Long non-coding RNA RMRP promotes human glioma tumor progression via miR-466/CXCR4 pathway

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Abstract

Purpose: Long non-coding RNA (lncRNA) RMRP has been confirmed to be associated with multiple tumor progression. However, the molecular regulatory mechanism of RMRP in human glioma was rarely elucidated.

Methods: The quantitative real-time polymerase chain reaction (qRT-PCR) was used to measure the expression levels of RMRP, microRNA-466 (miR-466) and CXCR4 in glioma tissues and cells. The ELISA-BrdU assay and flow cytometry analysis was used to perform the cell proliferation, apoptotic rate, cell cycle assay in human glioma cell lines A172 and U251 cells. The starBase v2.0 or TargetScan was used to predict the potential interactions among RMRP, miR-466 and CXCR4, and the luciferase reporter assay was performed to validate the relationship among these molecules. The western blot was used to determine the expression levels of protein associated with cell cycle and CXCR4.

Results: The levels of RMRP, CXCR4 were highly expressed, but miR-466 was decreased in glioma tissues and cells. And miR-466 was verified as a direct target of RMRP and directly regulated CXCR4. RMRP expression or miR-466 inhibitor promote cell proliferation and G1/S cell cycle transition but reduced apoptotic rate in A172 and U251 cells, while RMRP promote G1/S cell cycle transition through regulating CDK4 pathway, not CDK2 pathway. And that, the introduction of CXCR4 partly counteracted these impacts. In addition, RMRP silencing and miR-466 mimic impeded xenograft tumor growth *in vivo*.

Conclusion: RMRP enhanced glioma progression, partially through miR-466/CXCR4 pathway. The molecular regulation mechanism based on RMRP/miR-466/CXCR4 might contribute to the diagnosis of glioma and provide the effective therapeutic targets for its treatment.

Introduction

Glioma is the most common tumor of the nervous system [1]. In addition, gliomas are typically characterized by malignant hyperplasia and aggressive growth with a high recurrence rate and poor prognosis. Therefore, it is difficult to cure glioma completely with traditional treatment methods. Hence, Therefore, it is urgent to explore the mechanism of glioma development and identify new therapeutic targets.

Non-coding RNAs (ncRNAs) are known to influence tumor progression through a variety of regulatory pathways. [2]. Long non-coding RNAs (lncRNAs, > 200 nts) are non-translational ncRNAs that regulate gene expression in tumorigenesis and progression [3]. Previous studies have identified multiple lncRNAs up-regulated in gliomas. For instance, lncRNA H19 was increased in glioma and promoted glioma angiogenesis via binding miR-138 and regulating HIF-1 α /VEGF axis [4]. Another study demonstrated that lncRNA PVT1 facilitated tumorigenesis and progression in human glioma, and confirmed a regulatory relationship between PVT1 and miR-128-3p as well as that between miR-128-3p and GREM1 [5]. The RNA component of mitochondrial RNA processing endoribonuclease (RMRP) is a 277 nt-long lncRNA molecule, and increasing evidence has demonstrated that it is a novel oncogene, the expression level of

which is increased in bladder cancer and non-small cell lung cancer [6, 7]. For example, Zhao et al. reported that lncRNA RMRP knockdown could suppress hepatocellular carcinoma and targeted miR-206 to regulate TACR1 to inhibit cell proliferation [8]. However, lncRNAs often target multiple miRNAs and thus have multiple regulatory functions. Therefore, it is necessary to further elucidate the regulatory mechanisms of the oncogene RMRP in the progression of human glioma.

MicroRNAs (miRNAs) are another type of non-coding RNAs that primarily perform RNA silencing functions [9]. The miRNA is involved in almost all the developmental and pathological processes in animals, and its disturbance is associated with many human diseases, including tumor progression [10]. lncRNAs can inhibit miRNA expression through competitive binding of miRNAs, and thus exert different biological functions in negative regulation of downstream target genes [11]. Overexpression of lncRNA or interrupting the expression of miRNAs can promote the proliferation, invasion, migration, and apoptosis of tumors. Some previous researches have indicated that miR-466 was significantly downregulated, which might be potential cancer suppressors in different types of cancers, such as neuroblastoma, colorectal cancer, prostate carcinoma, and hepatocellular carcinoma [12–14]. Nevertheless, the regulatory function of lncRNA RMRP/miR-466 axis in glioma cells needs to be further elucidated.

Herein, our study aims to investigate the explicit role of lncRNA RMRP in the pathogenesis of glioma as well as the potential regulatory mechanism between lncRNA RMRP and miR-466 in human glioma progression. We delved into the lncRNA–miRNA–mRNA networks in occurrence and development of human glioma and provide a potential novel biomarker or therapeutic target for the prognosis and treatment of human glioma.

Materials And Methods

Patients and Clinical tissue samples

Fifty-eight patients who underwent surgery at the Department of Neurosurgery in the First Affiliated of Chengdu Medical College from July 2015 to September 2016 were included in the study, and classified according to the WHO guidelines for the classification of tumours of the central nervous system [15]. This study mainly collected the information from the patients' medical records, and the detail information was showed in Table 1. The survival rate was calculated from the data of surgical resection to Dec 31, 2020. The human glioma tissue of the patients or the control group was immediately immersed in liquid nitrogen until RNA extraction.

The written informed consents were collected from all patients, and this study was approved by the Ethics Committee of the First Affiliated of Chengdu Medical College (IRB number: 20140332).

Table 1
Clinicopathological characteristics of RMRP expression in glioma patients.

Clinical feature	RMRP expression [#]		<i>p</i> value
	Low	High	
Age			0.076*
< 60	18	13	
≥ 60	10	17	
Gender			0.368
Male	14	16	
Female	14	14	
Histologic Type			0.041*
Astrocytoma	2	7	
Glioblastoma	20	16	
Oligoastrocytoma	1	4	
Oligodendroglioma	5	3	
WHO Grade			0.024*
Grade II	5	5	
Grade III	10	6	
Grade IV	13	19	
Recurrence Status			0.434
No	19	20	
Yes	9	10	
Recurrence time in months [mean (sd)]^{&}	23.4 (25.3)	14.5 (16.4)	0.121
Survival Status			0.009*
Alive	20	9	

**p* < 0.05 was considered statistically significant.

[#] Low/high expression was determined by the sample mean. Pearson chi-square test was utilized to analyze the clinical data.

[&]*p*-value from t test.

Clinical feature	RMRP expression [#]		<i>p</i> value
	Low	High	
Deceased	8	21	
Survival time in months [mean (sd)] ^{&}	52 (32.2)	21.3 (23.5)	0.003*
IDH1 mutation			0.037*
Negative	15	19	
Positive	13	11	
* <i>p</i> < 0.05 was considered statistically significant.			
[#] Low/high expression was determined by the sample mean. Pearson chi-square test was utilized to analyze the clinical data.			
^{&} <i>p</i> -value from t test.			

Cell culture

The human glioma cell lines A172, SHG44, U251, LN229 as well as the normal human astrocytes (NHAs) were obtained from the American Type Culture Collection (Manassas, VA, USA). All cell lines were maintained in DMEM medium (Gibco, Thermo Fisher Scientific, Waltham, MA, USA) containing 10% FBS (Gibco) and cultured in a 5% CO₂ incubator at 37°C.

RNA extraction and quantitative RT-PCR analysis

The total RNA was isolated from human glioma tissues and cell lines using TRIzol reagent (Invitrogen). For quantitative RT-PCR, the total RNA was reverse-transcribed into complementary DNA using the reverse transcription kit (Invitrogen), according to the manufacturer's instruction. Further, the SYBR green PCR kit (Toyobo, Nipro, Osaka, Japan) was used to conduct real-time PCR with the 7500 Fast Real-time PCR system (Applied Biosystems, Waltham, MA, USA). The 2^{-ΔΔCt} method was used for calculating the target gene expression levels, and GAPDH or U6, as endogenous control, was used to normalize the target gene expression levels, respectively. The primer sequences were as follows: RMRP, F-5'-CACTGCCTATGTGCACGACT-3', R-5'-AGAGTCCGGCAAGAAGAACA-3'; miR-466, F-5'-GGGATTCCCCTGGGTGTCAAACTGGC-3', R-5'-TGCGCAGCACAGTTCCACGGGTC-3'; CXCR4, F-5'-ACCCCTTCATTGACCTCAACTA-3', R-5'-TCTCGCTCCTGGAAGATGGTGA-3'; GAPDH: F-5'-AATGGGCAGCCGTTAGGAAA-3', R-5'-TGAAGGGGTCATTGATGGCA-3'; and U6: F-5'-CTCGCTTCGGCAGCACATATA-3', R-5'-ACGCTTCACGAATTTGAGTGTC-3'. All assays were performed three times.

Cell transfection

The small short hairpin RNA (shRNA) targeting RMRP, control shRNA, pcDNA-RMRP, and the control plasmid pcDNA3.1 were provided by Novogene (Beijing, China). The detailed sequences of all targeted genes were used: shRMRP: (F) 5'GGCCAAACCCUCAAUGAAUTT-3' and (R) 5'AUUCAUUGAGGGUUUGGCCTG-3'; shCtrl: (F) 5'UUCUCCGAACGUGUCACGUTT-3' and (R) 5'ACGUGACACGUUCGGAGAATT-3'; pcDNA-RMRP: (F) 5'CGATCTTAATTAAGGGGTACCAAAGTCCACTCTG-3' and (R) 5'TCAGTGGCGCGCCTTTTCGTGAGTACACAATAGTCATC-3'. The miR-466 mimic (5'-ACCUGGCAUACAAUGUAGAUUU-3'), miR-negative control (NC) mimic (5'-UUCUCCGAACGUGUCACGUTT-3'), miR-466 inhibitor (5'-UGAGGUAGUAGGUUGUAUAGUU-3) and miR-NC inhibitor (5'-CAGUACUUUUGUGUAGUACAA-3') were provided by Shanghai Sangon Biotech Corporation. The corresponding plasmid or miRNAs (50 nM) were transfected into A172 and U251 cell lines with a density of 10^5 /well in 6-well plates by Lipofectamine 2000 reagent (GE Healthcare Life Sciences, Lafayette, CO, USA), and the cells were collected for analysis at 48 h after transfection.

MTT assays

A172 and U251 cell lines were added to a 96-well dish at a density of 10^3 cells per well and then transfected with pcDNA-RMRP, sh-RMRP, miRNA, pcDNA-CXCR4, or shCXCR4. Subsequently, at 0, 24, 48, 72, and 96 h, 10 μ l MTT solution was added to each well. After 4 h of culture, the supernatant was discarded, and then 100 μ l of DMSO was added. The absorbance at 570 nm was determined using an enzyme-labeling instrument (EL808 BioTek Instruments, Winooski, VT, USA).

BrdU proliferation assay

A cell proliferation assay was performed using a cell proliferation ELISA-BrdU (colorimetric) Kit (Roche Diagnostics, CA, USA), according to the manufacturer's protocol. Briefly, the A172 and U251 cell lines were cultured into a 96-well plate at a density of 10^3 cells per well. Overnight, the cells were transfected with the corresponding pcDNA-RMRP, sh-RMRP, miRNA, pcDNA-CXCR4, or shCXCR4 at 37°C. After 24 h of incubation, cell proliferation was analyzed.

Apoptosis analysis

The A172 and U251 cells were seeded into 6-well plates at a density of 10^5 cells per well, and the pcDNA-RMRP, sh-RMRP, miRNA, pcDNA-CXCR4, or shCXCR4 were transfected into cells. After that, a cell apoptosis assay was conducted via flow cytometry using the Annexin V-FITC Apoptosis Detection Kit (Beyotime, China). Briefly, cells were collected with annexin-V/FITC (5 μ l) and propidium iodide (10 μ l), and were incubated for 15 min at room temperature in the dark. The cell apoptosis rate was analyzed within 1 h using a FACS caliber cytometer (BD Biosciences, Franklin Lakes, NJ, USA).

Cell cycle analysis

The A172 and U251 cells were transfected with pcDNA-RMRP, sh-RMRP, miRNA, pcDNA-CXCR4, or shCXCR4 for 24 h. After that, the cells were collected by trypsinization and washed with PBS that was precooled on ice. The cells were fixed in 70% methanol and incubated at 4 °C for 1 h. The cells were then centrifuged and added with RNase at 37 °C and incubated for 30 min. The cells were stained by

propidium iodide for 1 h. The cell cycle of pretreated cells was analyzed using FACScan flow cytometer, and the data were presented by CellQuest software.

Western blotting

The Western blot assay used in this study was according to the following previously described methods. Total proteins were extracted from A172 cells using a protein isolation kit (Tiangen, Beijing, China), and the protein concentrations were quantified through the Pierce BCA assay (Thermo Scientific, CA, USA). Equal amounts of protein samples were separated by SDS-PAGE and then electrophoretically transferred to a polyvinylidene fluoride membrane (Milipore, CA, USA). The antibodies against CDK4, Cyclin-D1, CDK2, Cyclin-E, β -actin, and CXCR4 (Santa Cruz Biotechnology, CA, USA) as the primary antibody (all obtained from Cell signaling Technology, Beverly, MA, USA) were used in the Western blot assay, and peroxidase-conjugated anti-IgG (Abcam) was also used as the secondary antibody. Monoclonal β -actin or GAPDH antibody (Sigma) was used as the control. The blots were detected using an enhanced chemiluminescent kit (Pierce, Rockford, IL, USA). The data of Western blot bands were quantified by Image J software.

Tumorigenesis analysis

The nude mice were used to determine the tumorigenesis as described previously. Briefly, 4-week-old female BALB/c nude mice were subcutaneously injected with U373MG cells (10^5 cell in 200 μ L/mouse) transfected with shRMRP, shCtrl, miR-466 mimic or mimic control at a single site under their right front leg. After 2 weeks, the tumor sizes were measured every 3 days. After 5 tests (14, 17, 20, 23 and 26 days), the tumor volumes were calculated. The average weight of the excised tumor was measured at the time of sacrifice at 5 weeks after the initial injection.

All the procedures were approved by the Animal Management rule of the First Affiliated of Chengdu Medical College.

Statistical analysis

All experiments were carried out in triplicate, and the GraphPad Prism 5 software (GraphPad Software Inc., San Diego, CA, USA) was used for all statistical analyses. Data for each group of each experiment were presented as mean $\pm$ standard deviation (SD). The overall survival rate was calculated using the Kaplan–Meier method. Two-tailed Student's *t*-test or analysis of variance followed by Turkey's post hoc test was applied to analyze significance, and the differences were considered statistically significant at $P < 0.01$.

Results

The expression level of lncRNA RMRP is upregulated in human glioma

To investigate the correlation between RMRP and human glioma, RT-qPCR was used to detect the levels of RMRP in 58 pairs of human glioma tissue samples and corresponding non-tumor tissue samples. As shown in Fig. 1A, the expression of RMRP was clearly higher in human glioma tissues relative to non-tumor tissues (Fig. 1A). The data of overall survival rate also showed that human glioma patients with high RMRP level had worse survival than those with low RMRP level (Fig. 1B). In addition, the clinical data confirmed that the expression level of RMRP was significantly related to WHO grades, histological type but not with patient's age and gender (Table 1). Further, the increased expression levels of RMRP were also confirmed in human glioma cell lines A172, SHG44, U251, LN229 and the normal human astrocytes (NHAs) (Fig. 1C). Taken together, our findings indicate that RMRP expression is upregulated in human glioma, and closely related to the development of this cancer.

RMRP increases cell proliferation, reduces apoptosis of glioma cells and promotes cell cycle via regulating CDK4/Cyclin-D1 expression

To determine the relationship between RMRP and human glioma progression, the pcDNA-RMRP and shRMRP were transfected into the human glioma cell lines A172 and U251 (the levels of RMRP in these cell lines was higher than other cell lines in previous experiment) to cause overexpression or silencing of RMRP, respectively. In addition, the comparative analysis result is that the control data of pcDNA3.1 and shCtrl are taken as negative control. Analysis showed that, compared to the negative control, RMRP promoted cell viability and proliferation (Fig. 2A and B). In contrast, RMRP decreased the apoptosis of human glioma cells (Fig. 2C). Collectively, these data suggest that RMRP promotes cell proliferation and attenuates apoptosis in human glioma.

Because the RMRP effectively promoted human glioma cell proliferation, we then evaluated the effect of RMRP on the cell cycle of human glioma cells and confirmed this using flow cytometry. As shown in Fig. 2D, compared with cells transfected with shRMRP, pcDNA-RMRP significantly reduced the percentage of G1/G0 peak cells and increased the percentage of S peak cells, which suggested that RMRP may promote the cell proliferation by accelerating the G1/S cell cycle transition. And then, we investigated the mechanisms by which RMRP regulates the cell cycle. As we know, G1/S cell cycle transition is governed by cyclin D-CDK4/6 and cyclin E-CDK2 complexes [16, 17]. Therefore, we examined the effects of the RMRP on cyclin D-CDK4/6 and cyclin E-CDK2 expression. As shown in Fig. 2E and F, CDK4 and Cyclin-D1 protein expressions were upregulated in human glioma cells transfected with the pcDNA-RMRP compared with those cells transfected with shRMRP. Besides, CDK2 and Cyclin-E protein expression levels did not change much in pcDNA-RMRP-transfected cells compared with those in the control. These data revealed that RMRP could accelerate cell cycle by regulating cyclin D-CDK4 complex expression.

miR-466 is a direct target of RMRP

Further, we found that miR-466 was predicted as a direct target of RMRP via the DIANA tool (Fig. 3A). And that, the expression levels of miR-466 was decreased in various human glioma cell lines (Fig. 3B). The data of luciferase reporter assay showed that miR-466 mimic could direct interact with RMRP (Fig. 3C). Moreover, the expression levels of miR-466 in cells transfected with the pcDNA-RMRP or shRMRP was

examined by RT-qPCR. The data showed that miR-466 levels was obviously decreased by the pcDNA-RMRP but increased by the shRMRP (Fig. 3D). And that, the expression levels of RMRP also affect by the miR-466 mimic or miR-466 inhibitor (Fig. 3E).

To confirm the biological function of miR-466 targeting RMRP in the progression of human glioma, miR-466 mimics and pcDNA-RMRP were co-transfected into glioma cells and cell proliferation assay and flow cytometry were performed. As shown in Fig. 3F **and G**, miR-466 mimics reversed the reduction of cell viability and proliferation induced by overexpressing RMRP. Subsequently, miR-466 rescued the apoptosis induced by the overexpression of RMRP (Fig. 3H). Similarly, miR-23b mimics also largely reversed the block effects of RMRP on human glioma cell cycle (Fig. 3I). Taken together, these results further confirmed that RMRP could promote human glioma progression via targeting miR-466.

CXCR4 bound to miR-466, and promoted cell proliferation but reduced apoptosis to human glioma cells

Similar with the RMRP target to miR-466, miR-466 could target to CXCR4 via the DIANA tool (Fig. 4A). And that, the expression levels of CXCR4 was increased in various human glioma cell lines (Fig. 4B). The dual-luciferase reporter gene assay also confirmed this interaction between miR-466 and CXCR4 (Fig. 4C). After verifying the transfection efficacy of miR-466 mimic, transfection of miR-466 mimic remarkably downregulated mRNA and protein levels of CXCR4 (Fig. 4D). To confirm that CXCR4 regulates human glioma cell proliferation and apoptosis, we transfected pcDNA-CXCR4, shCXCR4, or a negative control into glioma cells and analyzed the cells using MTT, BrdU proliferation, flow cytometry assay, and cell cycle assay. Distinctly higher cell viability and proliferation, as well as clearly reduced apoptosis, and G1/S cell cycle transition were observed in the shCXCR4 group relative to the pcDNA-CXCR4 or control groups (Fig. 4E–H).

RMRP silencing or miR-466 mimic inhibits tumor growth in vivo

In order to determine the therapeutic potential of shRMRP or miR-466 mimic for the treatment of human glioma, nude mice were subcutaneously injected with U373MG cells that were successfully transfected with corresponding shRNA or miRNA mimic. Our data showed that the tumors were isolated from nude mice and measured grew more slowly in the shRMRP and miR-466 mimic group as compared to the control group (Fig. 5A–C). Notably, the tumor volume and size of mice injected with the miR-466 mimic transfected cells were smaller as compared to that of mice injected with the shRMRP-transfected cells (Fig. 5A–C). This result confirmed that RMRP could be potential for the therapeutic target for human glioma *in vivo*.

Discussion

The dysregulated expression of lncRNA may provide a favorable landscape for cell growth by affecting epigenetic information, leading to the uncontrolled growth of tumor cells, and thus to the occurrence and development of tumors. Moreover, effective control of cell survival and cell proliferation is the key to the prevention and treatment of cancer [18]. Therefore, identifying cancer-related lncRNAs and studying their clinical significance and molecular regulatory mechanisms may be able to fill a missing piece in the well-known mystery of carcinogenesis and tumor inhibition network. The RNA component of mitochondrial RNA processing endoribonuclease (RMRP), a lncRNA, was first discovered in cartilage-hair hypoplasia (CHH), an autosomal recessive inherited disease [19]. RMRP is primarily expressed in a variety of human tissues, and existed in the nucleus, nucleolus and mitochondria, which contributes to early stages of embryonic development [20, 21]. Recent study have found that RMRP silencing can significantly inhibit the proliferation and differentiation of gastric cancer cells *in vitro* and *in vivo*, indicating that it plays an important role in the occurrence and development of gastric cancer, and has potential as a biomarker and drug target for the diagnosis and treatment of gastric cancer [22]. Therefore, our study systematically investigated the regulatory networks and molecular mechanisms of RMRP in the development and progression of human glioma.

Subsequently, our data revealed that lncRNA RMRP promoted cell proliferation but reduced apoptosis in human glioma cells via targeting miR-466. Amounting evidence indicated that miR-466 was associated with tumor progression in various cancers. For example, Li and colleagues reported that miR-466 was downregulated in tumor tissues in prostate carcinoma patients, and overexpression of miR-466 resulted in decreased rates of cell migration and invasion [23]. Another study in hepatocellular carcinoma revealed that miR-466 was obviously decreased in tissues and cell lines, and restoration of miR-466 expression could inhibit the cell proliferation but induced apoptosis *in vitro*, as well as impeded xenograft tumor growth *in vivo* [13]. Consistent with these studies, our data also demonstrated that miR-466 was significantly decreased in glioma tissues and cells, while miR-466 overexpression suppressed cell viability, proliferation even tumor growth whereas promoted apoptotic rate in experiments *in vitro* and *in vivo*. These results confirmed that RMRP was negatively interacted with miR-466, contributed to the promotion effect on glioma progression.

In addition, several lncRNA have been described as regulators of cell proliferation and cell cycle. Most of the studies are related to lncRNA-induced cell cycle arrest, and these lncRNA have been confirmed to target multiple components of the cell cycle, including cyclin D1, cyclin E, CDK4, CDK2, and E2F3. However, the molecular mechanisms of lncRNA promoting cell cycle and eliciting G1/S transition have not been reported, and the biological relevance of such a lncRNA-mediated regulation of the cell cycle machinery *in vivo* remains unclear. CDK4 and CDK6 are activated by D-type cyclin complex formation that acts as a cell cycle growth sensor, whereas CDK2 binds to cyclin E or cyclin A, and also regulates G1-S phase [24]. In this study, our results found that lncRNA RMRP could induce G1/S cell cycle transition through cyclin D-CDK4/6 complex upregulation but not cyclin E-CDK2 complexes. Notably, most previous studies have observed that CDK4/6 and CDK2 are upregulated and downregulated respectively and simultaneously in the cell cycle [25, 26]. However, RMRP-promoted cell cycle only upregulated CDK4

expression, whereas CDK2 expression was unchanged. This may indicate that RMRP promotes cell proliferation via targeting CDK4, but the detailed mechanisms need to be further elucidated.

Further experiments showed that CXCR4 was a candidate of miR-46a. Previous studies manifested that the increased expression of CXCR4 was confirmed in many tumors. For example, CXCR4 has been reported that as an oncogene fulfill the function in cell proliferation, migration, as well as metastasis in breast cancer [27]. Also, the meta-analysis demonstrated that CXCR4 over-expression was highly correlated with poor survival in various types of cancers, including esophageal cancer, renal cancer, gynecologic cancer, and liver cancer [28]. Consistent with the previous documented, our data also revealed that CXCR4 was apparently increased in human glioma cell lines. Furthermore, the functional experiments indicated that CXCR4 overexpression partly counteracted the promotion impacts on cell proliferation as well as facilitate to G1/S cell cycle transition, but reduce the apoptosis, indicating the promoting role of CCT6A in glioma progression. These data implied that RMRP promoted human glioma progression via sponging miR-466 and positively regulating CXCR4 expression. However, the deficiency of our study is that we only evaluated RMRP and miR-466 as therapeutic targets for treating glioma *in vivo*, without the evaluation of CXCR4, since CXCR4 has been widely reported as a cancer therapeutic target [29, 30]. In future experimental design, we will design more and more detailed experiments to comprehensively evaluate the treatment potential of these targets for human glioma.

In conclusion, our study provides a new insight on the regulatory mechanisms of lncRNA RMRP on human glioma progression. Our data were first to highlight high RMRP expression levels in human glioma and demonstrate that RMRP promotes cell proliferation, G1/S cell cycle transition but inhibits apoptosis in human glioma by targeting miR-466/CXCR4 axis. Overall, our data revealed that RMRP and miR-466 may be the potential targets of human glioma treatment in the future.

Declarations

Compliance with Ethical Standards

Funding

Not applicable

Conflict of interest

The authors have completed the ICMJE uniform disclosure form, and confirmed that there is no conflict of interests.

Ethics approval

This study was approved by the Institutional Research Ethics Committee of the First Affiliated of Chengdu Medical College (IRB number: 20140332)

Informed consent

All participants provided written informed consent.

Author Contributions

Jie Zhang conceived and designed the experiments; Jie Zhang, Ling He, Xun Xia, Lie Zhang and Enren Wang performed the experiments and analyzed the data; Jie Zhang and Ling He wrote the manuscript.

Data availability statement

The authors confirm that all data underlying the findings are fully available without restriction. All relevant data are within the paper files.

Abbreviations

Long non-coding RNA, lncRNA; RNA component of mitochondrial RNA processing endoribonuclease, RMRP; cartilage-hair hypoplasia, CHH; microRNA-466, miR-466; normal human astrocytes, NHAs; Non-coding RNAs, ncRNAs.

Consent for publication

Not applicable.

Acknowledgements

Not applicable.

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Figures

Figure 1

The increased expression levels of lncRNA RMRP is determined in human glioma tissues. The expression levels of RMRP in 58 pairs of human glioma tissues and their adjacent, non-cancerous normal tissues (Normal) (A) and the human glioma cell lines A172, SHG44, U251, LN229 and the normal human astrocytes (NHAs) (C) are detected by qRT-PCR. U6 is used as the internal control. (C) Overall survival based on RMRP expression levels in glioma patients was represented by Kaplan–Meier survival analysis. All results were presented as the mean $\pm$ SD and experiments were conducted in triplicate. **P < 0.01 and ***P < 0.001.

Figure 2

lncRNA RMRP promotes proliferation, G1/S cell cycle transition and inhibits apoptosis in human glioma cells. (A) pcDNA-RMRP is transfected into A172 and U251 cells and induces an obviously higher proliferation rate than shRMRP transfected group and negative control group. (B) The significantly increased cell proliferation levels in A172 and U251 cells transfected with pcDNA-RMRP plasmid and the obviously down-regulated cell proliferation of shRMRP transfected group compared with that in the controls were observed by ELISA-BrdU assay. (C) pcDNA-RMRP or shRMRP is transfected into A172 and U251 cells and shRMRP triggers a significantly high apoptosis compared with controls. (D) The effects of pcDNA-RMRP or shRMRP transfection on cell cycle in A172 and U251 cells using flow cytometry. (E) and (F) The cyclin D-CDK4/6 and cyclin E-CDK2 complex protein expressions were determined by Western blotting using β -actin as an internal control. * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$.

Figure 3

miR-466 is a direct target of RMRP. (A) Bioinformatics-based target prediction analysis shows that a potential target of RMRP is called as miR-466. (B) The expression levels of miR-466 are markedly decreased in human glioma cell lines compared with those in NHAs. (C) Luciferase reporter assay shows that in the miR-466 mimic group, the significantly decreased luciferase activity driven by RMRP is observed compared with that in the negative control and RMRP-MUT group. (D) The expression levels of miR-466 were measured in A172 cells transfected with the pcDNA-RMRP or shRMRP. (E) The expression levels of RMRP were measured in A172 cells transfected with the miR-466 mimic or miR-466 inhibitor. MiR-466 reverses the biological function of RMRP on human glioma cell. (F) Cell MTT assay, (G) cell proliferation assay, (H) apoptosis assay and (I) cell cycle assay were performed in human glioma cells transfected with pcDNA-RMRP + miR-466 mimics, pcDNA-RMRP + mimic control, pcDNA3.1 + miR-466 mimics and pcDNA3.1 + mimic control. All results were presented as the mean $\pm$ SD and experiments were conducted in triplicate, ** $p < 0.01$ and *** $p < 0.001$.

Figure 4

CXCR4 is a direct target of miR-466. (A) Bioinformatics-based target prediction analysis shows that a potential target of miR-466 is called as CXCR4. (B) The expression levels of CXCR4 are markedly increased in human glioma cell lines compared with those in NHAs. (C) Luciferase reporter assay shows that in the miR-466 mimic group, the significantly decreased luciferase activity driven by CXCR4 is observed compared with that in the negative control and CXCR4-MUT group. (D) The expression levels of CXCR4 were measured in A172 cells transfected with the miR-466 mimic or miR-466 inhibitor. CXCR4 expression promotes cell proliferation and decreases apoptosis on human glioma cells. (E) An obviously higher cell viability rate is observed in the pcDNA-CXCR4 group than that transfected with shCXCR4 as control. (F) A significantly higher cell proliferation is observed in the pcDNA-CXCR4 group than control. (G) The significantly down-regulated apoptosis rate is detected in the pcDNA-CXCR4 group compared with control. (H) Cell cycle of various groups was detected using flow cytometry. Expression of CXCR4 could block cell cycle in S phase. ** $p < 0.01$.

Figure 5

RMRP knockdown and miR-466 expression suppresses the growth of human glioma cells in vivo. (A) Tumor volume after injection of U373MG cells transfected with shRMRP, shCtrl, miR-466 or mimic control. (B) After 2 weeks, the tumor volumes were calculated after 5 tests by measure the tumor sizes (day14, 17, 20, 23 and day 26. (C) The average weight of the tumor at 5 weeks after cell injection. ** $P < 0.05$.