figure 3D). What's more, miR-628-5p expression was evidently lower in GBM tumors and cells than that of the normal ones, suggesting the repressive role of miR-628-5p in GBM (Figure 3E-F). There was

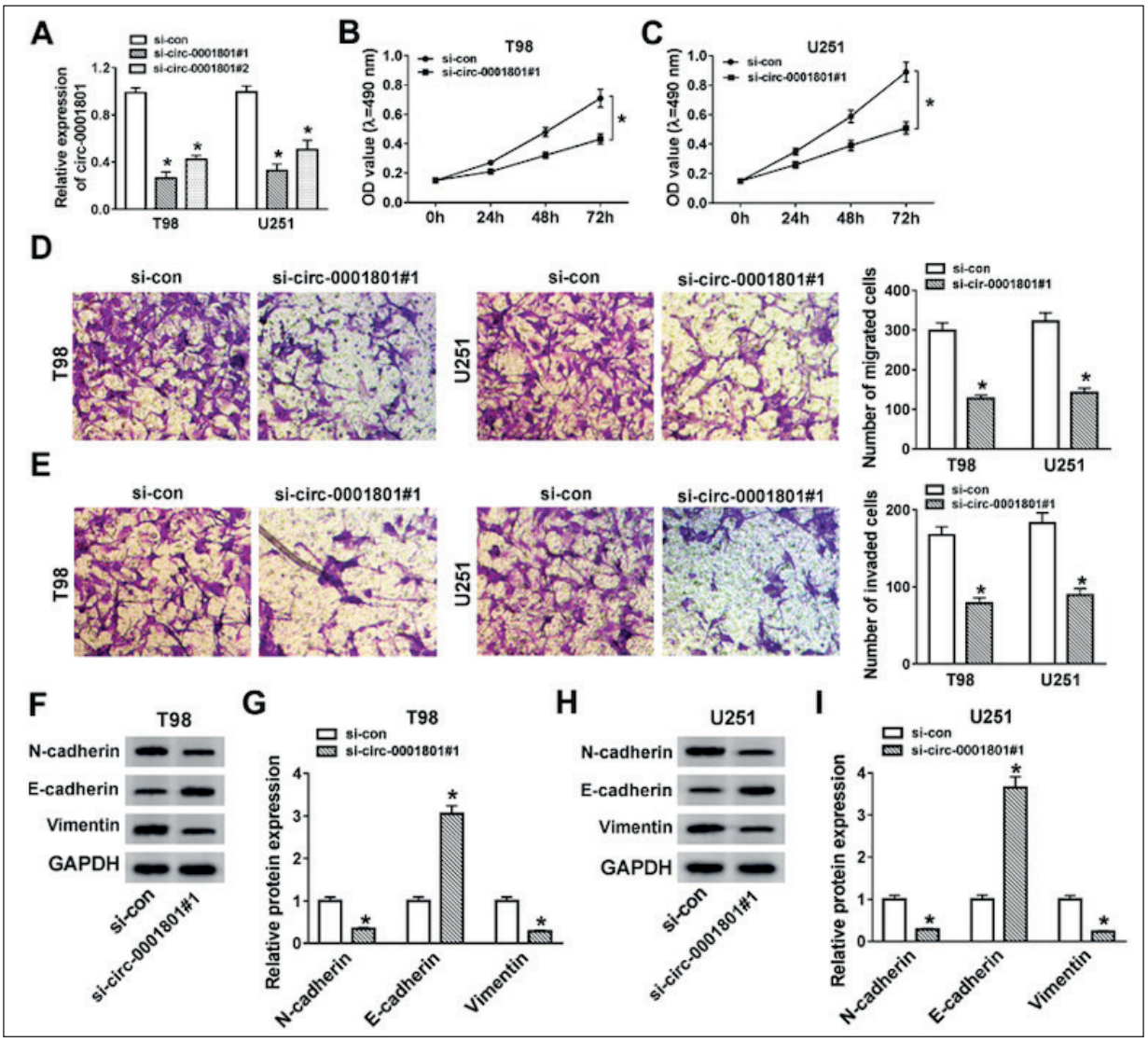

Figure 2. Circ-0001801 knockdown inhibited proliferation, migration, invasion and EMT of GBM cells. T98 and U251 cells were transfected with si-con, si-circ-0001801#1 and si-circ-0001801#2. **A,** Detection of circ-0001801 expression in transfected GBM cells using qRT-PCR. **B-C,** Cell viability was assessed by MTT assay. **D-E,** Cell migration and invasion were examined by transwell assay (× 100). **F-I,** Protein expression of N-cadherin, E-cadherin and Vimentin was analyzed by Western blot. *$p<0.05$.

negative linear relationship between circ-0001801 and miR-628-5p (r=-0.5145, p=0.0036) (Figure 3G). Collectively, circ-0001801 acted as a sponge of miR-628-5p in GBM.

MiR-628-5p Inhibitor Abrogated Circ-0001801 Silencing-Induced Suppression on GBM Cell Proliferation, Migration, Invasion and EMT

The biological mechanism of circ-0001801 for GBM cell development was further explored by rescue experiment. As exhibited in Figure 4A, the expression of miR-628-5p was enhanced by circ-0001801 silencing and reduced by miR-628-5p inhibitor. MTT result revealed that miR-628-5p inhibitor could rescue circ-0001801 silencing-mediated inhibition of GBM cell proliferation (Figure 4B-C). As expected, overexpression of miR-628-5p repressed, whereas low expression of miR-628-5p facilitated cell migration and invasion (Figure 4D-E). Moreover, circ-0001801 knockdown boosted the expression of EMT marker E-cadherin and blocked the expression of N-cadherin and Vimentin. However, the reg-

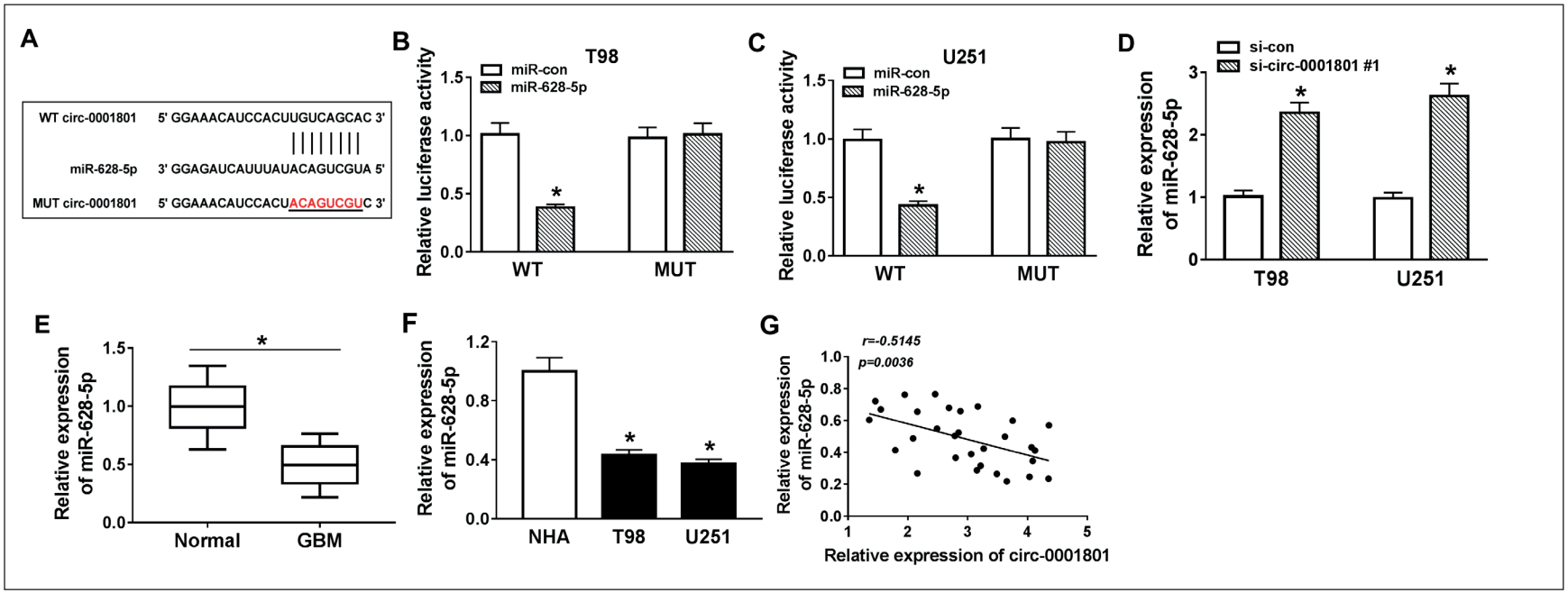

Figure 3. Circ-0001801 directly interacted with miR-628-5p. (A) The potential binding sites between circ-0001801 and miR-628-5p were predicted by starBase v2.0. **B-C,** Luciferase activity of T98 and U251 cells co-transfected with circ-0001801 WT or MUT and miR-628-5p or miR-con was evaluated by dual-luciferase reporter assay. **D,** The expression of miR-628-5p in GBM cells transfected with si-con and si-circ-0001801#1 was detected by qRT-PCR. **E-F,** The expression of miR-628-5p in GBM tumors and cells compared with normal tissues and cells was detected by qRT-PCR. **G,** The correlation between circ-0001801 and miR-628-5p was analyzed by Pearson's correlation coefficient (r=-0.5145, p=0.0036). *p<0.05.

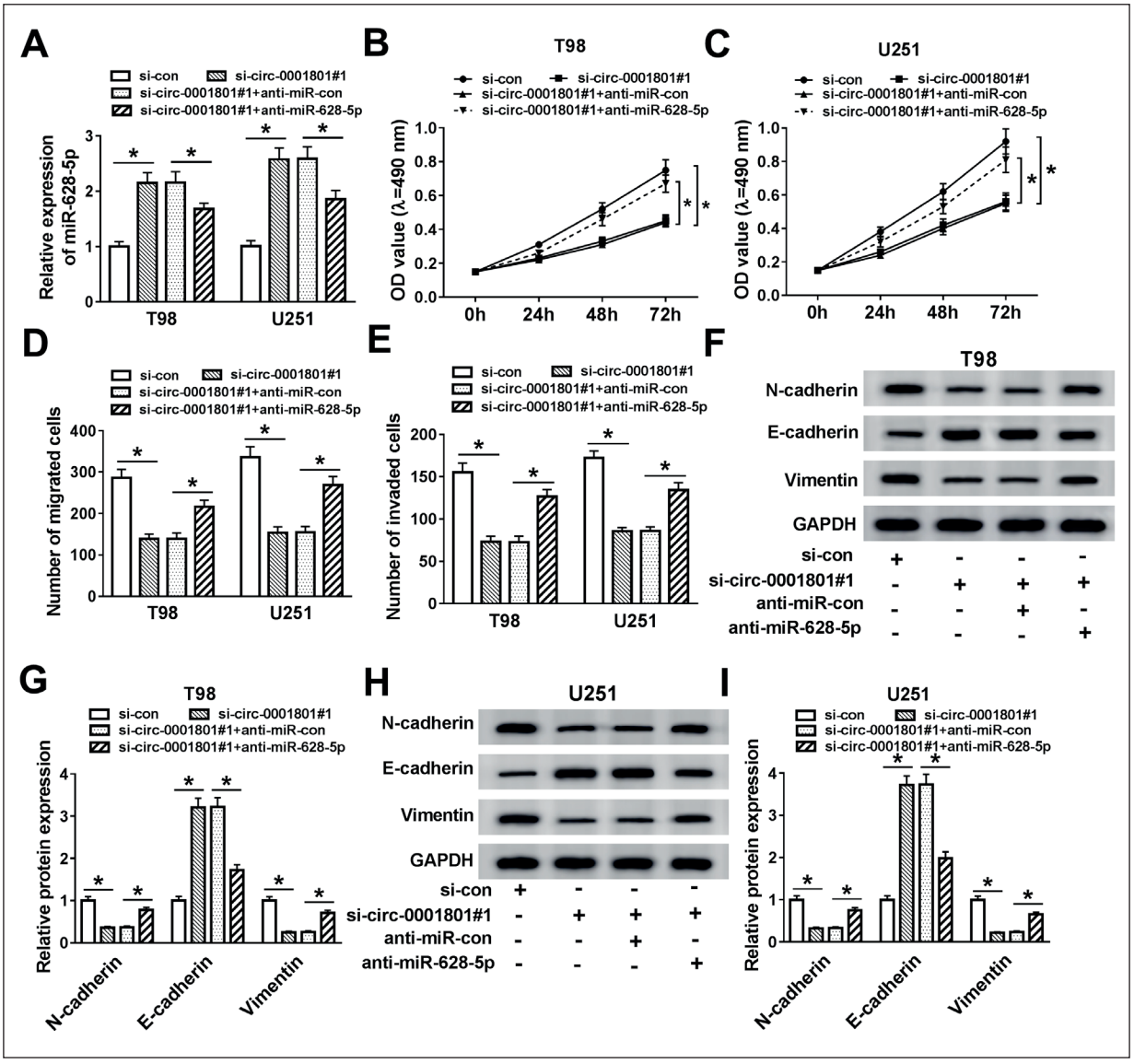

Figure 4. MiR-628-5p inhibitor attenuated circ-0001801 silencing-mediated inhibition of cell proliferation, migration, invasion and EMT in GBM. T98 and U251 cells were transfected with si-con, si-circ-0001801#1, si-circ-0001801#1+anti-miR-con and si-circ-0001801#1+anti-miR-628-5p. **A,** Evaluation of miR-628-5p expression in transfected GBM cells by qRT-PCR. **B-C,** Cell viability was detected by MTT. **D-E,** Transwell assay was exploited to analyze cell migration and invasion. **F-I,** Western blot was used to analyze the expression of protein N-cadherin, E-cadherin and Vimentin. *$p<0.05$.

ulative effect on EMT marker expression was inversed by miR-628-5p inhibitor (Figure 4F-I). Altogether, circ-0001801 modulated cell progression in GBM by sponging miR-628-5p.

Identification of the Interaction Between HMGB3 and MiR-628-5p

As predicted by starBase v2.0, miR-628-5p could specifically bind to HMGB3 (Figure 5A). Luciferase activity was decreased in T98 and U251 cells co-transfected with WT-HMGB3 and miR-628-5p compared with cells co-transfected with MUT-HMGB3 and miR-628-5p (Figure 5B-C). Next, the potential role of HMGB3 was assessed by qRT-PCR and Western blot. As shown in Figure 5D-E, HMGB3 mRNA and protein expression were up-regulated in GBM tumors in comparison with normal tissues. Consistently, the expression of HMGB3 protein was distinctly higher in GBM cells than that of normal cells (Figure 5F). After Pearson's correlation coefficient was carried out, we discovered that HMGB3 was negatively cor-

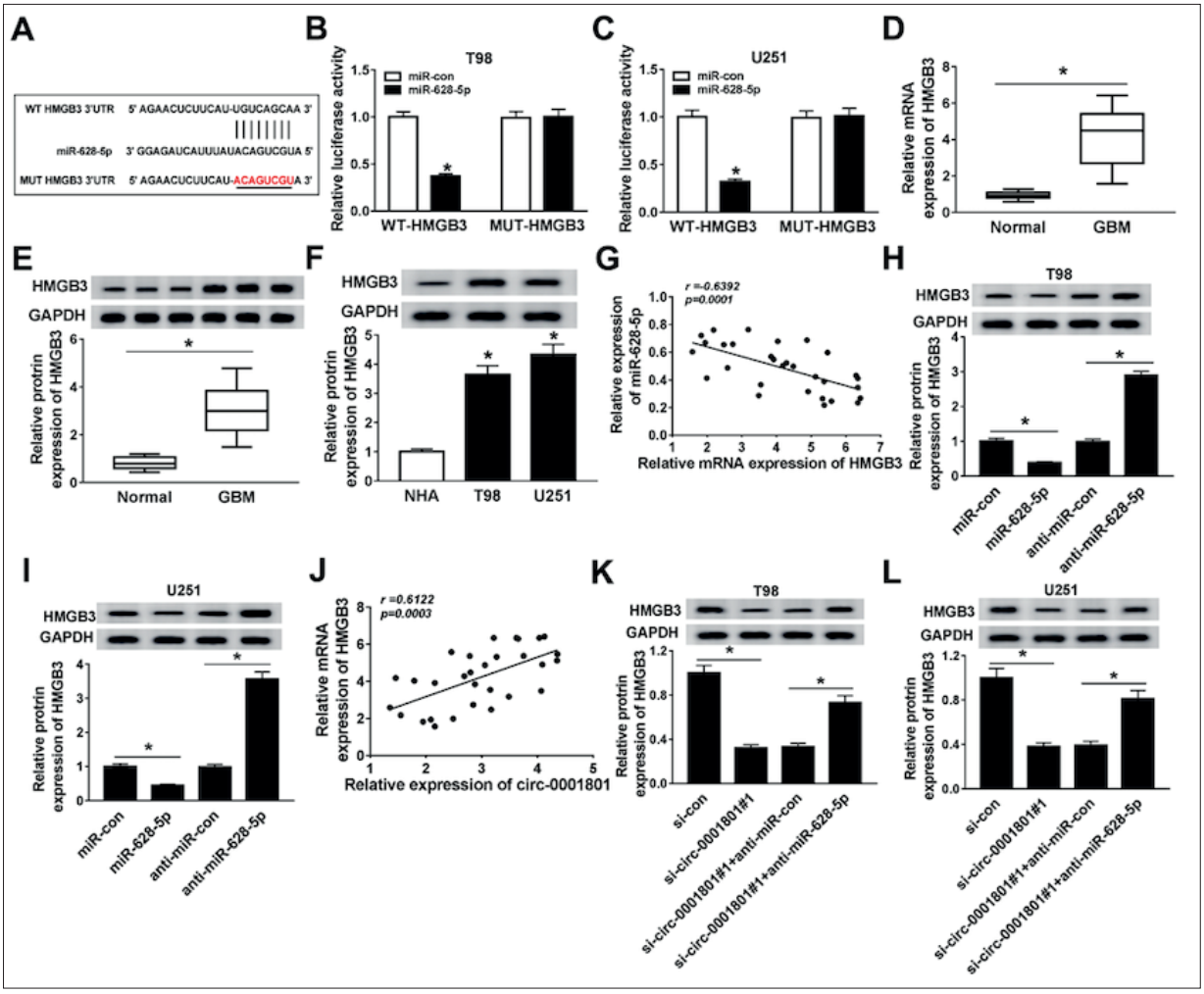

Figure 5. HMGB3 was a target of miR-628-5p. **A,** The potential binding sites between HMGB3 and miR-628-5p were predicted by starBase v2.0. **B-C,** Luciferase activity of T98 and U251 cells co-transfected with WT-HMGB3 or MUT-HMGB3 and miR-628-5p or miR-con was determined by dual-luciferase reporter assay. **D-E,** The expression of HMGB3 mRNA and protein in GBM tumors compared with normal tissues was detected by qRT-PCR and Western blot. **F,** HMGB3 protein expression in GBM cells compared with normal cells was measured by Western blot. **G,** The correlation between HMGB3 and miR-628-5p was analyzed by Pearson's correlation coefficient (r=-0.6392, p=0.0001). **H-I,** Detection of HMGB3 protein expression in GBM cells transfected with miR-con, miR-628-5p, anti-miR-con and anti-miR-628-5p using Western blot. **J,** The correlation between circ-0001801 and HMGB3 was determined by Pearson's correlation coefficient (r=0.6122, p=0.0003). (K-L) Analysis of HMGB3 protein expression in GBM cells transfected with si-con, si-circ-0001801#1, si-circ-0001801#1+anti-miR-con and si-circ-0001801#1+anti-miR-628-5p by Western blot. *p<0.05.

related with miR-628-5p (r=-0.6392, p=0.0001) (Figure 5G). By comparison, circ-0001801 was positively correlated with HMGB3 (r=0.6122, p=0.0003) (Figure 5J). In addition, HMGB3 protein expression in GBM cells was reduced by miR-628-5p and enhanced by miR-628-5p inhibitor (Figure 5H-I). Furthermore, miR-628-5p inhibitor counteracted circ-0001801 silencing induced inhibition of HMGB3 protein expression (Figure 5K-L). All the data revealed that circ-0001801 could regulate HMGB3 expression by targeting miR-628-5p in GBM.

Restoration of HMGB3 Reversed Circ-0001801 Silencing-Mediated Repression on Cell Progression in GBM

To elucidate the regulatory effects of circ-0001801/HMGB3 axis on GBM cell progression, T98 and U251 cells were transfected with si-con, si-circ-0001801#1, si-circ-0001801#1+pcDNA and si-circ-0001801#1+pcDNA-HMGB3. The expression of HMGB3 protein was blocked by circ-0001801 silencing. However, the blockage of HMGB3 protein expression was attenuated by HMGB3 (Figure 6A-B). Cell growth was low-

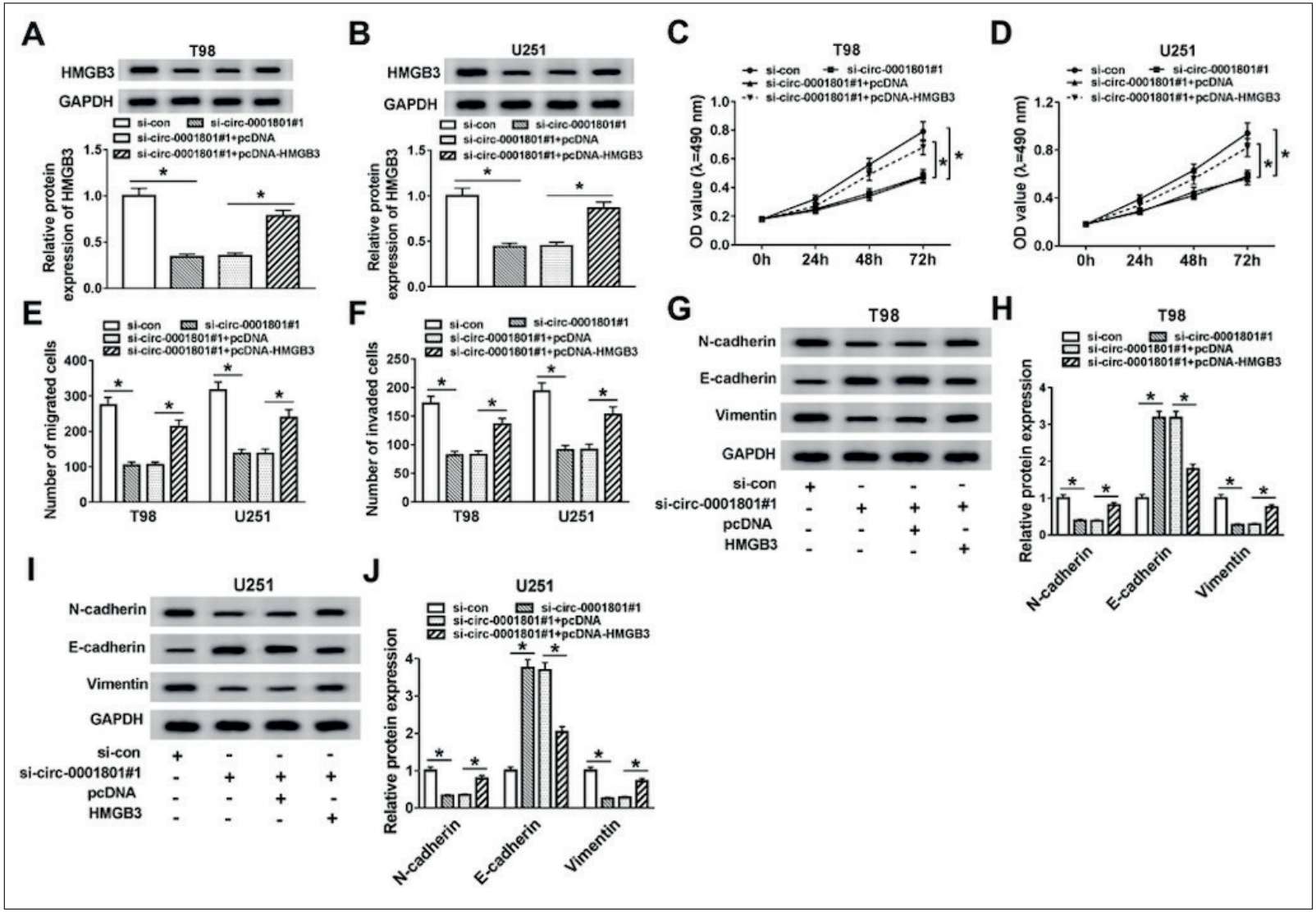

Figure 6. HMGB3 restored circ-0001801 silencing induced suppression on cell proliferation, migration, invasion and EMT in GBM. T98 and U251 cells were transfected with si-con, si-circ-0001801#1, si-circ-0001801#1+pcDNA and si-circ-0001801#1+pcDNA-HMGB3. **A-B,** Assessment of HMGB3 protein expression in transfected GBM cells using Western blot. **C-D,** Cell viability was analyzed by MTT. **E-F,** Cell migration and invasion were examined by transwell assay. **G-J,** Western blot was used to analyze the expression of protein N-cadherin, E-cadherin and Vimentin. *$p<0.05$.

ered by circ-0001801 silencing and accelerated by HMGB3 (Figure 6C-D). Likewise, restoration of HMGB3 neutralized circ-0001801 silencing-induced suppressive effect on cell migration and invasion (Figure 6E-F). Protein expression of N-cadherin and Vimentin was repressed by circ-0001801 silencing and promoted by HMGB3. However, the expression of protein E-cadherin exhibited the opposite trend (Figure 6G-J). Therefore, circ-0001801 promoted GBM cell progression by enhancing HMGB3 expression.

Discussion

Accumulating evidence has identified circRNAs as essential diagnostic biomarkers in a variety of diseases, such as cardiovascular diseases, Alzheimer's disease and cancer[21-23]. For instance, hsa_circRNA_102958 served as oncogene to accelerate cell progression by absorbing miR-585 and up-regulating CDC25B expression in colorectal cancer[24]. Consistently, abundance of circRNA_102171 facilitated tumor growth by affecting CTNNBIP1-mediated activation of Wnt/β-catenin pathway in papillary thyroid cancer[25]. What's more, Yu et al[26] reported that circRNA-104718 functioned as ceRNA in hepatocellular carcinoma to promote cell development *in vitro* and *in vivo* by targeting microRNA-218-5p to increase TXNDC5 expression. In addition, circRNA_101505 could improve the sensitivity of hepatocellular carcinoma cells against cisplatin by enhancing NOR1 expression via sponging miR-103[27]. However, the regulatory role of circ-0001801 in GBM remains poorly understood.

Bioinformatics analysis prediction by starBase v2.0 showed that circ-0001801 could potentially bind to miR-628-5p. Dual-Luciferase reporter assay and qRT-PCR further clarified that circ-0001801 could regulate miR-628-5p expression in GBM. Favreau et al[28] have demonstrated that miR-628-5p was involved in the oncogenesis and progression of various cancers. However, the role of miR-628-5p is still controversial in different cancers. For instance, enhanced expression of miR-628-5p improved cell survival and migration by absorbing IFI44L in osteosarcoma[29]. Conversely, miR-628-5p attenuated the deterioration of ovarian cancer by inhibiting cell growth and inducing cell apoptosis via targeting FGFR2[30]. Likewise, miR-628-5p functioned as tumor suppressor to repress cell progression in prostate cancer[31]. Therefore, clarification of the regulatory effects of miR-628-5p during GBM cell progression is greatly needed.

We speculated that circ-0001801 regulated GBM cell growth, migration, invasion and EMT by interacting with miR-628-5p. It is well acknowledged that EMT is an essential process for metastatic cancer cells migrating to blood and the adjacent tissues. During EMT process, epithelial cells acquire migratory properties and become mesenchymal cells, causing the alteration of EMT associated protein N-cadherin, E-cadherin and Vimentin[32,33]. Up-regulation of circ-0001801 in GBM tumors and cells implicated the promotive role of circ-0001801. As expected, cell proliferation, migration, invasion and EMT were attenuated by circ-0001801 knockdown. Next, the interaction between miR-628-5p and circ-0001801 or HMGB3 was certified by Dual-Luciferase reporter assay. Decreased expression of miR-628-5p in GBM tumors and cells proved the suppressive role of miR-628-5p. Further, the inhibition of miR-628-5p could rescue circ-0001801 silencing-induced suppression on GBM cell progression and EMT process. Consistently, up-regulation of HMGB3 could neutralize circ-0001801 silencing-mediated inhibition of GBM cell progression as well as EMT. We also discovered that circ-0001801 modulated GBM cell development by sponging miR-628-5p and altering HMGB3 expression.

Conclusions

Our research demonstrated that circ-0001801 contributed to cell survival, migration, invasion and EMT by enhancing HMGB3 expression via absorbing miR-628-5p in GBM. Our study provides prospective biomarkers for GBM diagnosis and treatment.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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Availability of Data and Materials

All original data and materials are available from the corresponding author upon request.

References

1) Olar A, Aldape KD. Using the molecular classification of glioblastoma to inform personalized treatment. J Pathol 2014; 232: 165-177.
2) Wang H, Pan JQ, Luo L, Ning XJ, Ye ZP, Yu Z, Li WS. NF-κB induces miR-148a to sustain TGF-β/Smad signaling activation in glioblastoma. Mol Cancer 2015; 14: 2.
3) Friesen C, Hormann I, Roscher M, Fichtner I, Alt A, Hilger R, Debatin KM, Miltner E. Opioid receptor activation triggering downregulation of cAMP improves effectiveness of anti-cancer drugs in treatment of glioblastoma. Cell Cycle 2014; 13: 1560-1570.
4) Da Ros M, De Gregorio V, Iorio AL, Giunti L, Guidi M, de Martino M, Genitori L, Sardi I. Glioblastoma chemoresistance: the double play by microenvironment and blood-brain barrier. Int J Mol Sci 2018; 19(10) pii: E2879.
5) Dunn GP, Rinne ML, Wykosky J, Genovese G, Quayle SN, Dunn IF, Agarwalla PK, Chheda MG, Campos B, Wang A, Brennan C, Ligon KL, Furnari F, Cavenee WK, Depinho RA, Chin L, Hahn WC. Emerging insights into the molecular and cellular basis of glioblastoma. Genes Dev 2012; 26: 756-784.
6) Jin J, Chen A, Qiu W, Chen Y, Li Q, Zhou X, Jin D. Dysregulated circRNA_100876 suppresses proliferation of osteosarcoma cancer cells by targeting microRNA-136. J Cell Biochem 2019; 120: 15678-15687.
7) Okholm TLH, Nielsen MM, Hamilton MP, Christensen LL, Vang S, Hedegaard J, Hansen TB, Kjems J, Dyrskjot L, Pedersen JS. Circular RNA expression is abundant and correlated to aggressiveness in early-stage bladder cancer. NP J Genom Med 2017; 2: 36.
8) Yang J, Cong X, Ren M, Sun H, Liu T, Chen G, Wang Q, Li Z, Yu S, Yang Q. Circular RNA hsa_circRNA_0007334 is predicted to promote mmp7 and col1a1 expression by functioning as a mirna sponge in pancreatic ductal adenocarcinoma. J Oncol 2019; 2019: 7630894.
9) Chen G, Shi Y, Zhang Y, Sun J. CircRNA_100782 regulates pancreatic carcinoma proliferation through the IL6-STAT3 pathway. Onco Targets Ther 2017; 10: 5783-5794.
10) Song T, Xu A, Zhang Z, Gao F, Zhao L, Chen X, Gao J, Kong X. CircRNA hsa_circRNA_101996 increases cervical cancer proliferation and invasion through activating TPX2 expression by restraining miR-8075. J Cell Physiol 2019; 234: 14296-14305.
11) Chen X, Yu J, Tian H, Shan Z, Liu W, Pan Z, Ren J. Circle RNA hsa_circRNA_100290 serves as a ceRNA for miR-378a to regulate oral squamous cell carcinoma cells growth via Glucose transporter-1 (GLUT1) and glycolysis. J Cell Physiol 2019; 234: 19130-19140.
12) Zhan W, Liao X, Chen Z, Li L, Tian T, Yu L, Wang W, Hu Q. Circular RNA hsa_circRNA_103809 promoted hepatocellular carcinoma development by regulating miR-377-3p/FGFR1/ERK axis. J Cell Physiol. 2019 Jul 17. doi: 10.1002/jcp.29092. [Epub ahead of print]
13) Zheng SQ, Qi Y, Wu J, Zhou FL, Yu H, Li L, Yu B, Chen XF, Zhang W. CircPCMTD1 acts as the sponge of miR-224-5p to promote glioma progression. Front Oncol 2019; 9: 398.
14) Li X, Zhang Q, Yang Z. Silence of MEG3 intensifies lipopolysaccharide-stimulated damage of human lung cells through modulating miR-4262. Artif Cells Nanomed Biotechnol 2019; 47: 2369-2378.
15) Gao F, Wu H, Wang R, Guo Y, Zhang Z, Wang T, Zhang G, Liu C, Liu J. MicroRNA-485-5p suppresses the proliferation, migration and invasion of small cell lung cancer cells by targeting flotillin-2. Bioengineered 2019; 10: 1-12.
16) Tu J, Zhao Z, Xu M, Chen M, Weng Q, Ji J. LINC00460 promotes hepatocellular carcinoma development through sponging miR-485-5p to up-regulate PAK1. Biomed Pharmacother 2019; 118: 109213.
17) Tian Z, Luo Y, Zhu J, Hua X, Xu J, Huang C, Jin H, Huang H, Huang C. Transcriptionally elevation of miR-494 by new ChlA-F compound via a HuR/JunB axis inhibits human bladder cancer cell invasion. Biochim Biophys Acta Gene Regul Mech 2019; 1862: 822-833.
18) Liu K, Liu S, Zhang W, Jia B, Tan L, Jin Z, Liu Y. miR-494 promotes cell proliferation, migration and invasion, and increased sorafenib resistance in hepatocellular carcinoma by targeting PTEN. Oncol Rep 2015; 34: 1003-1010.
19) Neumann A, Napp LC, Kleeberger JA, Benecke N, Pfanne A, Haverich A, Thum T, Bara C. MicroRNA 628-5p as a novel biomarker for cardiac allograft vasculopathy. Transplantation 2017; 101: e26-e33.
20) Xie P, Wang Y, Liao Y, Han Q, Qiu Z, Chen Y, Zuo X. MicroRNA-628-5p inhibits cell proliferation in glioma by targeting DDX59. J Cell Biochem 2019; 120: 17293-17302.
21) Bei Y, Yang T, Wang L, Holvoet P, Das S, Sluijter JPG, Monteiro MC, Liu Y, Zhou Q, Xiao J. Circular RNAs as potential theranostics in the cardiovascular system. Mol Ther Nucleic Acids 2018; 13: 407-418.
22) Wang Z, Xu P, Chen B, Zhang Z, Zhang C, Zhan Q, Huang S, Xia ZA, Peng W. Identifying circRNA-associated-ceRNA networks in the hippocampus of Aβ1-42-induced Alzheimer's disease-like rats using microarray analysis. Aging (Albany NY) 2018; 10: 775-788.
23) Wei J, Wei W, Xu H, Wang Z, Gao W, Wang T, Zheng Q, Shu Y, De W. Circular RNA hsa_circRNA_102958 may serve as a diagnostic marker for gastric cancer. Cancer Biomark 2019. doi: 10.3233/CBM-182029. [Epub ahead of print]
24) Li R, Wu B, Xia J, Ye L, Yang X. Circular RNA hsa_circRNA_102958 promotes tumorigenesis of col-

orectal cancer via miR-585/CDC25B axis. Cancer Manag Res 2019; 11: 6887-6893.

25) Bi W, Huang J, Nie C, Liu B, He G, Han J, Pang R, Ding Z, Xu J, Zhang J. CircRNA circRNA_102171 promotes papillary thyroid cancer progression through modulating CTNNBIP1-dependent activation of beta-catenin pathway. J Exp Clin Cancer Res 2018; 37: 275.

26) Yu J, Yang M, Zhou B, Luo J, Zhang Z, Zhang W, Yan Z. CircRNA-104718 acts as competing endogenous RNA and promotes hepatocellular carcinoma progression through microRNA-218-5p/TXNDC5 signaling pathway. Clin Sci (Lond) 2019; 133: 1487-1503.

27) Luo Y, Fu Y, Huang R, Gao M, Liu F, Gui R, Nie X. CircRNA_101505 sensitizes hepatocellular carcinoma cells to cisplatin by sponging miR-103 and promotes oxidored-nitro domain-containing protein 1 expression. Cell Death Discov 2019; 5: 121.

28) Favreau AJ, Sathyanarayana P. miR-590-5p, miR-219-5p, miR-15b and miR-628-5p are commonly regulated by IL-3, GM-CSF and G-CSF in acute myeloid leukemia. Leuk Res 2012; 36: 334-341.

29) Wang JY, Lu SB, Wang JQ. miR-628-5p promotes the growth and migration of osteosarcoma by targeting IFI44L. Biochem Cell Biol 2019. doi: 10.1139/bcb-2019-0001. [Epub ahead of print]

30) Li M, Qian Z, Ma X, Lin X, You Y, Li Y, Chen T, Jiang H. MiR-628-5p decreases the tumorigenicity of epithelial ovarian cancer cells by targeting at FGFR2. Biochem Biophys Res Commun 2018; 495: 2085-2091.

31) Srivastava A, Goldberger H, Dimtchev A, Marian C, Soldin O, Li X, Collins SP, Suy S, Kumar D. Circulatory miR-628-5p is downregulated in prostate cancer patients. Tumour Biol 2014; 35: 4867-4873.

32) Santamaria PG, Moreno-Bueno G, Portillo F, Cano A. EMT: present and future in clinical oncology. Mol Oncol 2017; 11: 718-738.

33) De Seta D, Mancini P, Minni A, Prosperini L, De Seta E, Attanasio G, Covelli E, De Carlo A, Filipo R. Bell's palsy: symptoms preceding and accompanying the facial paresis. ScientificWorldJournal 2014; 2014: 801971.