European Review for Medical and Pharmacological Sciences 2018; 22: 8399-8405

Low expression of miR-1231 in patients with glioma and its prognostic significance

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Abstract. – OBJECTIVE: MiR-1231 has been reported to be down-regulated in glioma tissues and to act as a negative regulator in glioma progression. However, the clinical significance of miR-1231 remains unclear. In this study, we aimed to further demonstrate the expression pattern and prognostic value of miR-1231 in glioma patients.

PATIENTS AND METHODS: We determined the expression level of miR-1231 in 154 cases of paired glioma and adjacent non-tumor tissues by quantitative Real Time-PCR (qRT-PCR). The association between miR-1231 expression levels and clinicopathological factors was examined by the χ^2 test. The Kaplan-Meier survival analysis was performed to analyze the association of miR-1231 expression with overall survival (OS) and progression-free survival (PFS) of patients. The significance of survival variables was analyzed using the Cox multivariate proportional hazards model.

RESULTS: We found that the expression level of miR-1231 in human glioma tissues was significantly lower than that in the adjacent nontumorous tissues ($p<0.01$). The expression levels of miR-1231 in glioma tissues with high grades were significantly lower than those with low grades. Decreased miR-1231 expression was significantly associated with advanced WHO grade ($p=0.001$) and KPS score ($p=0.023$). The Kaplan-Meier analysis indicated that low miR-1231 expression had a significant impact on OS ($p=0.0103$) and PFS ($p=0.0019$). Cox proportional hazards risk analysis demonstrated that miR-1231 was an independent prognostic factor for glioma.

CONCLUSIONS: Our study, for the first time, provides evidence that evaluating miR-1231 in glioma may have prognostic and predictive value in the clinical management of glioma.

Key Words
MiR-1231, Glioma, Prognosis.

Introduction

Glioma is one of the most common types of primary brain tumor in the central nervous system and represents highly lethal tumors associated with significant morbidity and mortality[1,2]. Gliomas are subdivided into four stages with a growing level of malignancy according to the World Health Organization (WHO) classification[3]. Although remarkable advances have been made in the management of glioma, the prognosis for patients with high-grade gliomas remains poor, with the 5-year survival rate lower than 10%[4,5]. Treatment effect for glioma is hampered due to the infiltrative growth and inherent resistance to both chemotherapy and radiotherapy[6]. Moreover, similar histological features of glioma may exhibit different clinical characteristics and response to therapy[7]. Therefore, it poses an emergency for us to identify a new potential biomarker for early diagnosis, accurate prognosis prediction and novel therapeutic target. MicroRNAs (miRNAs) are a group of non-coding, single-stranded RNAs that are approximately 22 nucleotides in length[8]. These small molecules have been found to inhibit gene expression by binding to target mRNAs at their 3'-untranslated region[9]. Since one miRNA targets many mRNAs, miRNAs have been found to participate in various biological processes, including cell growth, development, proliferation and metabolism[10,11]. Growing studies[12,13] show that miRNAs affect cancer initiation, promotion and progression, represent potential diagnostic markers and therapeutic targets. Other works have also reported that miRNAs such as miR-135a[14], miR-616[15], miR-130a[16] and miR-205[17] serve as oncogenes or tumor suppressors in glioma. However, a large number of miRNAs remains to be identified. Recently, a newly identified miRNA, miR-1231, attracted our attention. Unlike other well-studied

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miRNAs, only a few researches were reported about the expression and effect of miR-1231 in diseases, including cancers[18,19]. Recently, Zhang et al[20] reported that the miR-1231 expression was significantly up-regulated in glioma and serve as a tumor suppressor, indicating that miR-1231 had the potential to act as a diagnostic biomarker and treatment target. However, the clinical significance of miR-1231 in glioma has not been investigated. In this study, we further confirmed the expression pattern of miR-1231 in glioma and explored its prognostic value in glioma patients.

Patients and Methods

Patients and Clinical Samples

Tumorous and normal tissues were collected from 154 glioma patients during the surgery from March 2008 to December 2011 at the Shenzhen People's Hospital, The Second Clinical Medical College of Jinan University. All of the samples were reassessed by two pathologists. According to the WHO 2007 classification, the glioma specimens were classified into four grades, and then they were divided into a low-grade glioma group (WHO I-II) and a high-grade glioma group (WHO III-IV). All samples were frozen in liquid nitrogen immediately after surgery until the total RNA extraction, and were stored at -80 °C until use. No patients had received any therapy before surgery. The clinicopathological features of patients were summarized in Table I. Written informed consent was obtained from all patients, and the study was approved by the Institutional Review Board of Shenzhen People's Hospital, The Second Clinical Medical College of Jinan University.

RNA Extraction and qRT-PCR Analysis

Total RNA from glioma tissues and matched normal brain tissues was extracted using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. The isolated total RNA was reversed transcribed into cDNA using SuperScript Reverse Transcriptase II (Invitrogen, Carlsbad, CA, USA). The quantitative Real Time-Polymerase Chain Reaction (qRT-PCR) was performed using the SYBR Select Master Mix (Applied Biosystems, Foster City, CA, USA) on ABI 7500 system (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's instructions. The results were normalized to the expression of glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The PCR primers for miR-1231 or GAPDH were shown in Table I. The differences in gene expression, expressed as fold-changes, were calculated using the $2^{-\Delta\Delta Ct}$ method.

Table I. The primer sequences used in this study.

Gene	Sequences (5′-3′)
miR-1231 (F)	GCCAGTGTCTGGGCGGAC
miR-1231 (R)	GTGCAGGGTCCGAGGT
GAPDH (F)	GTCAACGGATTTGGTCTGTATT
GAPDH (R)	AGTCTTCTGGGTGGCAGTGAT

Statistical Analysis

All data were carried out using SPSS 17.0 software package (SPSS Inc, Chicago, IL, USA). The expression differences between glioma tissues and matched normal tissues were analyzed using paired samples *t*-test. Chi-square test was performed to analyze the correlation between the miR-1231 expression and clinicopathological parameters. Survival curves were obtained using the Kaplan-Meier method and assessed by the log-rank test. A Cox proportional hazards model was used for the multivariate analysis. A $p<0.05$ was considered to be statistically significant.

Results

Downregulation of MiR-1231 in Human Glioma Tissues

To explore the role of miR-1231 in the progression of glioma, the miR-1231 expression levels were investigated in 154 paired glioma samples and adjacent normal tissues using qPCR assays. As shown in Figure 1A, we found that miR-1231 expression was significantly down-regulated in glioma tissues compared to matched normal tissues ($p<0.01$). Moreover, we also observed that the expression of miR-1231 in glioma with high grades was significantly lower than those with low grades ($p<0.01$). Thus, these results indicated that miR-1231 may be involved in the progression of glioma.

Correlations Between MiR-1231 Expression and Clinical Characteristics

Next, we explored the correlation of the miR-1231 expression level with the clinicopathological factors in glioma patients. The median value of miR-1231 in all glioma tissues was 4.32 and was used as a cutoff value, and all glioma

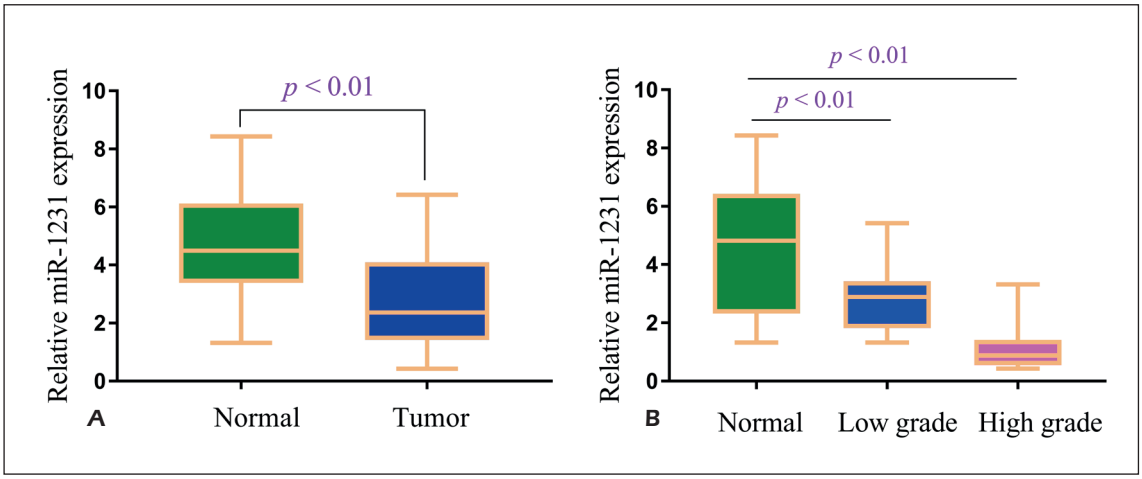

Figure 1. The expression of miR-1231 was significantly down-regulated in glioma tissues. **A**, The expression of miR-1231 in glioma tissues and normal brain tissues was measured by qRT-PCR. **B**, The expression of miR-1231 in normal brain tissues, low-grade glioma tissues and high-grade glioma tissues by qRT-PCR. Low-grade glioma refers to World Health Organization (WHO) grade I and WHO grade II, high-grade glioma refers to WHO grade III and WHO grade IV.

patients were divided into two groups: the high-miR-1231 expression group (n=79) and the low-miR-1231 expression group (n=75). As shown in Table II, we found that high miR-1231 was significantly associated with WHO grade (p=0.001) and KPS score (p=0.023). However, no significant difference was observed between miR-1231 expression levels and patient age, gender, the extent of resection and the tumor size (p>0.05). Our findings supported the notion that the miR-1231 down-regulation may be associated with tumor progression and development.

Prognostic Values of MiR-1231 Expression in Glioma

To further explore the correlation of miR-1231 expression with overall survival (OS) and progression-free survival (PFS) of glioma patients, Kaplan-Meier analyses were performed. As shown in Figure 2, the results showed that the patients with low miR-1231 level had worse OS than those with high miR-1231 level (p<0.0103). Moreover, the patients in the high miR-1231 group had a higher PFS rate than those in the low miR-1231 group (p=0.0019). Additionally, univariate and multivariate analyses

Table II. Clinicopathological features and the expression of lncRNA MINCR in HCC patients.

Parameter	No. of cases	miR-1231 expression		*p*-value
		High	Low	
Age				0.516
< 50	76	35	41	
≥ 50	78	40	38	
Gender				0.780
Male	88	42	46	
Female	66	33	33	
WHO grade				0.001
I–II	113	46	67	
III–IV	41	29	12	
KPS score				0.023
< 80	52	32	20	
≥ 80	102	43	59	
Extent of resection				0.199
< 98 %	112	51	61	
≥ 98 %	42	24	18	
Tumor size				0.150
< 5 cm	107	47	59	
≥ 5 cm	47	27	20	

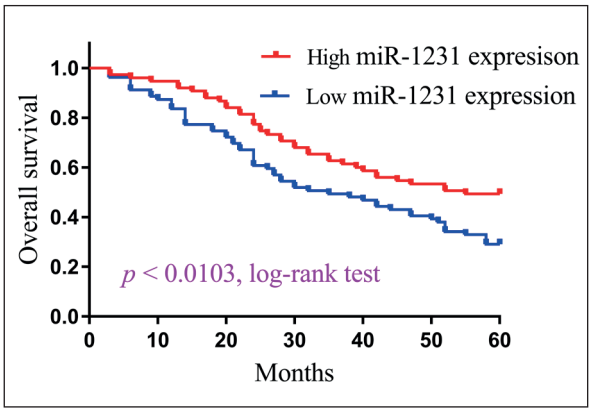

Figure 2. Kaplan-Meier survival curves of the 154 glioma patients. Overall survival rate in patients with high miR-1231 expression level was markedly higher than those with low miR-1231 expression level (p<0.0103, log-rank test).

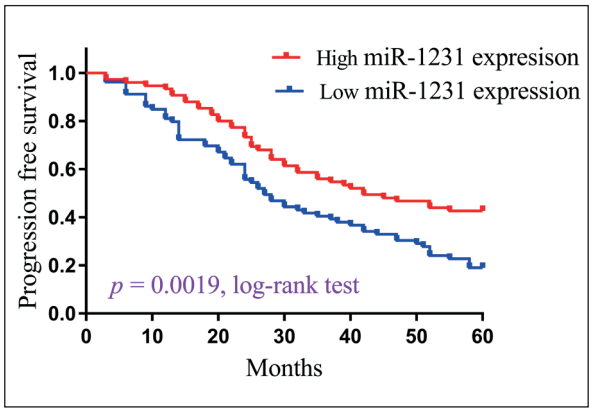

Figure 3. Kaplan-Meier survival curves of the 154 glioma patients. Progression-free survival rate in patients with high miR-1231 expression level was markedly higher than those with low miR-1231 expression level (p=0.0019, log-rank test).

were conducted to assess the prognostic variables in all patients. The results of the univariate analysis showed that the miR-1231 expression, WHO grade and KPS score were significantly related to OS and PFS rate in patients with glioma (Table III and IV). More importantly, in the multivariate analysis, miR-1231, WHO grade and KPS were confirmed to have the potential to independently predicate OS and PFS in glioma (Table III and IV).

Discussion

Glioma is the most common type of primary malignant brain tumor in the central nervous system[21]. Enormous achievements have been made in the treatment of advanced glioma using microsurgery, radiotherapy and chemotherapy. However, the prognosis of advanced glioma remains poor[22,23]. The dismal prognosis and shorter long-term survival of glioma are due to few symptoms in early-stage and lack of sensitive molecular biomarkers; patients are generally diagnosed with advanced-stage tumors[24,25]. It is urgent to develop novel biomarkers for its early diagnosis, accurate assessment, targeted therapy and prognosis evaluation. Dysregulation of miRNAs has been frequently observed in tumors, including glioma[26]. More and more evidence highlighted the importance of miRNAs as potential prognostic and diagnostic biomarkers for various tumors[27,28]. In this study, we aimed to explore the possibili-

Table III. Univariate and multivariate Cox regression analyses miR-1231 for overall survival of glioma patients.

Variable	Univariate analysis			Multivariate analysis		
	RR	95% CI	*p*-value	RR	95% CI	*p*-value
Age < 50 *vs.* ≥ 50	0.431	0.671-1.889	0.153	–	–	–
Gender Male *vs.* Female	0.331	0.472-1.742	0.114	–	–	–
WHO grade I–II *vs.* III–IV	3.673	1.334-5.723	**0.001**	3.174	1.183-4.273	**0.004**
KPS score < 80 *vs.* ≥ 80	3.135	1.468-4.114	**0.006**	2.562	1.169-3.216	**0.028**
Extent of resection < 98 % *vs.* ≥ 98 %	1.213	0.556-1.684	0.132	–	–	–
Tumor size(cm) < 5 *vs.* ≥ 5	0.754	0.834-1.947	0.155	–	–	–
miR-1231 expression High *vs.* Low	4.327	1.467-6.328	0.001	3.356	1.169-4.467	0.003

Table IV. Univariate and multivariate Cox regression analyses miR-1231 for progression-free survival of glioma patients.

Variable	Univariate analysis			Multivariate analysis		
	RR	95% CI	*p*-value	RR	95% CI	*p*-value
Age < 50 *vs.* ≥ 50	0.543	0.723-1.667	0.124	–	–	–
Gender Male *vs.* Female	0.674	0.561-1.445	0.159	–	–	–
WHO grade I–II *vs.* III–IV	3.943	1.255-6.238	**0.001**	3.321	1.155-4.785	**0.003**
KPS score < 80 *vs.* ≥ 80	3.342	1.334-4.423	**0.004**	2.782	1.199-3.762	**0.015**
Extent of resection < 98 % *vs.* ≥ 98 %	1.315	0.742-1.231	0.114	–	–	–
Tumor size(cm) < 5 *vs.* ≥ 5	1.354	0.834-2.542	0.125	–	–	–
miR-1231 expression High *vs.* Low	4.774	1.562-6.962	**0.001**	3.763	1.234-5.421	**0.001**

ty of miRNA-1231 as a novel biomarker for the prediction of prognosis of glioma patients. Growing evidence has shown that miRNAs is involved in human glioma development and progression. For instance, Qi et al[29] reported that miRNA-491 expression was significantly down-regulated in both glioma tissues and cell lines, and its low expression was significantly poor prognosis of glioma patients. In their functional assay, it was observed that the overexpression of miR-491 inhibits glioma cell proliferation by modulating TRIM28. Xue et al[30] found that miR-584-3p was lowly expressed in glioma and its overexpression reduces the migration and invasion of human glioma cells by targeting hypoxia-induced ROCK1. In their clinical assay, it was found that low miR-584-3p expression is correlated with poor prognosis. MiR-1231 was identified to be an important regulator in several diseases. Zhou et al[31] reported that miR-1231 was correlated with the susceptibility of hepatocellular carcinoma. Kohno et al[32] showed that miR-1231 was involved in the inhibition of hepatitis B virus replication by targeting core mRNA. Zhang et al[20] showed that miR-1231 was lowly expressed in glioma and its forced expression could inhibit glioma cell proliferation by regulating the EGFR/PI3K/AKT axis in glioma both i*n vitro* and *in vivo*. However, the clinical significance of miR-1231 remains unclear. In this study, we further detected the expression levels of miR-1231 in glioma samples, finding that miR-1231 expression was significantly down-regulated in glioma tissues compared to matched normal tissues. In addition, high-grade glioma tissues also exhibited a lower miR-1231 level. These results were consistent with previous findings. Then, we further explore the association between miR-1231 and clinical progression; low expression of miR-1231 was significantly associated advanced WHO grade and KPS score, indicating that miR-1231 may play a negative regulator in the progression of glioma. Moreover, based on the Kaplan-Meier survival analysis, we demonstrated that low miR-1231 expression was associated with poorer PFS and OS. In addition, univariate and multivariate analyses indicated that the miR-1231 level was identified to be an independent prognostic factor for glioma. Thus, miR-1231 may serve as a potential molecular marker for the prognosis of glioma.

Conclusions

We investigated the prognostic value of miR-1231 in human glioma for the first time. The miR-1231 expression may have significant value as a favorable progression indicator for glioma patients.

Conflict of Interests

The authors declare no conflicts of interest.

Acknowledgments

This study was supported by grants from the Science and Technology Program of Shenzhen No: (JCYJ20140416122812008).

References

1) Miller KD, Siegel RL, Lin CC, Mariotto AB, Kramer JL, Rowland JH, Stein KD, Alteri R, Jemal A. Cancer treatment and survivorship statistics, 2016. CA Cancer J Clin 2016; 66: 271-289.
2) Ferlay J, Steliarova-Foucher E, Lortet-Tieulent J, Rosso S, Coebergh JW, Comber H, Forman D, Bray F. Cancer incidence and mortality patterns in Europe: estimates for 40 countries in 2012. Eur J Cancer 2013; 49: 1374-1403.
3) Rooney AG, Brown PD, Reijneveld JC, Grant R. Depression in glioma: a primer for clinicians and researchers. J Neurol Neurosurg Psychiatry 2014; 85: 230-235.
4) Claus EB, Walsh KM, Wiencke JK, Molinaro AM, Wiemels JL, Schildkraut JM, Bondy ML, Berger M, Jenkins R, Wrensch M. Survival and low-grade glioma: the emergence of genetic information. Neurosurg Focus 2015; 38: E6.
5) Hardell L, Carlberg M. Use of mobile and cordless phones and survival of patients with glioma. Neuroepidemiology 2013; 40: 101-108.
6) Li W, Holsinger RM, Kruse CA, Flugel A, Graeber MB. The potential for genetically altered microglia to influence glioma treatment. CNS Neurol Disord Drug Targets 2013; 12: 750-762.
7) Juratli TA, Cahill DP, McCutcheon IE. Determining optimal treatment strategy for diffuse glioma: the emerging role of IDH mutations. Expert Rev Anticancer Ther 2015; 15: 603-606.
8) Kim VN. Small RNAs: classification, biogenesis, and function. Mol Cells 2005; 19: 1-15.
9) Bhaskaran M, Mohan M. MicroRNAs: history, biogenesis, and their evolving role in animal development and disease. Vet Pathol 2014; 51: 759-774.
10) Tian T, Wang J, Zhou X. A review: microRNA detection methods. Org Biomol Chem 2015; 13: 2226-2238.
11) Hammond SM. An overview of microRNAs. Adv Drug Deliv Rev 2015; 87: 3-14.
12) Tutar Y. miRNA and cancer; computational and experimental approaches. Curr Pharm Biotechnol 2014; 15: 429.
13) McGuire A, Brown JA, Kerin MJ. Metastatic breast cancer: the potential of miRNA for diagnosis and treatment monitoring. Cancer Metastasis Rev 2015; 34: 145-155.
14) Gomez Zubieta DM, Hamood MA, Beydoun R, Pall AE, Kondapalli KC. MicroRNA-135a regulates NHE9 to inhibit proliferation and migration of glioblastoma cells. Cell Commun Signal 2017; 15: 55.
15) Bai QL, Hu CW, Wang XR, Shang JX, Yin GF. MiR-616 promotes proliferation and inhibits apoptosis in glioma cells by suppressing expression of SOX7 via the Wnt signaling pathway. Eur Rev Med Pharmacol Sci 2017; 21: 5630-5637.
16) Tang C, Yang Z, Chen D, Xie Q, Peng T, Wu J, Qi S. Downregulation of miR-130a promotes cell growth and epithelial to mesenchymal transition by activating HMGB2 in glioma. Int J Biochem Cell Biol 2017; 93: 25-31.
17) Li FF, Xing C, Wu LL, Xue F. MiR-205 enhances cisplatin sensitivity of glioma cells by targeting E2F1. Eur Rev Med Pharmacol Sci 2018; 22: 299-306.
18) Hayes CN, Chayama K. MicroRNAs as biomarkers for liver disease and hepatocellular carcinoma. Int J Mol Sci 2016; 17: 280.
19) Tan YG, Zhang YF, Guo CJ, Yang M, Chen MY. Screening of differentially expressed microRNA in ulcerative colitis related colorectal cancer. Asian Pac J Trop Med 2013; 6: 972-976.
20) Zhang J, Zhang J, Qiu W, Zhang J, Li Y, Kong E, Lu A, Xu J, Lu X. MicroRNA-1231 exerts a tumor suppressor role through regulating the EGFR/PI3K/AKT axis in glioma. J Neurooncol 2018; 139: 547-562.
21) Mathiesen T, Peredo I, Lonn S. Two-year survival of low-grade and high-grade glioma patients using data from the Swedish Cancer Registry. Acta Neurochir (Wien) 2011; 153: 467-471.
22) Stark AM, van de Bergh J, Hedderich J, Mehdorn HM, Nabavi A. Glioblastoma: clinical characteristics, prognostic factors and survival in 492 patients. Clin Neurol Neurosurg 2012; 114: 840-845.
23) Sooman L, Freyhult E, Jaiswal A, Navani S, Edqvist PH, Ponten F, Tchougounova E, Smits A, Elsir T, Gullbo J, Lennartsson J, Bergqvist M, Ekman S. FGF2 as a potential prognostic biomarker for proneural glioma patients. Acta Oncol 2015; 54: 385-394.
24) Siegel EM, Nabors LB, Thompson RC, Olson JJ, Browning JE, Madden MH, Han G, Egan KM. Prediagnostic body weight and survival in high grade glioma. J Neurooncol 2013; 114: 79-84.
25) Li M, Deng H, Peng H, Wang Q. Functional nanoparticles in targeting glioma diagnosis and therapies. J Nanosci Nanotechnol 2014; 14: 415-432.
26) Romero-Cordoba SL, Salido-Guadarrama I, Rodriguez-Dorantes M, Hidalgo-Miranda A. miRNA biogenesis: biological impact in the development of cancer. Cancer Biol Ther 2014; 15: 1444-1455.
27) Casanova-Salas I, Rubio-Briones J, Calatrava A, Mancarella C, Masia E, Casanova J, Fernandez-Serra A, Rubio L, Ramirez-Backhaus M, Arminan A, Dominguez-Escrig J, Martinez F, Garcia-Casado Z, Scotlandi K, Vicent MJ, Lopez-Guerrero JA. Identification of miR-187 and miR-182 as biomarkers of early diagnosis and prognosis in patients with prostate cancer treated with radical prostatectomy. J Urol 2014; 192: 252-259.
28) Bai QL, Hu CW, Wang XR, Yin GF, Shang JX. Association between downexpression of miR-1301 and poor prognosis in patients with glioma. Eur Rev Med Pharmacol Sci 2017; 21: 4298-4303.
29) Qi Z, Cai S, Cai J, Chen L, Yao Y, Chen L, Mao Y. miR-491 regulates glioma cells proliferation by targeting TRIM28 in vitro. BMC Neurol 2016; 16: 248.
30) Xue H, Guo X, Han X, Yan S, Zhang J, Xu S, Li T, Guo X, Zhang P, Gao X, Liu Q, Li G. MicroRNA-584-3p, a novel tumor suppressor and prognostic marker, reduces the migration and invasion of human glioma cells by targeting hypoxia-induced ROCK1. Oncotarget 2016; 7: 4785-4805.

31) Zhou C, Yu Q, Chen L, Wang J, Zheng S, Zhang J. A miR-1231 binding site polymorphism in the 3'UTR of IFNAR1 is associated with hepatocellular carcinoma susceptibility. Gene 2012; 507: 95-98.

32) Kohno T, Tsuge M, Murakami E, Hiraga N, Abe H, Miki D, Imamura M, Ochi H, Hayes CN, Chayama K. Human microRNA hsa-miR-1231 suppresses hepatitis B virus replication by targeting core mRNA. J Viral Hepat 2014; 21: 89-97.