Upregulation of miRNA-202 promotes apoptosis and inhibits migration of glioma cell line via downregulation of ROCK1 gene

Mousa Behzadi
Shaheed Beheshti University of Medical Sciences
Hamed Hatami
Mashhad University of Medical Sciences
Fatemeh Alian
University of Tehran
Maryam Shojaee
Payame Noor University
Masoumeh Alimohammadi
Tehran University of Medical Sciences
Shahin Alizadeh-Fanalou
Iran University of Medical Sciences
jafar karami (karami.j@tak.iums.ac.ir)
Iran University of Medical Sciences https://orcid.org/0000-0002-2554-1669

Research article

Keywords: Glioma, miR-202, ROCK1, Proliferation, Invasion.

Posted Date: March 10th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-16674/v1

Abstract

Background: We evaluated role(s) of miR-202 in glioma cell lines, its effect on ROCK1 expression, and also evaluation of apoptosis and migration of human glioma cell line after transfection with miR-202 mimics and inhibitors. Material and methods: The cell lines were transfected with mimic, inhibitor and NC of miR-202. Reverse transcription polymerase chain reaction (RT-PCR) was conducted to evaluate the expression of miR-202 and ROCK1 . Western blot was performed to detect the protein level of ROCK1. Furthermore, MTT and wound healing assay were performed to evaluate the effects of miR-202 on apoptosis and migration of human glioma cell line, respectively. Results: miR-202 showed a significantly decrease in human glioma cell lines, compared with the NHA cell line (P<0.05). The ROCK1 expression was significantly upregulated in glioma cell lines, compared with the NHA cell line (P <0.05). Furthermore, a negative correlation was observed between expression of ROCK1 and miR-202 (P =0.01, r=-0.426). The mRNA and protein levels of ROCK1 were decreased in U87 cell line in miR-202 mimics group, compared with mimic NC group (P<0.05). In addition, apoptosis was significantly increased in miR-202 mimics, compared with the NC group in U87 cell line at 72 and 96 h (P<0.05). Furthermore, invasion showed a significant decrease in miR-202 mimic group, compared with U87 cell line at 24 and 48 h (P<0.05). Conclusions: The miR-202 could serve as a tumour-suppressor miRNA in glioma. Therefore, targeting ROCK1 by miR-202 may increase improve disease outcome and could be considered as a potential therapeutic target for glioma patients.

Background

Glioma is an aggressive and a common type of malignant brain tumour [1], which is characterized with hypoxia, angiogenesis, invasiveness and high rates of recurrence [2–5]. The main treatments, which is currently used for glioma patients, are; radiation, chemotherapy, and surgical excision [6]. These types of treatments are not satisfactory for both the patients and physicians [7, 8]. Despite the considerable efforts and progress in glioma treatment, the average survival time of patients is less than a year [9, 10]. Therefore, in order to develop new and efficient strategies for treatment of glioma patients, we need to clarify and understand the cellular and molecular mechanisms of progression and tumourigenesis of glioma.

MicroRNAs (miRNAs) are endogenous, evolutionarily conserved and non-coding RNAs, which have ~ 19–22 nucleotides [11]. miRNAs through binding to 3' untranslated regions (3'UTRs) of the target mRNAs, negatively regulates gene expression by two different mechanisms 1) degradation of the target mRNA and 2) delay translation of the target mRNA [12]. Through decades of researches, it has been evident that miRNAs have been participated in the regulation of different biological processes such as angiogenesis, metastasis, invasion, apoptosis, cell division, cell cycle, and cell growth [13–16]. Owing to their important roles, investigations on these short RNAs revealed that they are implicated in various human diseases [17, 18]. The balance of miRNA expression is disturbed in many diseases, some of these miRNAs are upregulated, which are considered as a tumour promoters owing to their suppressive roles for tumour suppressor genes. On the other hand, some of these miRNAs act as tumour suppressors owing to their

roles in downregulation of oncogenes [19, 20]. These data illustrated that miRNAs could be considered as potential therapeutic targets for prognosis, diagnosis, and treatment.

Recently, investigations have shown that the miR-202 is downregulated and implicated in biological processes such as apoptosis and proliferation in various cancers including colorectal carcinoma [20], lung carcinoma [21], and gastric cancer [22]. However, data concerning about role of miR-202 in glioma is insufficient. We evaluated role(s) of miR-202 in glioma cell lines, its effect on ROCK1 expression, and also evaluation of apoptosis and migration of human glioma cell line after transfection with miR-202 mimics and inhibitors.

According to our work, we clarified that the miR-202 expression is downregulated in glioma cell lines and the ROCK1 could be the potential target of this miRNA. In addition, the related molecular mechanisms were investigated to reveal the exact role of this miRNA in pathogenesis of glioma. Based on our present study, this information may lead to introducing a new therapeutic marker for treatment of glioma patients.

Materials And Methods

Cell culture

Five human glioma cell lines (LN229, U373, A172, U251, and U87) were cultured in Dulbecco's modified Eagle medium (DMEM), which was supplemented with 10% heat inactivated fetal bovine serum (FBS) and 1% penicillin/streptomycin (all from Gibco, USA). The normal human astroglia (NHA) cell line was cultured in astrocyte medium with 10% FBS and 1% astrocyte growth supplement (all from Gibco, USA). The cell lines were incubated in a humidified atmosphere at 37˚C and 5% CO2. For subsequent investigations, cell lines were in their logarithmic growth phase. For transfection of cell lines, cell lines were implanted in 96-well plates with 3000 cells per well. Afterward, cells were transfected with mimic, inhibitor and NC of miR-202 by Lipofectamine 2000 (Invitrogen) according to the protocol.

Reverse Transcription-quantitative Polymerase Chain Reaction (rt-qpcr)

Total RNA from the six cell lines (LN229, U373, A172, U251, U87, and NHA) was extracted by TRIzol reagent (Invitrogen). After RNA extraction, purity and yield of total RNA were measured by NanoDrop spectrophotometer (NanoDrop ND-2000C Spectrophotometer, Thermo Fisher Scientific, USA). TaqMan miRNA reverse transcription kit and TaqMan miRNA PCR kit (both from Applied Biosystems, USA) were used for cDNA synthesis and quantify the miR-202 expression, respectively. PrimeScript RT reagent kit and SYBR® Premix Ex Taq (both from Takara, Japan) were used for reverse transcription and quantify the ROCK1 expression, respectively. Each PCR mixture contained a set of specific forward and reverse primers, SYBR® Green Master Mix (Applied Biosystems, USA) and cDNA. In order to perform the Real-time

PCR analyses, StepOnePlus™ instrument (Applied Biosystems) was applied. U6 and β-actin were used as internal controls for miR-202 and ROCK1 expression, respectively. The $2^{-\Delta\Delta Ct}$ method was applied to measure the fold changes [23].

Western Blot

To analysis protein level of ROCK1 in the glioma cell lines, western blot analysis was carried out. The glioma cell lines were transfected with mimic, inhibitor and NC of miR-202, then harvested and washed with ice-cold phosphatebuffered saline (PBS) after 48 h. The Radio Immunoprecipitation Assay (RIPA) buffer with protease inhibitors (Cell Signaling, USA) was used to lyse the cell lines and then, total proteins were extracted. The BCA Protein Assay Kit (Thermo Scientific, USA) was used to measure the protein level. Sodium dodecyl sulfatepolyacrylamide gel electrophoresis (SDS-PAGE) was applied to separate protein extracts and transferred to polyvinylidene fluoride (PVDF) membranes (Millipore, USA). In order to block free spaces, 5% skim milk in Tris-buffered saline with Tween (TBST) was used (room temperature, 1 h). Then, the membrane was incubated with primary antibodies (overnight, 4˚C). The primary antibodies were mouse anti-human monoclonal GAPDH (1:1,000 dilution; sc-137179) and mouse anti-human monoclonal ROCK1 (1:1,000 dilution; sc-517220) (all from Santa Cruz Biotechnology, Santa Cruz, CA, USA). After washing, the membrane was incubated with goat anti-mouse horseradish peroxidase (HRP)-conjugated secondary antibody (1:4,000 dilution; Santa Cruz Biotechnology) (room temperature, 1 h). To visualize protein bands enhanced chemiluminescence detection kit (ECL, Amersham) was used. ImageJ software was used to semi quantify the results and β-actin was used as internal control to normalize protein level of ROCK1.

Mtt Assay

Cells (3000 cells per well) were seeded into a 96-well plate with 100 µl of DMEM. After medium replacement, cells were treated with mimic, inhibitor and NC of miR-202 for 24, 48, 72, and 96 h. After transfection, MTT assay solution (20 µL) was added into each well and incubated at 37 °C for 4 h. The medium was removed and dimethyl sulfoxide (DMSO) solutions (100 µL) was added into each well. The plate was covered and placed on shaker for 15 min. To evaluate the optical density (OD), a microplate reader (Bio-Tek, USA) with wavelength of 490 nm was used. All experiments were triplicate.

Scratch–wound healing migration assays

The U87 cell line was seeded into a 6-well plate. When confluency reached 80%, a 200-µL pipette tip was used to scratch across the center. Then, the medium was replaced with an FBS-free DMEM. To analysis migration capability of the cell line, pictures were taken at the time of wounding (0 h) and after 48 h. We used the Image J Software to measure migration capability of the cell line. All the experiments were triplicate and the mean values were reported.

Statistical analysis

The SPSS software (version 25.0, SPSS Inc.) was used to analysis data and the GraphPad Prism software (Version 8.0 for Windows) was used to generate graphs. According to normality test (KS), our data was normally distributed. In order to compare the results between two groups, the Student t-test was carried out and in cases to compare the results among multiple groups, the one-way Analysis of Variance (ANOVA) following post hoc Bonferroni test was operated. The Spearman's rank correlation coefficient test was operated to measure the correlation between ROCK1 and miR-202 levels of expression. P values less than 0.05 ($P < 0.05$) were considered statistically significant. Data are shown as mean ± standard deviation (S.D.).

Results

Targeting ROCK1 by miR-202 in glioma cell lines

miRNA targets prediction databases such as Targetscan, mirWalk, and miRanda were searched to identify the miR-202 targets. The bioinformatic analysis showed highly conserved binding sites in ROCK1 for miR-202. Firstly, the miR-202 expression in glioma cell lines (LN229, U373, A172, U251, and U87) and the NHA cell line was assessed and our results illustrated that the expression of miR-202 is significantly downregulated in glioma cell lines, compared with the NHA cell line ($P < 0.05$) (Fig. 1). Furthermore, relative expression of ROCK1 was evaluated in glioma and the NHA cell lines. ROCK1 expression was significantly increased in glioma cell lines, compared with the NHA cell line ($P < 0.05$) (Fig. 2). On one hand, the miR-202 expression was downregulated and on other hand, the ROCK1 expression was upregulated in glioma cell lines in comparison to the NHA cell line. The correlation between miR-202 and ROCK1 mRNA expression was analyzed in U87 cell line using Spearman's correlation analysis and the result showed a negative correlation ($P = 0.01$, $r=-0.426$) (Fig. 3). The U87glioma cell lines was transfected with mimic, inhibitor and NC of miR-202. After transfection, total mRNA and protein were extracted from the cell line and qRT-PCR and western blot assay were performed to measure the expression and protein level of ROCK1, respectively. We documented that the miR-202 may modulate the ROCK1 at transcriptional and translational level in U87glioma cell line. We illustrated that the miR-202 upregulation, miR-202 mimic group, could significantly decrease both the protein and mRNA levels of ROCK1 (Figs. 4 and 5). Nonetheless, downregulation of miR-202, miR-202 inhibitor group, could not significantly increase both the protein and mRNA levels of ROCK1 in U87 glioma cell lines. Indeed, our data showed that the ROCK1 could be a potential target of miR-202 and also, the miR-202 regulate the ROCK1 in the human glioma cell line at mRNA and protein level.

Overexpression Of Mir-202 And Apoptosis

The MTT assay was performed to evaluate cell viability. For this purpose, U87 glioma cell line was transfected by mimic, inhibitor and NC of miR-202. Our data revealed that the mimic group, low level of

ROCK1, showed significantly higher apoptosis than the miR-202 NC groups at 72 and 96 h ($P < 0.05$) (Fig. 6). Indeed, downregulation of miR-202 could be associated with high level of ROCK1, which is linked with low rate of apoptosis in cancerous cells. The bottom line is that high level of miR-202 may reduce the ROCK1 expression and leads to apoptosis in cancerous cells such as human glioma cell lines.

Upregulation of miR-202 and migration capability of U87 cell line

The scratch test was carried out to evaluate role of miR-202 in migration capability of the U87 glioma cell line. For this purpose, the U87 cell line was transfected with mimic, inhibitor and NC of miR-202 and healing rate in different groups was measured after 24 h and 48 h of transfection. Our results revealed that the healing rate was significantly suppressed in the miR-202 mimic group in comparison to the miR-202 NC group ($P < 0.05$) (Fig. 7). Briefly, the closed area in miR-202 mimic group was 25% and 35% after 24 and 48 h, respectively. In miR-202 NC group, the closed area was 37% and 53% after 24 and 48 h, respectively. In miR-202 inhibitor group, the closed area was 42% and 59% after 24 and 48 h, respectively. Collectively, our data showed that migration capability of U87 cell line may be influenced by miR-202 level. Low level of miR-202 expression may lead to upregulation of ROCK1 and eventually causes invasion and migration of U87 cell line. The other side of the coin, high level of miR-202 could possibly downregulate the ROCK1 and migration and invasion of these cells could be suppressed.

Discussion

Many researchers are trying to clarify the important molecular mechanisms that are implicated in glioma pathogenesis. Through recent years, various genes and miRNAs are introduced to be participated in glioma tumorigenesis. From decades up to know, miRNAs are considered as an important biomarker, which are dysregulated in many types of disorders including glioma [24–26]. Change in expression of miRNAs is associated with various malignant biological behaviors in glioma such as cell survival, metastasis, proliferation, resistance to chemotherapy and radiation [11, 27].

It has been reported that the miR-202 is dysregulated in many cancers. There are reports, which has been shown that the expression of miR-202 is downregulated in cervical cancer [28], lung cancer [21], hepatocellular carcinoma [29], colorectal cancer [20], and multiple myeloma [30]. In oesophageal squamous cell carcinoma, the miR-202 showed low level of expression in tumour tissues and its expression was significantly correlated (negative correlation) with lymph node metastasis and cell differentiation [31]. It is documented that the miR-202 expression in gastric cancer is also downregulated in tumour tissues and showed an inverse correlation with age and tumour size [22]. In osteosarcoma, miR-202 was downregulated and upregulation of miR-202 increased apoptosis and decreased cell growth [32]. Another report in cervical cancer showed that overexpression of miR-202 could suppress metastasis and cell growth in cervical cancer cell line [28]. In lung cancer, restoration of miR-202 increased apoptosis and G0/G1 cell cycle arrest and suppressed cell proliferation in lung cancer cell line [21]. Studies on

oesophageal squamous cell carcinoma showed that upregulation of miR-202 could suppresses invasion, migration, proliferation and also, increase cell apoptosis [31, 33]. Together, these findings clarify that the miR-202 could be introduced as an important biomarker in these cancers and may serve as a potential therapeutic target.

ROCK1 is a member of the Rho-associated serine/ threonine kinase family and play an important role in cell movement through reorganization of actin cytoskeleton [34]. It has been reported that ROCK1 is overexpressed in different cancers including prostate cancer [35], osteosarcoma [36], and gastric cancer [37]. ROCK1 gene is implicated in metastasis, invasion, and migration of cancer cells and could be considered as an oncogene [37]. Recent researches reported that the upregulation of ROCK1 is associated with poor survival in various cancers [38, 39].

To clarify miR-202 effects in glioma, direct targets of miR-202 were evaluated through bioinformatic databases. Among potential targets of miR-202, the ROCK1 gene was selected for further evaluation owing to its upregulation in glioma. Moreover, it was documented that this gene is implicated in progression and tumourigenesis of glioma [40–42].

Our study revealed that the miR-202 is significantly downregulated in human glioma cell lines in comparison to the NHA cell line (Fig. 1). The ROCK1 expression is significantly upregulated in human glioma cell lines in comparison to the NHA cell line (Fig. 2). Indeed, the miR-202 level was decreased and the ROCK1 level was increased in glioma cell lines, compared to the NHA cell line. Furthermore, there was a negative correlation between expression level of miR-202 and ROCK1 (Fig. 3), which makes the ROCK1 as a possible target for miR-202 in glioma. Besides, the online bioinformatic databases showed that the ROCK1 could be one of miR-202 target. We further analysed mRNA and protein levels of ROCK1 in U87 glioma cell lines after transfection with mimic, inhibitor and NC of miR-202. We documented that the miR-202 may modulate the ROCK1 at transcriptional and translational level in the glioma cell line. We illustrated that miR-202 upregulation could significantly decrease both the protein and mRNA levels of ROCK1 (Figs. 4 and 5). Indeed, our data showed that the ROCK1 could be a potential target of miR-202.

We reported that apoptosis rate could be elevated via downregulation of ROCK1 through miR-202 upregulation, miR-202 mimic group, in U87 glioma cell line (Fig. 6). Indeed, apoptosis was significantly increased in miR-202 mimic group in comparison to the miR-NC group in U87 glioma cell lines. Since the miR-202 expression was low in glioma cell lines, the miR-202 inhibitor could not increase the ROCK1 expression significantly in the U87 glioma cell line. Together, ROCK1 suppression through high level of miR-202, miR-202 mimic group, could increase the apoptosis in glioma.

We showed that migration could be suppressed in situation with high level of miR-202, the mimic group. Upregulation of miR-202 was associated with downregulation of ROCK1 and since the ROCK1 plays an important role in invasion and migration of glioma, then its downregulation leads to suppression of invasion and metastasis in the U87 cell line. In the miR-202 inhibitory group, migration capability was increased in comparison to the miR-202 NC group but, difference was not significant. The miR-202 level

is low in glioma cell line and the inhibitory miR-202 did not make any differences. These results proposed that the miR-202 effects in glioma likely exerted through negative controlling of ROCK1.

Generally, the miR-202 expression was low in glioma cell lines, which makes the miR-202 as a tumour-suppressor miRNA in glioma. High level of miR-202 expression in glioma cell lines led to high apoptosis, low proliferation rate and also suppressed invasion and migration capability in glioma through negative regulation of ROCK1. The role of ROCK1 gene in glioma is known and served as an oncogene in glioma. Therefore, targeting ROCK1 by miR-202 may increase survival, improve disease outcome and could be considered as a potential therapeutic target for glioma patients.

Declarations

Ethics approval and consent to participate: Not applicable.

Consent to publish: All authors read the manuscript and consent for its publication.

Availability of data and materials: Not applicable.

Competing interests: The authors declare that they have no competing interests.

Funding: Not applicable

Authors' contributions: Jafar Karami and Shahin Alizadeh-Fanalou designed the study and developed the main idea. Mousa Behzadia and Hamed Hatami performed the practical experiments. Jafar Karami performed the statistical analysis and approved the manuscript drafting. Fatemeh Alian, Maryam Shojaee, and Masoumeh Alimohammadi participated in manuscript drafting. All authors have read and approved the manuscript.

Acknowledgements: The authors are grateful of Deputy of Research from Iran University of Medical Science.

References

1. Zhang C, Bao Z, Zhang W, Jiang T: **Progress on molecular biomarkers and classification of malignant gliomas**. *Frontiers of medicine* 2013, **7**(2):150-156.
2. Vaupel P: **Hypoxia and aggressive tumor phenotype: implications for therapy and prognosis**. *The oncologist* 2008, **13**(Supplement 3):21-26.
3. McNamara MG, Mason WP: **Antiangiogenic therapies in glioblastoma multiforme**. *Expert review of anticancer therapy* 2012, **12**(5):643-654.
4. Zhou M, Wang H, Zhou K, Luo X, Pan X, Shi B, Jiang H, Zhang J, Li K, Wang H-M: **A novel EGFR isoform confers increased invasiveness to cancer cells**. *Cancer research* 2013, **73**(23):7056-7067.

5. Katakowski M, Buller B, Wang X, Rogers T, Chopp M: **Functional microRNA is transferred between glioma cells**. *Cancer research* 2010, **70**(21):8259-8263.
6. Khan UA, Bhavsar A, Asif H, Karabatsou K, Leggate JR, Sofat A, Kamaly-Asl ID: **Treatment by specialist surgical neurooncologists improves survival times for patients with malignant glioma**. *Journal of neurosurgery* 2015, **122**(2):297-302.
7. Omuro A, DeAngelis LM: **Glioblastoma and other malignant gliomas: a clinical review**. *Jama* 2013, **310**(17):1842-1850.
8. Van Meir EG, Hadjipanayis CG, Norden AD, Shu HK, Wen PY, Olson JJ: **Exciting new advances in neuro-oncology: the avenue to a cure for malignant glioma**. *CA: a cancer journal for clinicians* 2010, **60**(3):166-193.
9. Davis FG, McCarthy BJ: **Current epidemiological trends and surveillance issues in brain tumors**. *Expert review of anticancer therapy* 2001, **1**(3):395-401.
10. Clarke J, Butowski N, Chang S: **Recent advances in therapy for glioblastoma**. *Archives of neurology* 2010, **67**(3):279-283.
11. Bartel DP: **MicroRNAs: genomics, biogenesis, mechanism, and function**. *cell* 2004, **116**(2):281-297.
12. Lee YS, Dutta A: **MicroRNAs in cancer**. *Annual Review of Pathological Mechanical Disease* 2009, **4**:199-227.
13. Cellini F, Morganti AG, Genovesi D, Silvestris N, Valentini V: **Role of microRNA in response to ionizing radiations: evidences and potential impact on clinical practice for radiotherapy**. *Molecules* 2014, **19**(4):5379-5401.
14. Magee P, Shi L, Garofalo M: **Role of microRNAs in chemoresistance**. *Annals of translational medicine* 2015, **3**(21).
15. Miska EA: **How microRNAs control cell division, differentiation and death**. *Current opinion in genetics & development* 2005, **15**(5):563-568.
16. Jovanovic M, Hengartner M: **miRNAs and apoptosis: RNAs to die for**. *Oncogene* 2006, **25**(46):6176.
17. Aslani S, Mahmoudi M, Karami J, Jamshidi AR, Malekshahi Z, Nicknam MH: **Epigenetic alterations underlying autoimmune diseases**. *Autoimmunity* 2016, **49**(2):69-83.
18. Aslani S, Jafari N, Javan MR, Karami J, Ahmadi M, Jafarnejad M: **Epigenetic modifications and therapy in multiple sclerosis**. *Neuromolecular medicine* 2017, **19**(1):11-23.
19. Josson S, Gururajan M, Hu P, Shao C, Chu GC-Y, Zhau HE, Liu C, Lao K, Lu C-L, Lu Y-T: **miR-409-3p/-5p promotes tumorigenesis, epithelial-to-mesenchymal transition, and bone metastasis of human prostate cancer**. *Clinical cancer research* 2014, **20**(17):4636-4646.
20. Wang Q, Huang Z, Guo W, Ni S, Xiao X, Wang L, Huang D, Tan C, Xu Q, Zha R: **microRNA-202-3p inhibits cell proliferation by targeting ADP-ribosylation factor-like 5A in human colorectal carcinoma**. *Clinical Cancer Research* 2014, **20**(5):1146-1157.
21. Jiang J, Huang J, Wang X, Quan Y: **MicroRNA-202 induces cell cycle arrest and apoptosis in lung cancer cells through targeting cyclin D1**. *Eur Rev Med Pharmacol Sci* 2016, **20**(11):2278-2284.

22. Zhao Y, Li C, Wang M, Su L, Qu Y, Li J, Yu B, Yan M, Yu Y, Liu B: **Decrease of miR-202-3p expression, a novel tumor suppressor, in gastric cancer**. *PloS one* 2013, **8**(7):e69756.
23. Livak KJ, Schmittgen TD: **Analysis of relative gene expression data using real-time quantitative PCR and the 2− ΔΔCT method**. *methods* 2001, **25**(4):402-408.
24. Gu J-J, Gao G-Z, Zhang S-M: **MiR-218 inhibits the tumorgenesis and proliferation of glioma cells by targeting Robo1**. *Cancer Biomarkers* 2016, **16**(3):309-317.
25. Liu H, Song Z, Liao D, Zhang T, Liu F, Zheng W, Luo K, Yang L: **miR-503 inhibits cell proliferation and invasion in glioma by targeting L1CAM**. *International journal of clinical and experimental medicine* 2015, **8**(10):18441.
26. Stojcheva N, Schechtmann G, Sass S, Roth P, Florea A-M, Stefanski A, Stühler K, Wolter M, Müller NS, Theis FJ: **MicroRNA-138 promotes acquired alkylator resistance in glioblastoma by targeting the Bcl-2-interacting mediator BIM**. *Oncotarget* 2016, **7**(11):12937.
27. Wang Z-y, Xiong J, Zhang S-s, Wang J-j, Gong Z-j, Dai M-h: **Up-regulation of microRNA-183 promotes cell proliferation and invasion in glioma by directly targeting NEFL**. *Cellular and molecular neurobiology* 2016, **36**(8):1303-1310.
28. Yi Y, Li H, Lv Q, Wu K, Zhang W, Zhang J, Zhu D, Liu Q, Zhang W: **miR-202 inhibits the progression of human cervical cancer through inhibition of cyclin D1**. *Oncotarget* 2016, **7**(44):72067.
29. Zhang Y, Zheng D, Xiong Y, Xue C, Chen G, Yan B, Ye Q: **miR-202 suppresses cell proliferation in human hepatocellular carcinoma by downregulating LRP6 post-transcriptionally**. *FEBS letters* 2014, **588**(10):1913-1920.
30. Yu J, Qiu X, Shen X, Shi W, Wu X, Gu G, Zhu B, Ju S: **miR-202 expression concentration and its clinical significance in the serum of multiple myeloma patients**. *Annals of clinical biochemistry* 2014, **51**(5):543-549.
31. Ma G, Zhang F, Dong X, Wang X, Ren Y: **Low expression of microRNA-202 is associated with the metastasis of esophageal squamous cell carcinoma**. *Experimental and therapeutic medicine* 2016, **11**(3):951-956.
32. Sun Z, Zhang T, Hong H, Liu Q, Zhang H: **miR-202 suppresses proliferation and induces apoptosis of osteosarcoma cells by downregulating Gli2**. *Molecular and cellular biochemistry* 2014, **397**(1-2):277-283.
33. Meng X, Chen X, Lu P, Ma W, Yue D, Song L, Fan Q: **MicroRNA-202 inhibits tumor progression by targeting LAMA1 in esophageal squamous cell carcinoma**. *Biochemical and biophysical research communications* 2016, **473**(4):821-827.
34. Wen X, Ding L, Wang J-J, Qi M, Hammonds J, Chu H, Chen X, Hunter E, Spearman P: **ROCK1 and LIM kinase modulate retrovirus particle release and cell-cell transmission events**. *Journal of virology* 2014, **88**(12):6906-6921.
35. Xu B, Huang Y, Niu X, Tao T, Jiang L, Tong N, Chen S, Liu N, Zhu W, Chen M: **Hsa-miR-146a-5p modulates androgen-independent prostate cancer cells apoptosis by targeting ROCK1**. *The Prostate* 2015, **75**(16):1896-1903.

36. Li CH, Yu TB, Qiu HW, Zhao X, Zhou CL, Qi C: **miR-150 is downregulated in osteosarcoma and suppresses cell proliferation, migration and invasion by targeting ROCK1**. *Oncology letters* 2017, **13**(4):2191-2197.
37. Kim Y-I, Cho S-J, Shin J-Y, Kook M-C, Ryu KW, Kim Y-W, Choi IJ: **943 MicroRNA 135a Suppresses Lymph Node Metastasis Through Down-Regulation of ROCK1 in Early Gastric Cancer**. *Gastroenterology* 2014, **146**(5):S-164.
38. Zhou F, Li Y, Hao Z, Liu X, Chen L, Cao Y, Liang Z, Yuan F, Liu J, Wang J: **MicroRNA-300 inhibited glioblastoma progression through ROCK1**. *Oncotarget* 2016, **7**(24):36529.
39. Luo D, Chen H, Li X, Lu P, Long M, Peng X, Lin S, Tan L, Zhu Y, Ouyang N: **Activation of the ROCK1/MMP-9 pathway is associated with the invasion and poor prognosis in papillary thyroid carcinoma**. *International journal of oncology* 2017, **51**(4):1209-1218.
40. Hu B, Emdad L, Bacolod MD, Kegelman TP, Shen X-N, Alzubi MA, Das SK, Sarkar D, Fisher PB: **Astrocyte elevated gene-1 interacts with Akt isoform 2 to control glioma growth, survival, and pathogenesis**. *Cancer research* 2014, **74**(24):7321-7332.
41. Emdad L, Sarkar D, Lee S-G, Su ZZ, Yoo BK, Dash R, Yacoub A, Fuller CE, Shah K, Dent P: **Astrocyte elevated gene-1: a novel target for human glioma therapy**. *Molecular cancer therapeutics* 2010, **9**(1):79-88.
42. He Z, He M, Wang C, Xu B, Tong L, He J, Sun B, Wei L, Chu M: **Prognostic significance of astrocyte elevated gene-1 in human astrocytomas**. *International journal of clinical and experimental pathology* 2014, **7**(8):5038.

Figures

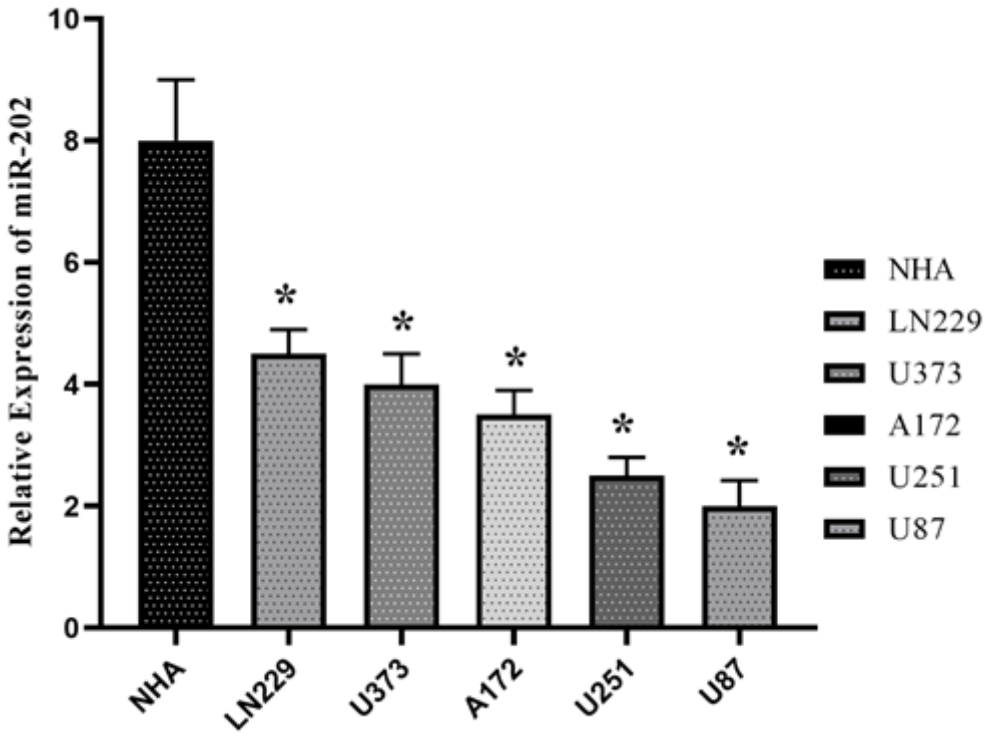

Figure 1

Relative expression of miR-202 was evaluated in glioma cell lines (LN229, U373, A172, U251, and U87) and normal human astrocytes (NHA) cell line by Real-time PCR. miR-202 expression was significantly decreased in glioma cell lines, compared with the NHA cell line. Data are represented as mean ± SD. *P<0.05.

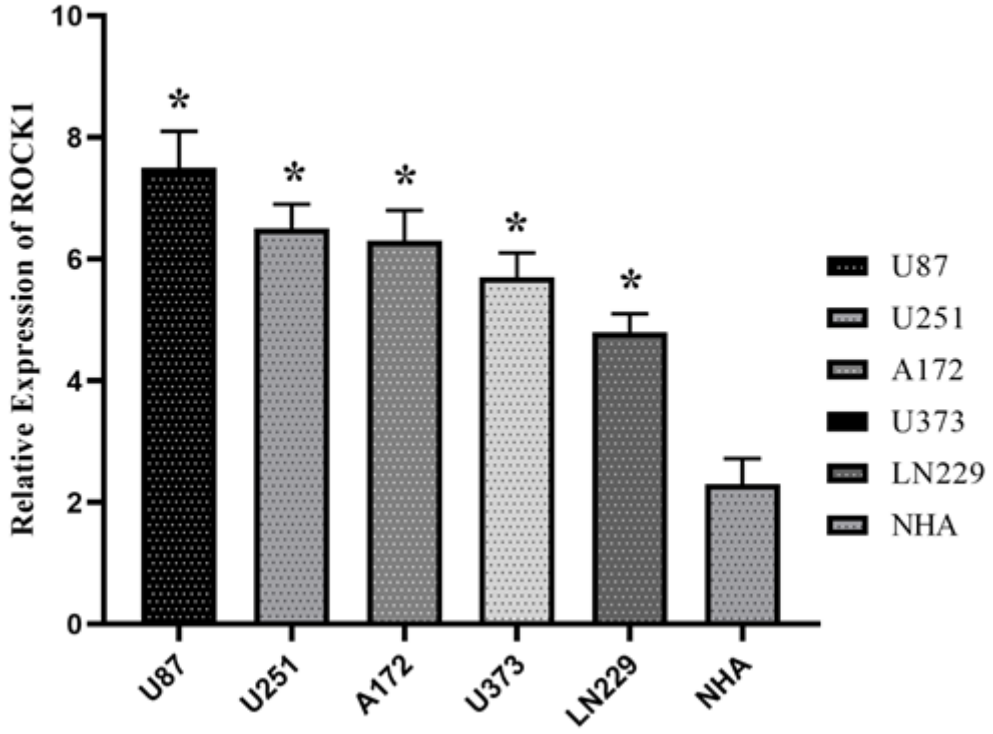

Figure 2

Relative expression of ROCK1 was measured in glioma cell lines (LN229, U373, U251, U87, and A172) and normal human astrocytes (NHA) cell line by Real-time PCR. ROCK1 expression was significantly increased in glioma cell lines, compared with NHA cell line. Data are represented as mean ± SD. *P<0.05.

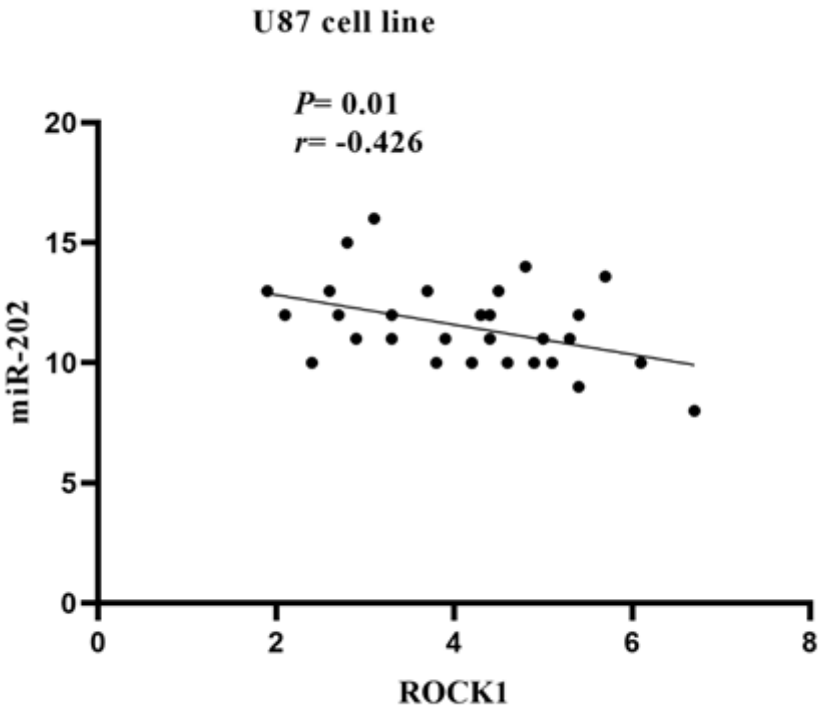

Figure 3

The correlation between miR-202 and ROCK1 mRNA expression was analyzed in U87 cell line using Spearman's correlation analysis and the result showed a negative correlation. (P=0.01, r=-0.426).

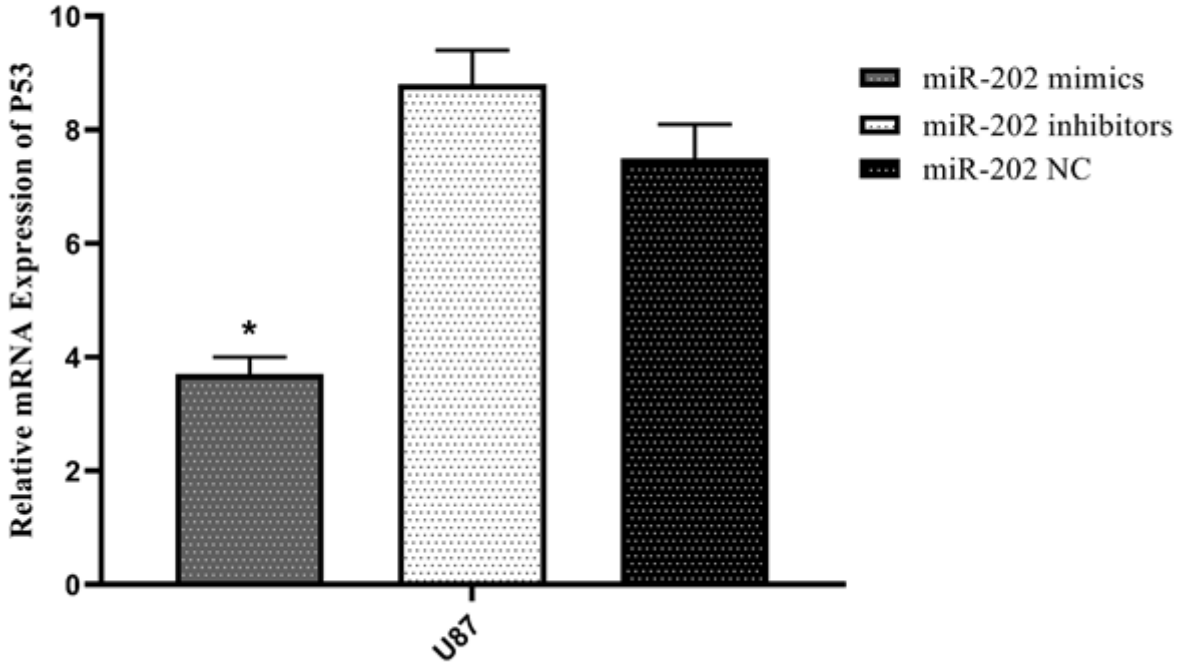

Figure 4

The impact of miR-202 on mRNA level of ROCK1 in U87 glioma cell lines. Regulatory effect of miR-202 on ROCK1 mRNA in the glioma cell line was detected by RT-qPCR. The mRNA expression of ROCK1 was significantly decreased in miR-202 mimics group, compared with miR-202 NC group at 24 h in U87 cell line (P<0.05). Data are represented as mean ± SD. *P<0.05, NC; negative control, miR; microRNA.

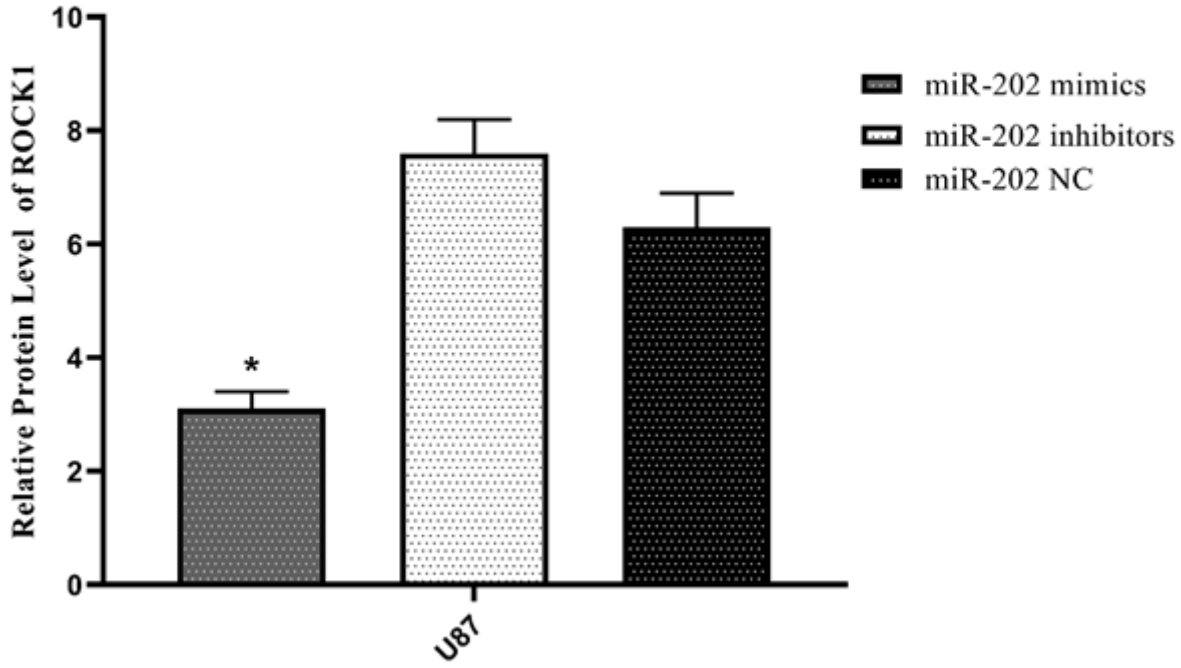

Figure 5

The impact of miR-202 on protein level of ROCK1 in U87 human glioma cell line. Regulatory effect of miR-202 on ROCK1 protein in U87 human glioma cell line was detected by Western blot. The protein level of ROCK1 was significantly decreased in miR-202 mimics group, compared with miR-202 NC group in U87 cell lines at 48 h (P<0.05). Data are represented as mean ± SD. *P<0.05, NC; negative control, miR; microRNA.

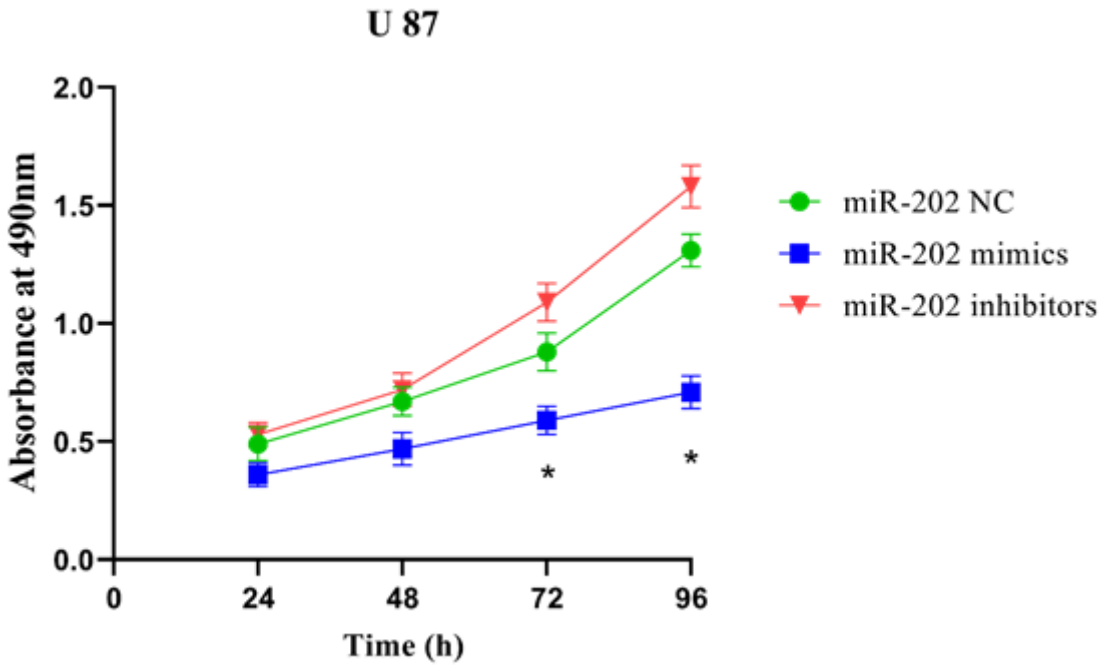

Figure 6

High level of miR-202 increased apoptosis in U87 glioma cell line. The effect of miR-202 on apoptosis of U87 glioma cell line was evaluated through MTT assay. Upregulation of miR-202 significantly increased apoptosis in miR-202 mimics group, compared with the NC group in U87 cell line at 72 and 96 h (P<0.05). Data are presented as the mean ± SD. *P<0.05, NC; negative control, miR; microRNA.

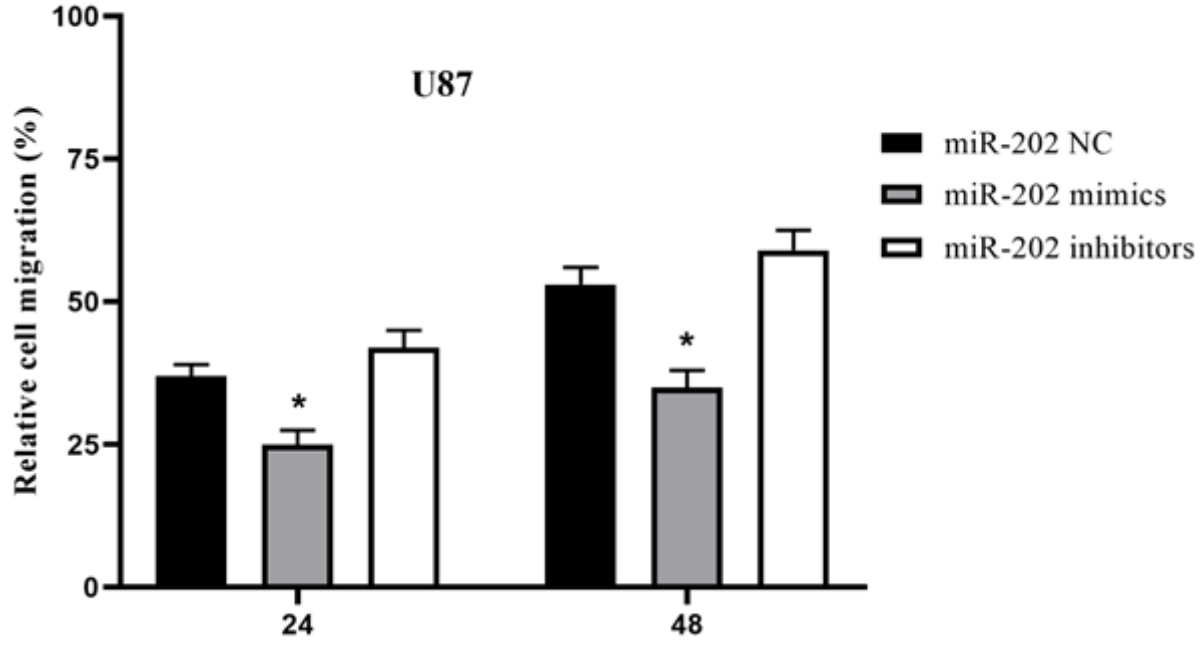

Figure 7

miR-202 decreased migration of the U87 glioma cell line. The effect of miR-202 on migration of U87 glioma cell line was evaluated through scratch–wound healing migration assay. Upregulation of miR-202 significantly decreased migration ability in miR-202 mimics group, compared with the NC group in U87 cell line at 24 and 48 h ($P<0.05$). Quantification of cell migration was conducted through measuring the wound width. The number of migrating cells was displayed as the percent of migrating cells at 24 and 48 h. Data are presented as mean ± SD. *$P<0.05$, NC, negative control; miR, microRNA.