European Review for Medical and Pharmacological Sciences 2018; 22: 8406-8414

Upregulation of long noncoding RNA MRCCAT1 predicts poor prognosis and functions as an oncogene in glioma

J. MA, L.-J. SU, Z.-J. ZHENG

Department of Neurology, Ningbo Yinzhou No. 2 Hospital, Ningbo, Zhejiang, China
Jia Ma and Li-Jing Su contributed equally

Abstract. – OBJECTIVE: This study aimed to investigate the expressions, clinical significances, roles, and mechanism of action of MRCCAT1 in glioma.

PATIENTS AND METHODS: The expression of MRCCAT1 in 103 glioma tissues with different grades and 21 normal brain tissues was measured by qPCR. The prognostic value of MRCCAT1 was investigated by Kaplan-Meier survival analysis. The biological roles of MRCCAT1 on glioma cell proliferation were assessed by Glo cell viability assays and ethynyl deoxyuridine incorporation assays. The roles of MRCCAT1 on glioma cell migration were evaluated by transwell assays. The effects of MRCCAT1 on p38-MAPK signaling were assessed by Western blot.

RESULTS: MRCCAT1 is upregulated in glioma tissues and positively associated with glioma grades. Increased expression of MRCCAT1 confers poor prognosis of glioma patients independent of glioma grades. Ectopic expression of MRCCAT1 promotes glioma cell proliferation and migration. Knockdown of MRCCAT1 inhibits glioma cell proliferation and migration. Mechanistically, we found that MRCCAT1 activates p38-MAPK signaling in glioma.

CONCLUSIONS: MRCCAT1 is upregulated in glioma. Increased expression of MRCCAT1 predicts poor outcome of glioma patients. MRCCAT1 promotes glioma cell proliferation and migration via activating p38-MAPK signaling. MRCCAT1 may be a potential prognostic biomarker and therapeutic target for glioma.

Key Words
Long noncoding RNA, Prognosis, Oncogene, Glioma.

Introduction

Glioma is the most common and aggressive primary brain tumor with poor prognosis[1,2]. Currently, the main therapeutic strategies for glioma are still surgical resection, radiotherapy, and chemotherapy[3]. Unfortunately, due to the diffuse brain invasion, surgery could not completely resect the gliomas[4]. Furthermore, most gliomas are low sensitive to radiotherapy and chemotherapy[5]. Therefore, the outcomes of gliomas are very dismal, particular for these gliomas with high grades[6]. To develop more effective therapy and improve the prognosis of glioma patients, a better understanding of molecular mechanisms underlying glioma tumorigenesis and progression is urgently needed[7,8]. Long noncoding RNA (lncRNA) is a class of transcript with more than 200 nucleotides in length. LncRNAs have no protein-coding potential[9,10]. Previously, lncRNAs are regarded as transcriptional noise. However, increasing evidence showed that lncRNAs were frequently deregulated in various diseases. LncRNAs were also reported to be implicated in many pathophysiological processes[11-14]. Many lncRNAs were revealed to function as oncogenes or tumor suppressors in a variety of cancers[15-18]. They regulate many cellular processes of cancers, such as proliferation, cell cycle, apoptosis, senescence, migration, invasion, and drug-sensitivity[19-23]. Furthermore, many lncRNAs were demonstrated to be biomarkers for the diagnosis and prognosis of different cancers[24-26]. Although the expressions and functions of several lncRNAs in glioma have been studied, most lncRNAs are less understood in glioma[27-29]. LncRNA MRCCAT1 was first identified in clear cell renal cell carcinoma (ccRCC), which is upregulated in metastatic ccRCC, associated with poor prognosis of ccRCC patients, and functions as an oncogene in ccRCC[30]. Whether the upregulation and oncogenic roles of MRCCAT1 is ccRCC specific or popular in various cancers need further investigation. The expression and role of MRCCAT1 in glioma are also unclear. We investigated the expression and clinical values of MRCCAT1 in glioma. We also explored the biological roles of MRCCAT1 in glioma. The mechanisms of action of MRCCAT1 in glioma were also investigated.

Corresponding Author: Zhen-Jie Zheng, MD; e-mail: zhengzhen_jie@126.com

Patients and Methods

Patients and Clinical Specimens

The use of clinical specimens was reviewed and approved by the Ethical Committee of Ningbo Yinzhou No. 2 Hospital (Ningbo, Zhejiang, China). The 103 glioma tissues and 21 normal brain tissues used in this study were obtained from Ningbo Yinzhou No. 2 Hospital (Ningbo, Zhejiang, China). The written informed consent was obtained from all subjects. All glioma patients had not received radiotherapy and/or chemotherapy before surgery. These 103 gliomas were categorized following the WHO classification, including 36 grade I-II gliomas and 67 grade III-IV gliomas. The 21 normal brain specimens were donated by patients with cerebral trauma. All clinical specimens were histopathologically diagnosed by two independent pathologists. All clinical specimens were immediately frozen in liquid nitrogen after surgery and stored at -80°C until use.

Cell Culture

The human normal glia cells NHA and glioma cells U87, LN229, and U251 were obtained from the Chinese Academy of Medical Sciences (Beijing, China). These cells were maintained in Dulbecco's Modified Eagle's Medium (DMEM; Invitrogen, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (FBS; Invitrogen, Carlsbad, CA, USA). All the cells were cultured at 37°C in an atmosphere containing 5% CO_2.

RNA Extraction and Quantitative Real-Time Polymerase Chain Reaction (qPCR)

Total RNA was extracted from tissues or cells using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) following the manufacturer's protocol. The RNA was treated with DNase I (TaKaRa, Dalian, Liaoning, China) to remove genomic DNA. Reverse transcription was performed using the M-MLV Reverse Transcriptase (Invitrogen, Carlsbad, CA, USA) following the manufacturer's protocol. Quantitative real-time polymerase chain reaction (qPCR) was carried out using the SYBR® Premix Ex Taq™ II (TaKaRa) on StepOnePlus™ Real-Time PCR Systems (Applied Biosystems, Foster City, CA, USA) following the manufacturer's protocols. β-actin was used as an endogenous control for the quantification of the expression of MRCCAT1. The primer sequences used were as follows: for MRCCAT1, 5'-CAGCCGTTCAGAATCTCCTGT-3' (sense), and 5'-AACCAGATGGCTTTGGGCAG-3' (anti-sense)[30]; for β-actin, 5'-GGGAAATCGTGCGTGACATTAAG-3' (sense) and 5'-TGTGTTGGCGTACAGGTCTTTG-3' (anti-sense). The quantification of the expression of MRCCAT1 was calculated with the comparative Ct method.

Plasmids Construction and Transfection

Full-length MRCCAT1 was PCR amplified with the PrimeSTAR HS DNA polymerase (TaKaRa) and subcloned into the *Nhe* I and *Xho* I sites of pcDNA3.1 plasmid with the primers 5'-CTAGCTAGCCTAGCCATCTCCGTTTTCAAATC-3' (sense) and 5'-CCGCTCGAGAGAGAGAGAGAGGGAAGACA-3' (antisense), named as pcDNA3.1-MRCCAT1. The oligonucleotides for shRNAs specifically targeting MRCCAT1 were synthesized and inserted into the shRNA expression plasmid pGPU6/Neo (GenePharma, Shanghai, China). The two MRCCAT1 specific shRNAs target sites were: 5'-CCACTACACAGCACTGCTT-3' and 5'-GCTTCCAGCCCAGAACTTT-3'. A scrambled non-targeting shRNA was used as negative control (shNC). The transfection of plasmids was performed using Lipofectamine 3000 (Invitrogen) following the manufacturer's protocol.

Stable Cell Lines Construction

To obtain MRCCAT1 stably overexpressed or silenced glioma cells, pcDNA3.1-MRCCAT1 or pcDNA3.1 was transfected into U87 cells, and MRCCAT1 specific shRNAs or shNC was transfected into U251 cells with Lipofectamine 3000 (Invitrogen). Then, these transfected cells were selected with 1000 μg/ml neomycin for four weeks.

Glo Cell Viability Assays

Three thousand indicated glioma cells per well were plated in 96-well plates. After culture for 0, 1, 2, and 3 days, cell viability was assessed with the CellTiter-Glo® Luminescent Cell Viability Assay (Promega, Madison, WI, USA) in accordance with the manufacturer's protocol.

Ethynyl Deoxyuridine (EdU) Incorporation Assays

Cell proliferation was also evaluated using ethynyl deoxyuridine (EdU) incorporation assays. Briefly, indicated glioma cells were seeded to perform EdU incorporation assays using an EdU Kit (RiboBio, Guangzhou, China) in accordance with the manufacturer's instruction. The results were measured with Zeiss photomicroscope (Carl Zeiss, Oberkochen, Germany) through counting at least five random fields.

Transwell Cell Migration Assays

Transwell assays were performed to evaluate cell migration. Indicated glioma cells suspended in FBS-free medium to inhibit cell proliferation were plated in the upper chamber of a 24-well poly-carbonate transwell insert (Millipore, Billerica, MA, USA). Complete medium with 10% fetal bovine serum (FBS) was added to the lower chamber. After culture for 48 hours, cells on the upper chamber of transwell inserts were scraped off, and cells on the lower surface were fixed with paraformaldehyde, stained with 0.1% crystal violet, and counted using Zeiss photomicroscope (Carl Zeiss, Jena, Germany) through counting at least five random fields.

Western Blot

Total protein was extracted from indicated glioma cells using RIPA Lysis Buffer (Beyotime, Jiangsu, China) and PMSF (Beyotime) in accordance with the manufacturer's instructions. The protein was separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, followed by being transferred to nitrocellulose filter membrane. After being blocked using bovine serum albumin, the membranes were incubated with primary antibodies for p-p38 (Cell Signaling Technology, Danvers, MA, USA), p38 (Cell Signaling Technology, Danvers, MA, USA), or β-actin (Proteintech, Rosemont, IL, USA). After being washed, the membranes were further incubated with IRdye 700-conjugated goat anti-mouse IgG or IRdye 800-conjugated goat anti-rabbit IgG (Li-Cor, Lincoln, NE, USA), followed by being detected using an Odyssey infrared scanner (Li-Cor).

Statistical Analysis

Statistical analyses were performed with the GraphPad Prism Software. Mann-Whitney U test, Student's *t*-test, Kaplan-Meier survival curve analysis, or log-rank test were carried out as indicated. $p<0.05$ were defined as statistically significant.

Results

MRCCAT1 is Upregulated in Glioma And Correlates With Glioma Grade

To examine the expression pattern of MRCCAT1 in glioma, we collected 21 normal brain tissues and 103 glioma tissues. Then, we measured the MRCCAT1 expression in these tissues by qPCR. As shown in Figure 1A, the MRCCAT1 expression was significantly upregulated in glioma tissues compared with that in normal brain tissues ($p<0.001$ by Mann-Whitney U test). The correlation between MRCCAT1 expression and glioma grade was analyzed. As shown in Figure 1B, the MRCCAT1 expression was also significantly increased in glioma tissues with grade III-IV (n=67) compared with that in glioma tissues with grade

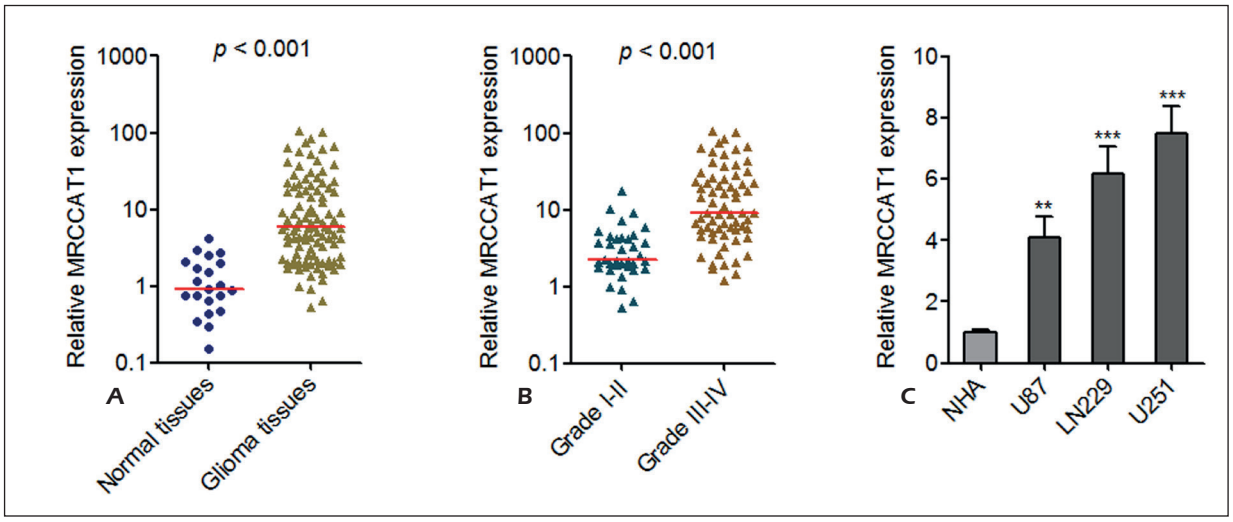

Figure 1. The expression of MRCCAT1 in glioma tissues and cells, and its association with glioma grade. **A**, The expression of MRCCAT1 in 21 normal brain tissues and 103 glioma tissues was measured by qPCR. $p<0.001$ by Mann-Whitney U test. **B**, The expression of MRCCAT1 in 36 grade I-II gliomas and 67 grade III-IV gliomas was measured by qPCR. $p < 0.001$ by Mann-Whitney U test. **C**, The expression of MRCCAT1 in normal glia cells NHA and glioma cells U87, LN229, and U251 was measured by qPCR. Results are shown as mean ± SD from three independent experiments. $^{**}p<0.01$, $^{***}p<0.001$ by Student's t-test.

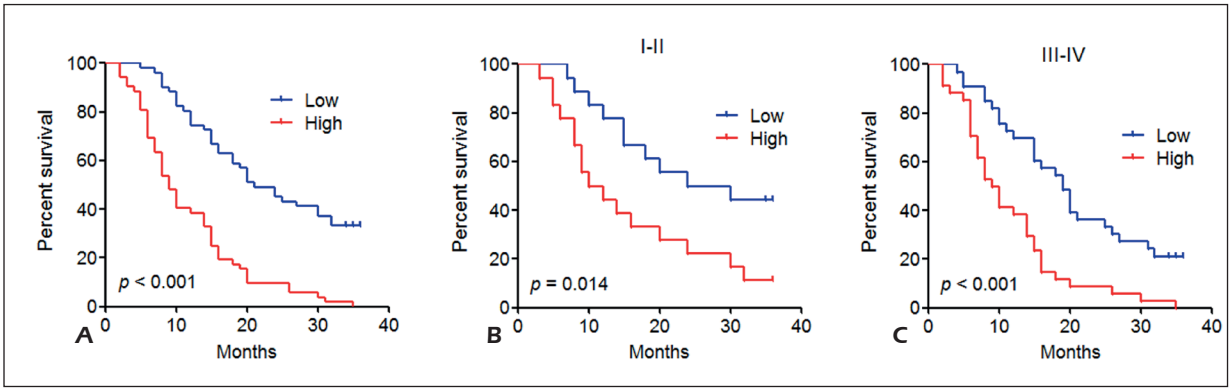

Figure 2. The correlation between MRCCAT1 expression and overall survival of glioma patients. **A**, Kaplan-Meier survival analyses of the correlation between the expression of MRCCAT1 and overall survival of these 103 gliomas. Median expression level of these 103 gliomas was used as the cutoff. $p<0.001$ by log-rank test. **B**, Kaplan-Meier survival analyses of the correlation between the expression of MRCCAT1 and overall survival of these 36 grade I-II gliomas. Median expression level of these 36 grade I-II gliomas was used as the cutoff. $p=0.014$ by log-rank test. **C**, Kaplan-Meier survival analyses of the correlation between the expression of MRCCAT1 and overall survival of these 67 grade III-IV gliomas. Median expression level of these 67 grade III-IV gliomas was used as the cutoff. $p<0.001$ by log-rank test.

I-II (n = 36) ($p < 0.001$ by Mann-Whitney U test). Furthermore, the expression of MRCCAT1 in normal glia cells NHA and glioma cells U87, LN229, and U251 was also measured by qPCR. The results revealed that the expression of MRCCAT1 was also significantly upregulated in U87, LN229, and U251 cells compared with that in NHA cells (Figure 1C). These results suggested that MRCCAT1 is upregulated in glioma tissues and cell lines, and correlates with glioma grade.

MRCCAT1 Upregulation Confers Poor Prognosis of Glioma Patients

Next, the correlation between the expression of MRCCAT1 and overall survival of these 103 glioma patients was investigated by Kaplan-Meier survival curve analysis. As shown in Figure 2A, high expression of MRCCAT1 correlated with poor survival of glioma patients ($p<0.001$ by log-rank test). In addition, high expression of MRCCAT1 correlated with poor survival in grade I-II gliomas ($p=0.014$ by log-rank test) (Figure 2B). In grade III-IV gliomas, high expression of MRCCAT1 also indicated poor survival ($p<0.014$ by log-rank test) (Figure 2C). These results suggested that MRCCAT1 upregulation confers poor prognosis of glioma patients, and implied that MRCCAT1 may have important roles in glioma.

MRCCAT1 Promotes Proliferation of Glioma Cells

To investigate whether MRCCAT1 has biological roles in glioma, MRCCAT1 stably overexpressed and control U87 cells were constructed via transfecting MRCCAT1 overexpression plasmid (pcDNA3.1-MRCCAT1) or control empty plasmid (pcDNA3.1). The overexpression efficacy was measured by qPCR (Figure 3A). Glo cell viability assays revealed that ectopic expression of MRCCAT1 increased cell viability of glioma cells (Figure 3B). EdU incorporation assays further verified that ectopic expression of MRCCAT1 promoted cell proliferation of glioma cells (Figure 3C).

MRCCAT1 stably silenced and control U251 cells were also constructed via transfecting MRCCAT1 specific shRNAs (shRNA1 and shRNA2) or negative control shRNA (shNC). The knockdown efficacy was verified by qPCR (Figure 3D). Glo cell viability assays revealed that knockdown of MRCCAT1 decreased cell viability of glioma cells (Figure 3E). Similarly, EdU incorporation assays verified that knockdown of MRCCAT1 inhibited cell proliferation of glioma cells (Figure 3F). Collectively, these results suggested that MRCCAT1 promotes glioma cell proliferation.

MRCCAT1 Promotes Migration of Glioma Cells

To further investigate the roles of MRCCAT1 in glioma cell migration, transwell assays were performed in MRCCAT1 stably overexpressed and control U87 cells, and MRCCAT1 stably silenced and control U251 cells. As shown in Figure 4A, the ectopic expression of MRCCAT1 promoted cell migration of glioma cells. Conversely, knockdown of MRCCAT1 inhibited cell migration of glioma cells (Figure 4B). These results suggested that MRCCAT1 promotes glioma cell migration.

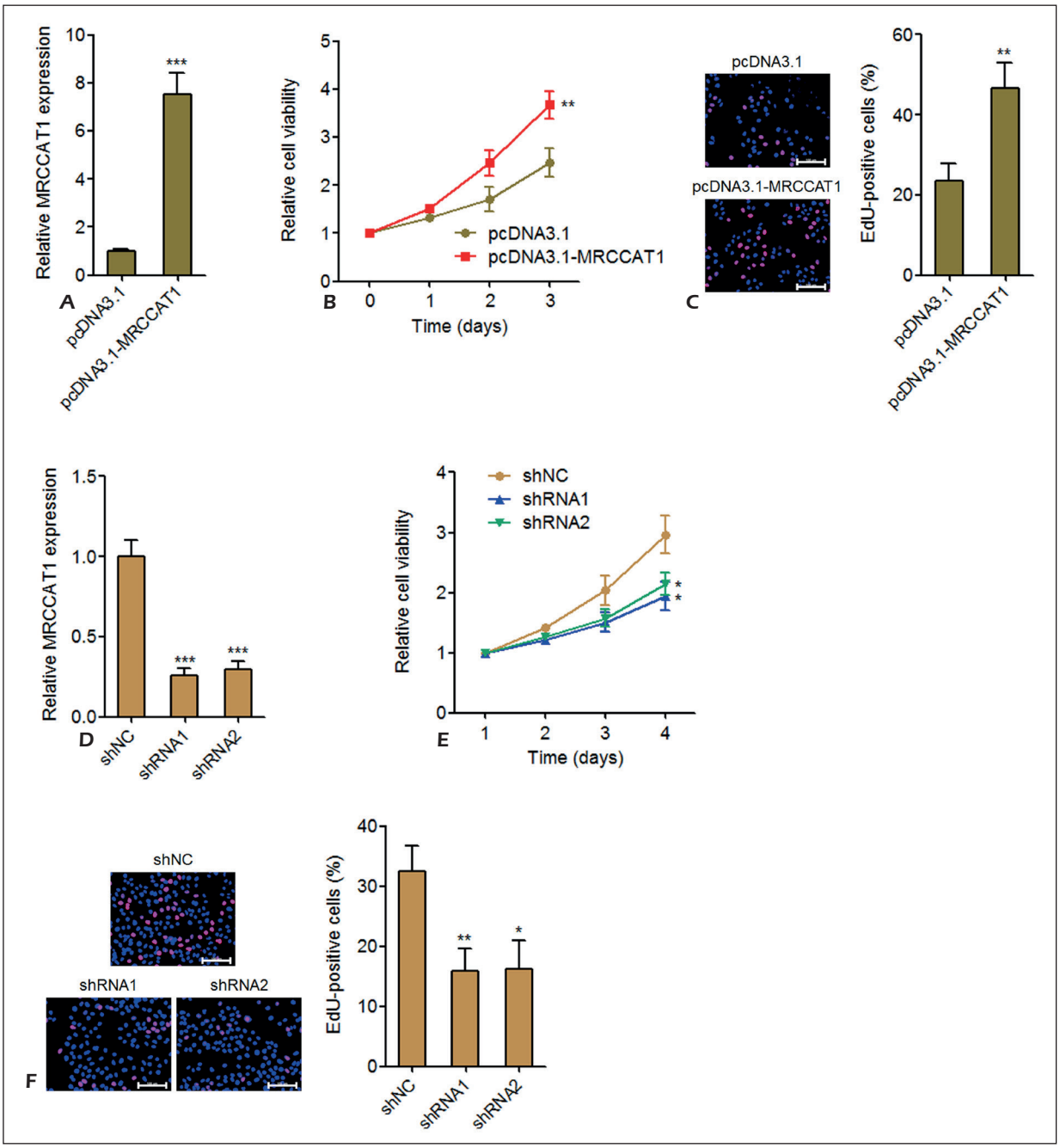

Figure 3. MRCCAT1 promotes glioma cell proliferation. ***A***, The expression of MRCCAT1 in MRCCAT1 stably overexpressed and control U87 cells was measured by qPCR. **B**, The roles of MRCCAT1 overexpression on cell viability of U87 cells were assessed using Glo cell viability assays. **C**, The roles of MRCCAT1 overexpression on cell proliferation of U87 cells were assessed using EdU incorporation assays. The red color represents EdU-positive cells. Scale bars, 100 μm. **D**, The expression of MRCCAT1 in MRCCAT1 stably silenced and control U251 cells was measured by qPCR. **E**, The roles of MRCCAT1 knockdown on cell viability of U251 cells were assessed using Glo cell viability assays. **F**, The roles of MRCCAT1 knockdown on cell proliferation of U251 cells were assessed using EdU incorporation assays. The red color represents EdU-positive cells. Scale bars, 100 μm. Results are shown as mean ± SD from three independent experiments. *p<0.05, **p<0.01, ***p<0.001 by Student's t-test.

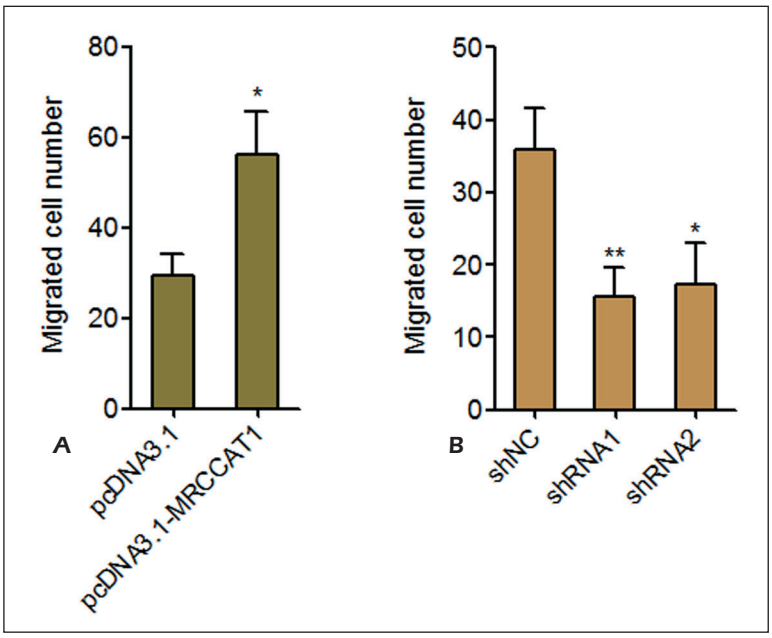

Figure 4. MRCCAT1 promotes glioma cell migration. **A**, The roles of MRCCAT1 overexpression on cell migration of U87 cells were assessed using transwell assays. **B**, The roles of MRCCAT1 knockdown on cell migration of U251 cells were assessed using transwell assays. Results are shown as mean ± SD from three independent experiments. *p<0.05, **p<0.01 by Student's t-test.

MRCCAT1 Activates p38-MAPK Signaling in Glioma Cells

MRCCAT1 was reported to activate p38-MAPK signaling in renal cell carcinoma. p38-MAPK signaling is well known to potentiate cell proliferation and migration in various cancers, including glioma[31-33]. To investigate whether MRCCAT1 also activates p38-MAPK signaling to promote cell proliferation and migration in glioma, p38 phosphorylation levels were measured by Western blot in MRCCAT1 stably overexpressed and control U87 cells, and MRCCAT1 stably silenced and control U251 cells. As shown in Figure 5A, the ectopic expression of MRCCAT1 increased phosphorylation levels of p38 in glioma cells. Conversely, knockdown of MRCCAT1 decreased phosphorylation levels of p38 in glioma cells (Figure 5B). These results suggested that MRCCAT1 activates p38-MAPK signaling in glioma cells.

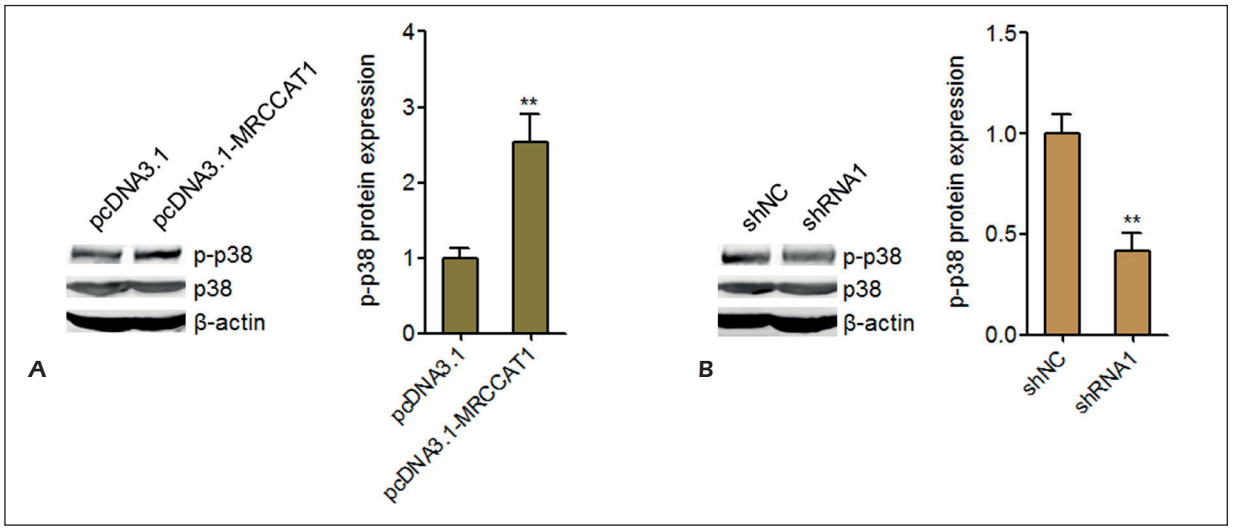

Figure 5. MRCCAT1 activates p38-MAPK signaling in glioma cells. **A**, The roles of MRCCAT1 overexpression on p38 phosphorylation levels in U87 cells were assessed using Western blot. **B**, The roles of MRCCAT1 knockdown on p38 phosphorylation levels in U251 cells were assessed using Western blot. Results are shown as mean ± SD from three independent experiments. **p<0.01 by Student's t-test.

Discussion

As the most common primary brain tumor, unfortunately, the prognosis of glioma is very poor with a median survival of about 15 months[34]. Although there are various clinical therapies for glioma, the efficacies are all unsatisfied[35]. Hence, identifying critical molecules mediating the initiation and progression of glioma and searching efficient prognostic biomarker and therapeutic target are urgent.

As a novel class of transcript with no protein-coding potential, lncRNAs gradually attract attention for their important roles in various pathophysiological processes[36,37]. LncRNA CRNDE was reported to promote progression of glioma via attenuating miR-384/PIWIL4/STAT3 Axis[38]. LncRNA CCAT1 was reported to promote tumorigenesis of glioma via sponging miR-181b[39]. lncRNA TUSC7 was reported to play tumor-suppressing roles in glioma[40]. LncRNA PTCSC3 was reported to inhibit glioma cell proliferation and invasion via suppressing the Wnt/β-catenin signaling pathway[41]. Although several lncRNAs have been revealed to be implicated in glioma, the expressions and roles of most other lncRNAs in glioma are less understood. Transcriptome sequencing of human cells has demonstrated that there are near 60000 lncRNAs existing in human cells[42]. Among the enormous sum of lncRNAs, many lncRNAs may also have important roles in glioma, and these need further investigation.

In this study, we investigated the expressions and roles of a newly identified lncRNA MRCCAT1 in glioma. Our results revealed that MRCCAT1 is upregulated in glioma tissues and cell lines. MRCCAT1 is also highly expressed in gliomas with high grade than that in gliomas with low grade. Furthermore, increased expression of MRCCAT1 indicates poor prognosis of glioma patients, which is independent of the grades of gliomas. In grade I-II gliomas, MRCCAT1 is associated with poor prognosis of glioma patients. In grade III-IV gliomas, MRCCAT1 is also associated with poor prognosis of glioma patients. Gain-of-function and loss-of-function assays showed that overexpression of MRCCAT1 promotes glioma cell proliferation and migration, and conversely, knockdown of MRCCAT1 inhibits glioma cell proliferation and migration. Mechanistically, we found that similar to that in ccRCC, MRCCAT1 also activates the p38-MAPK signaling pathway in glioma. Hence, our data revealed the important clinical significances and roles of MRCCAT1 in glioma, and it suggested that MRCCAT1 may be a potential prognostic biomarker and therapeutic target for glioma.

Conclusions

We identified MRCCAT1 as an oncogenic lncRNA in glioma. MRCCAT1 is upregulated in glioma, and it is positively associated with glioma grade and poor prognosis. MRCCAT1 promotes glioma cell proliferation and migration via activating p38-MAPK signaling pathway. Our data implied that MRCCAT1 would be a potential prognostic biomarker and therapeutic target for glioma.

Conflict of Interests

The authors declare that they have no conflicts of interest.

References

1) Mackay A, Burford A, Carvalho D, Izquierdo E, Fazal-Salom J, Taylor K R, Bjerke L, Clarke M, Vinci M, Nandhabalan M, Temelso S, Popov S, Molinari V, Raman P, Waanders A J, Han H J, Gupta S, Marshall L, Zacharoulis S, Vaidya S, Mandeville H C, Bridges L R, Martin A J, Al-Sarraj S, Chandler C, Ng H K, Li X, Mu K, Trabelsi S, Brahim D H, Kisljakov A N, Konovalov D M, Moore A S, Carcaboso A M, Sunol M, De Torres C, Cruz O, Mora J, Shats L I, Stavale J N, Bidinotto L T, Reis R M, Entz-Werle N, Farrell M, Cryan J, Crimmins D, Caird J, Pears J, Monje M, Debily M A, Castel D, Grill J, Hawkins C, Nikbakht H, Jabado N, Baker S J, Pfister S M, Jones D T W, Fouladi M, Von Bueren A O, Baudis M, Resnick A, Jones C. Integrated molecular meta-analysis of 1,000 pediatric high-grade and diffuse intrinsic pontine glioma. cancer Cell 2017; 32: 520-537. e525.
2) Ricard D, Idbaih A, Ducray F, Lahutte M, Hoang-Xuan K, Delattre J Y. Primary brain tumours in adults. Lancet 2012; 379: 1984-1996.
3) Fan Q, Aksoy O, Wong R A, Ilkhanizadeh S, Novotny C J, Gustafson W C, Truong A Y, Cayanan G, Simonds E F, Haas-Kogan D, Phillips J J, Nicolaides T, Okaniwa M, Shokat K M, Weiss W A. A kinase inhibitor targeted to mTORC1 drives regression in glioblastoma. Cancer Cell 2017; 31: 424-435.
4) Weller M, Van Den Bent M, Tonn J C, Stupp R, Preusser M, Cohen-Jonathan-Moyal E, Henriksson R, Le Rhun E, Balana C, Chinot O, Bendszus M, Reijneveld J C, Dhermain F, French P, Marosi C, Watts C, Oberg I, Pilkington G, Baumert B G, Taphoorn M J B, Hegi M, Westphal M, Reifenberger G, Soffietti R, Wick W, European Association for Neuro-Oncology Task Force On G. European Association for Neuro-Oncology (EANO) guideline on the diagnosis and treatment of adult astrocytic and oligodendroglial gliomas. Lancet Oncol 2017; 18: e315-e329.

5) Huang T, Kim C K, Alvarez A A, Pangeni R P, Wan X, Song X, Shi T, Yang Y, Sastry N, Horbinski C M, Lu S, Stupp R, Kessler J A, Nishikawa R, Nakano I, Sulman E P, Lu X, James C D, Yin X M, Hu B, Cheng S Y. MST4 phosphorylation of ATG4B regulates autophagic activity, tumorigenicity, and radioresistance in glioblastoma. Cancer Cell 2017; 32: 840-855 e848.
6) Saha D, Martuza R L, Rabkin S D. Macrophage polarization contributes to glioblastoma eradication by combination immunovirotherapy and immune checkpoint blockade. Cancer Cell 2017; 32: 253-267 e255.
7) Miroshnikova Y A, Mouw J K, Barnes J M, Pickup M W, Lakins J N, Kim Y, Lobo K, Persson A I, Reis G F, Mcknight T R, Holland E C, Phillips J J, Weaver V M. Tissue mechanics promote IDH1-dependent HIF1alpha-tenascin C feedback to regulate glioblastoma aggression. Nat Cell Biol 2016; 18: 1336-1345.
8) Zhou A, Lin K, Zhang S, Chen Y, Zhang N, Xue J, Wang Z, Aldape K D, Xie K, Woodgett J R, Huang S. Nuclear GSK3beta promotes tumorigenesis by phosphorylating KDM1A and inducing its deubiquitylation by USP22. Nat Cell Biol 2016; 18: 954-966.
9) Ponting C P, Oliver P L, Reik W. Evolution and functions of long noncoding RNAs. Cell 2009; 136: 629-641.
10) Fatica A, Bozzoni I. Long non-coding RNAs: new players in cell differentiation and development. Nat Rev Genet 2014; 15: 7-21.
11) Grelet S, Link L A, Howley B, Obellianne C, Palanisamy V, Gangaraju V K, Diehl J A, Howe P H. A regulated PNUTS mRNA to lncRNA splice switch mediates EMT and tumour progression. Nat Cell Biol 2017; 19: 1105-1115.
12) Liu X, Xiao Z D, Han L, Zhang J, Lee S W, Wang W, Lee H, Zhuang L, Chen J, Lin H K, Wang J, Liang H, Gan B. LncRNA NBR2 engages a metabolic checkpoint by regulating AMPK under energy stress. Nat Cell Biol 2016; 18: 431-442.
13) Yuan J H, Yang F, Wang F, Ma J Z, Guo Y J, Tao Q F, Liu F, Pan W, Wang T T, Zhou C C, Wang S B, Wang Y Z, Yang Y, Yang N, Zhou W P, Yang G S, Sun S H. A long noncoding RNA activated by TGF-beta promotes the invasion-metastasis cascade in hepatocellular carcinoma. Cancer Cell 2014; 25: 666-681.
14) Su S, Liu J, He K, Zhang M, Feng C, Peng F, Li B, Xia X. Overexpression of the long noncoding RNA TUG1 protects against cold-induced injury of mouse livers by inhibiting apoptosis and inflammation. FEBS J 2016; 283: 1261-1274.
15) Schmitt A M,Chang H Y. Long noncoding RNAs in cancer pathways. Cancer Cell 2016; 29: 452-463.
16) Lin A, Li C, Xing Z, Hu Q, Liang K, Han L, Wang C, Hawke D H, Wang S, Zhang Y, Wei Y, Ma G, Park P K, Zhou J, Zhou Y, Hu Z, Zhou Y, Marks J R, Liang H, Hung M C, Lin C, Yang L. The LINK-A lncRNA activates normoxic HIF1alpha signalling in triple-negative breast cancer. Nat Cell Biol 2016; 18: 213-224.
17) Parasramka M, Yan I K, Wang X, Nguyen P, Matsuda A, Maji S, Foye C, Asmann Y, Patel T. BAP1 dependent expression of long non-coding RNA NEAT-1 contributes to sensitivity to gemcitabine in cholangiocarcinoma. Mol Cancer 2017; 16: 22.
18) Jiang C, Li X, Zhao H, Liu H. Long non-coding RNAs: potential new biomarkers for predicting tumor invasion and metastasis. Mol Cancer 2016; 15: 62.
19) Yuan J H, Liu X N, Wang T T, Pan W, Tao Q F, Zhou W P, Wang F, Sun S H. The MBNL3 splicing factor promotes hepatocellular carcinoma by increasing PXN expression through the alternative splicing of lncRNA-PXN-AS1. Nat Cell Biol 2017;
20) Lin A, Hu Q, Li C, Xing Z, Ma G, Wang C, Li J, Ye Y, Yao J, Liang K, Wang S, Park P K, Marks J R, Zhou Y, Zhou J, Hung M C, Liang H, Hu Z, Shen H, Hawke D H, Han L, Zhou Y, Lin C, Yang L. The LINK-A lncRNA interacts with PtdIns(3,4,5)P3 to hyperactivate AKT and confer resistance to AKT inhibitors. Nat Cell Biol 2017; 19: 238-251.
21) Zhu X T, Yuan J H, Zhu T T, Li Y Y, Cheng X Y. Long noncoding RNA glypican 3 (GPC3) antisense transcript 1 promotes hepatocellular carcinoma progression via epigenetically activating GPC3. FEBS J 2016; 283: 3739-3754.
22) Chen Q N, Chen X, Chen Z Y, Nie F Q, Wei C C, Ma H W, Wan L, Yan S, Ren S N, Wang Z X. Long intergenic non-coding RNA 00152 promotes lung adenocarcinoma proliferation via interacting with EZH2 and repressing IL24 expression. Mol Cancer 2017; 16: 17.
23) Montes M,Lund A H. Emerging roles of lncRNAs in senescence. FEBS J 2016; 283: 2414-2426.
24) Zhou M, Zhao H, Xu W, Bao S, Cheng L, Sun J. Discovery and validation of immune-associated long non-coding RNA biomarkers associated with clinically molecular subtype and prognosis in diffuse large B cell lymphoma. Mol Cancer 2017; 16: 16.
25) Niu Y, Ma F, Huang W, Fang S, Li M, Wei T, Guo L. Long non-coding RNA TUG1 is involved in cell growth and chemoresistance of small cell lung cancer by regulating LIMK2b via EZH2. Mol Cancer 2017; 16: 5.
26) Zhu X, Tian X, Yu C, Shen C, Yan T, Hong J, Wang Z, Fang J Y, Chen H. A long non-coding RNA signature to improve prognosis prediction of gastric cancer. Mol Cancer 2016; 15: 60.
27) Su R, Cao S, Ma J, Liu Y, Liu X, Zheng J, Chen J, Liu L, Cai H, Li Z, Zhao L, He Q, Xue Y. Knockdown of SOX2OT inhibits the malignant biological behaviors of glioblastoma stem cells via up-regulating the expression of miR-194-5p and miR-122. Mol Cancer 2017; 16: 171.
28) Yu M, Xue Y, Zheng J, Liu X, Yu H, Liu L, Li Z, Liu Y. Linc00152 promotes malignant progression of glioma stem cells by regulating miR-103a-3p/FEZF1/CDC25A pathway. Mol Cancer 2017; 16: 110.
29) Liu S, Mitra R, Zhao M M, Fan W, Eischen C M, Yin F, Zhao Z. The potential roles of long noncoding RNAs (lncRNA) in glioblastoma development. Mol Cancer Ther 2016; 15: 2977-2986.
30) Li J K, Chen C, Liu J Y, Shi J Z, Liu S P, Liu B, Wu D S, Fang Z Y, Bao Y, Jiang M M, Yuan J H, Qu L, Wang L H. Long noncoding RNA MRCCAT1 promotes metastasis of clear cell renal cell carcinoma via inhibiting NPR3 and activating p38-MAPK signaling. Mol Cancer 2017; 16: 111.

31) Girnius N, Davis RJ. TNFalpha-mediated cytotoxic responses to IAP inhibition are limited by the p38alpha MAPK pathway. Cancer Cell 2016; 29: 131-133.
32) Lalaoui N, Hanggi K, Brumatti G, Chau D, Nguyen N Y, Vasilikos L, Spilgies L M, Heckmann D A, Ma C, Ghisi M, Salmon J M, Matthews G M, De Valle E, Moujalled D M, Menon M B, Spall S K, Glaser S P, Richmond J, Lock R B, Condon S M, Gugasyan R, Gaestel M, Guthridge M, Johnstone R W, Munoz L, Wei A, Ekert P G, Vaux D L, Wong W W, Silke J. Targeting p38 or MK2 enhances the anti-leukemic activity of smac-mimetics. Cancer Cell 2016; 29: 145-158.
33) Chen Y, Gao F, Jiang R, Liu H, Hou J, Yi Y, Kang L, Liu X, Li Y, Yang M. Down-regulation of AQP4 expression via p38 MAPK signaling in temozolomide-induced glioma cells growth inhibition and invasion impairment. J Cell Biochem 2017; 118: 4905-4913.
34) Jansen M, Yip S, Louis D N. Molecular pathology in adult gliomas: diagnostic, prognostic, and predictive markers. Lancet Neurol 2010; 9: 717-726.
35) Zhang B, Chang M, Wang J, Liu Y. Neuroglobin functions as a prognostic marker and promotes the tumor growth of glioma via suppressing apoptosis. Biomed Pharmacother 2017; 88: 173-180.
36) Zhang L, Yang F, Yuan J H, Yuan S X, Zhou W P, Huo X S, Xu D, Bi H S, Wang F, Sun S H. Epigenetic activation of the MiR-200 family contributes to H19-mediated metastasis suppression in hepatocellular carcinoma. Carcinogenesis 2013; 34: 577-586.
37) Bi H S, Yang X Y, Yuan J H, Yang F, Xu D, Guo Y J, Zhang L, Zhou C C, Wang F, Sun S H. H19 inhibits RNA polymerase II-mediated transcription by disrupting the hnRNP U-actin complex. Biochim Biophys Acta 2013; 1830: 4899-4906.
38) Zheng J, Liu X, Wang P, Xue Y, Ma J, Qu C, Liu Y. CRNDE promotes malignant progression of glioma by attenuating miR-384/PIWIL4/STAT3 axis. Mol Ther 2016; 24: 1199-1215.
39) Cui B, Li B, Liu Q, Cui Y. lncRNA CCAT1 promotes glioma tumorigenesis by sponging miR-181b. J Cell Biochem 2017; 118: 4548-4557.
40) Shang C, Guo Y, Hong Y, Xue Y X. Long non-coding RNA TUSC7, a target of miR-23b, plays tumor-suppressing roles in human gliomas. Front Cell Neurosci 2016; 10: 235.
41) Xia S, Ji R, Zhan W. Long noncoding RNA papillary thyroid carcinoma susceptibility candidate 3 (PTCSC3) inhibits proliferation and invasion of glioma cells by suppressing the Wnt/beta-catenin signaling pathway. BMC Neurol 2017; 17: 30.
42) Iyer M K, Niknafs Y S, Malik R, Singhal U, Sahu A, Hosono Y, Barrette T R, Prensner J R, Evans J R, Zhao S, Poliakov A, Cao X, Dhanasekaran S M, Wu Y M, Robinson D R, Beer D G, Feng F Y, Iyer H K, Chinnaiyan A M. The landscape of long noncoding RNAs in the human transcriptome. Nat Genet 2015; 47: 199-208.