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Downregulation of miR-154 in human glioma and its clinicopathological and prognostic significance

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Abstract

Objective: MicroRNA-154 (miR-154) was previously reported to be downregulated in several types of human cancers and may act as a tumour suppressor. This study aimed to measure miR-154 levels and determine its clinical significance in human glioma.

Methods: This retrospective study analysed fresh human glioma specimens and non-neoplastic brain tissues using real-time quantitative reverse transcription–polymerase chain reaction to determine the relative levels of miR-154. The association between miR-154 levels and various clinicopathological characteristics and survival was analysed.

Results: A total of 115 patients with gliomas and 115 non-neoplastic brain tissues were examined. MiR-154 levels were significantly downregulated in gliomas compared with non-neoplastic brain tissues. Low levels of miR-154 were associated with high World Health Organization grade, large tumour size ($\geq$ 5 cm), a low Karnofsky performance status score ($<$ 80), and a shorter overall survival. Multivariate analyses using the Cox proportional hazards regression model confirmed that decreased miR-154 level was an independent predictor of a poor prognosis.

Conclusions: These results suggest that miR-154 downregulation may be involved in glioma formation and progression, and that miR-154 might serve as a potential prognostic biomarker for patients with this disease.

Keywords

MicroRNA-154, glioma, real-time quantitative reverse transcription–polymerase chain reaction, prognosis

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Introduction

Gliomas, arising from glial or precursor cells, represent the most common primary brain tumours in adults.[1] Despite recent improvements in multimodal therapy including neurosurgery, radiotherapy, chemotherapy, and photodynamic therapy, the prognosis for patients with high-grade gliomas remains poor, with the 5-year survival rate lower than 10%.[2] Hence, there is an urgent need to understand the mechanisms underlying glioma tumorigenesis and aggressiveness at the molecular level, and to identify novel biomarkers and therapeutic targets for this life-threatening disease.

MicroRNAs (miRNAs) are small non-coding RNAs that are 18–25 nucleotides in length, which negatively regulate gene expression by binding to the 3'-UTR region of the target mRNA, leading to mRNA degradation or suppression of translation.[3] Deregulation or dysfunction of miRNAs has been suggested to be involved in a variety of human diseases including cancer.[4–6] Emerging evidence demonstrates that miRNAs participate in the regulation of cancer cell growth, apoptosis, tumour angiogenesis, epithelial–mesenchymal transition (EMT), tumour invasion and metastasis, and chemotherapy resistance.[7–9] In glioma, aberrant expression of several miRNAs and their function as either oncogenes or tumour suppressors have been reported. For example, decreased miR-203 levels in glioma cells was responsible for cell migration and invasion.[10] Overexpression of miR-34a *in vitro* suppressed the proliferation and induced apoptosis of U87 glioma cells.[11] Moreover, low miR-326 levels in glioma was significantly associated with advanced pathological grade and a low Karnofsky performance status (KPS) score.[12] miR-203 downregulation and miR-372 upregulation were reported as unfavourable prognostic factors in patients with gliomas.[13,14] These findings indicate that miRNAs play important roles in glioma initiation and development, and may have applications in its diagnosis, prognosis, and gene therapy.

One of the cancer-related miRNAs is miR-154. Recent studies demonstrated that miR-154 was downregulated and might act as a potential tumour suppressor in some human tumours, such as non-small cell lung cancer (NSCLC),[15] hepatocellular carcinoma,[16] colorectal cancer,[17,18] and prostate cancer.[19,20] However, the levels of miR-154 and their clinical significance in human glioma have not yet been evaluated. The aim of the present study was to measure miR-154 levels in human glioma tissue samples and investigate the correlation between miR-154 levels and clinicopathological characteristics as well as patient survival.

Patients and methods

Patients and tissue samples

This retrospective study analysed fresh glioma specimens collected from consecutive patients who underwent surgery at the Department of Neurology, First Hospital of Jilin University, Changchun, Jilin Province, China between January 2007 and December 2010. The diagnosis of glioma was confirmed by histological examination. The gliomas were classified according to the World Health Organization (WHO) classification.[21] Eligibility criteria included: (i) histologically-proven glioma specimens; (ii) patients aged 18–70 years. Patients who had previously received preoperative cytotoxic chemotherapy or radiotherapy treatment were excluded from the study. Non-neoplastic brain specimens were obtained from age- and sex-matched patients undergoing surgery for other reasons such as epilepsy or cerebral haemorrhage at the same institution during the same time period. These non-neoplastic brain specimens provided the control group. Immediately after surgical resection, the tissue specimens were

stored at −80°C until use. The follow-up data were available and complete for each study participant. Overall survival was calculated from the day of primary surgery to death or last follow-up. The KPS score was evaluated as previously described.[22] The Research Ethics Committee of Jilin University provided approval for the study (no. 2015032) and all study participants provided written informed consent.

RNA extraction and quantitative real-time RT–PCR

Total RNA was isolated from 20 mg of tissue sample using TRIzol® reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. RNA concentration and purity were measured using a NanoDrop 1000 spectrophotometer (NanoDrop, Wilmington, DE, USA). Only samples with an OD A260/A280 ratio close to 2.0, which indicates that the RNA is pure, were subsequently analysed. cDNA was synthesized from 10 ng of total RNA using a TaqMan® MicroRNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA). Quantitative real-time reverse transcription–polymerase chain reaction (qRT–PCR) was performed on an ABI 7900 Fast System with a TaqMan® MicroRNA Assay kit (both from Applied Biosystems). Small nuclear RNA U6 was used as an endogenous control. The primer sequences used were as follows: miR-154, 5′-CGCGAATTCGCATCTAGGACCTCCA-TCAC-3′ (forward) and 5′- ACGGGATCC GAACCATCCCTTCACTTACC-3′ (reverse); U6, 5′- CTCGCTTCGGCAGC ACA-3′ (forward) and 5′- AACGCTTCA CGAATTTGCGT-3′ (reverse). The PCR amplification conditions were as follows: 95°C for 10 min, followed by 40 cycles of denaturation at 95°C for 15 s and annealing at 60°C for 1 min; followed by a final elongation step at 72°C for 10 min. Each sample was examined in triplicate. The cycle threshold (CT) was recorded and the amount of miR-154 relative to RNA U6 was calculated using the $2^{-\Delta CT}$ method ($\Delta CT = CT_{miR\text{-}154} - CT_{U6}$). The median miR-154 level was used as a cut-off value to divide all patients with glioma into two groups: high miR-154 level group and low miR-154 level group.

Statistical analyses

All statistical analyses were performed using the SPSS® statistical package, version 15.0 (SPSS Inc., Chicago, IL, USA) for Windows®. Data are presented as mean ± SD. The differences between the two groups were analysed using Student's *t*-test. The relationship between miR-154 levels and clinicopathological characteristics was analysed using χ^2-test. Overall survival was estimated using the Kaplan–Meier method and compared statistically using the log-rank test. Cox regression analysis was performed to analyse the prognostic significance of each variable. A P-value < 0.05 was considered statistically significant.

Results

Fresh glioma specimens were collected from 115 patients (66 males and 49 females; median age 50 years; range 26–75 years). Non-neoplastic brain specimens were obtained from 115 age- and sex-matched patients (66 males and 49 females; median age 50 years; range 26–75 years) undergoing surgery for other reasons such as epilepsy or cerebral haemorrhage. According to the WHO classification, 38 patients with gliomas were classified as low-grade (14 pilocytic astrocytomas [WHO I] and 24 diffuse astrocytomas [WHO II]) and 77 were classified as high-grade (30 anaplasia astrocytomas [WHO III] and 47 primary glioblastomas [WHO IV]).

The qRT-PCR demonstrated that the miR-154 level in glioma specimens was significantly lower compared with non-neoplastic brain specimens (mean ± SD,

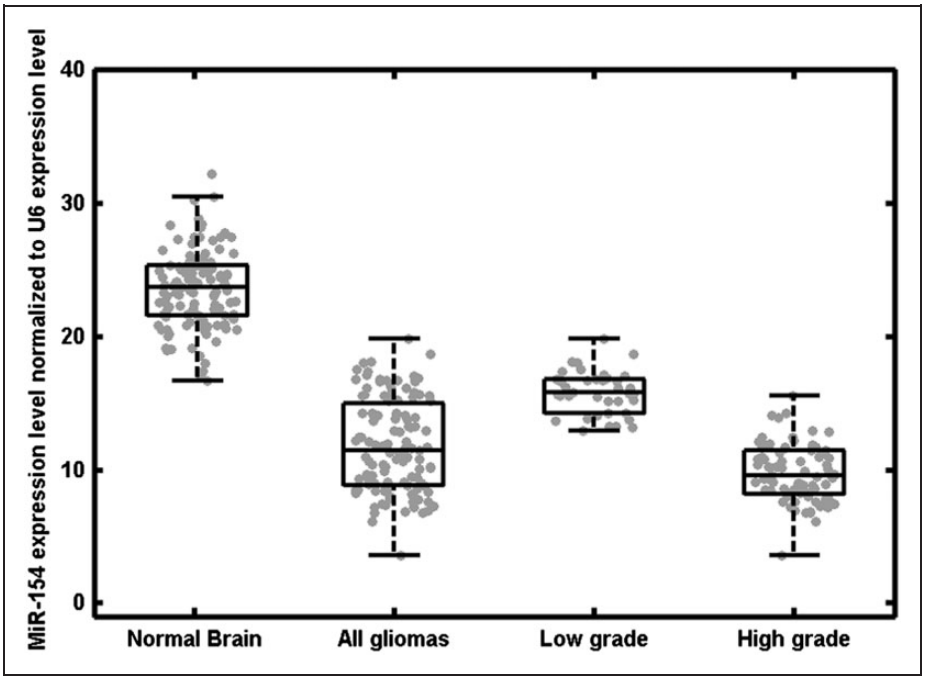

Figure 1. The relative levels of microRNA-154 (miR-154) in 115 glioma tissues and 115 non-neoplastic brain tissues (normal brain). Low-grade glioma refers to World Health Organization (WHO) grade I and II and high-grade glioma refers to WHO grade III and IV. The central black horizontal lines in each box are the mean values. The extremities of the box are the 25th and 75th percentiles. The error bars represent the minimum and maximum outliers.

11.83 ± 3.27 versus 23.01 ± 3.69, respectively; $P < 0.001$; Figure 1). In addition, there was a significant difference in miR-154 level between high-grade (WHO grades III–IV) and low-grade (WHO grades I–II) glioma specimens (9.96 ± 1.79 versus 15.61 ± 2.15, respectively; $P < 0.001$).

Table 1 presents details of the clinical and pathological characteristics of the patients with glioma and their relationship to the levels of miR-154. The median miR-154 level of 11.25 was used as a cut-off value to divide all 115 patients into two groups: a high miR-154 level group ($n = 58$) and a low miR-154 level group ($n = 57$). Decreased miR-154 levels were found to be significantly associated with high WHO grade ($P = 0.001$), large tumour size ($\geq$ 5 cm) ($P = 0.019$), and low KPS score ($<$ 80) ($P < 0.001$). There were no significant correlations between miR-154 levels and age and sex.

The prognostic significance of miR-154 levels in patients with glioma was investigated using a Kaplan–Meier survival analysis, which showed that patients with low miR-154 levels had a significantly shorter overall survival than those with high miR-154 levels ($P < 0.001$; Figure 2). Univariate Cox regression analysis demonstrated that low WHO grade ($P = 0.003$) and high KPS score ($P = 0.014$) were predictive factors for a favourable outcome (Table 2). Multivariate analyses using the Cox proportional hazards regression model revealed that miR-154 level (relative risk [RR] 5.86; $P = 0.005$), KPS score (RR 2.19; $P = 0.032$), and WHO grade (RR 5.15; $P = 0.008$) were

Table 1. Relationship between microRNA-154 (miR-154) levels and various clinicopathological characteristics in the 115 patients with gliomas who were studied.

Clinicopathological characteristic	*n*	MiR-154 levels		Statistical significance[a]
		High, $n = 58$	Low, $n = 57$	
Age at diagnosis, years				
< 50	57	32 (56.1)	25 (43.9)	NS
≥ 50	58	26 (44.8)	32 (55.2)	
Sex				
Male	66	35 (53.0)	31 (47.0)	NS
Female	49	23 (46.9)	26 (53.1)	
WHO grade				
I + II	38	28 (73.7)	10 (26.3)	$P = 0.001$
III + IV	77	30 (39.0)	47 (61.0)	
Tumour size				
< 5 cm	41	27 (65.9)	14 (34.1)	$P = 0.019$
≥ 5 cm	74	31 (41.9)	43 (58.1)	
KPS score				
< 80	68	24 (35.3)	44 (64.7)	$P < 0.001$
≥ 80	47	34 (72.3)	13 (27.7)	

Data presented as *n* of patients (%).
[a]χ^2-test.
WHO, World Health Organization; KPS, Karnofsky performance status; NS, no significant between-group difference ($P \geq 0.05$).

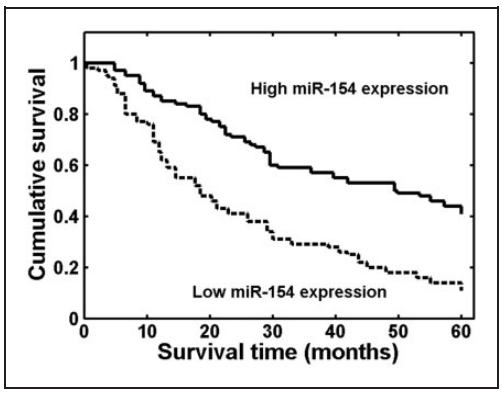

Figure 2. Kaplan–Meier survival curves for patients with gliomas that had low miR-154 levels ($n = 57$) compared with those that had high miR-154 levels ($n = 58$). $P < 0.001$ for low level group compared with high level group; log-rank test.

independently correlated with overall survival of patients with glioma.

Discussion

Identifying novel molecules that take part in glioma formation and progression may be helpful for improving the diagnosis, prevention and treatment of this life-threatening disease. The relationship between miRNAs and tumours has become a focus of considerable research. This present study demonstrated that miR-154 levels were downregulated in glioma specimens compared with non-neoplastic brain tissues. Reduced miR-154 levels were significantly correlated with high WHO grade, large tumour size, low KPS score, and shorter overall survival. Multivariate Cox

Table 2. Univariate and multivariate Cox regression analyses of the clinicopathological characteristics associated with overall survival in 115 patients with glioma.

Clinicopathological characteristic	Univariate Cox regression analysis, *P*-value	Multivariate Cox proportional hazards regression model analysis, *P*-value	Relative risk
Age at diagnosis, years			
< 50 versus ≥ 50	NS	–	–
Sex			
Male versus female	NS	–	–
Tumour size, cm			
< 5 versus ≥ 5	NS	–	–
WHO grade			
I + II versus III + IV	$P = 0.003$	$P = 0.008$	5.15
KPS			
< 80 versus ≥ 80	$P = 0.014$	$P = 0.032$	2.19
MiR-154 level			
High versus low	$P < 0.001$	$P = 0.005$	5.86

WHO, World Health Organization; KPS, Karnofsky performance status; MiR, microRNA; NS, no significant difference ($P \geq 0.05$).

proportional hazards regression model analysis identified low miR-154 levels as an independent indicator of an unfavourable prognosis. Thus, loss of miR-154 might be involved in glioma development and serve as a potential biomarker for a poor prognosis.

The results of the present study were consistent with previous findings in other cancers. For example, miR-154 was significantly downregulated in NSCLC, and decreased miR-154 levels were significantly associated with metastasis, larger tumour size, and advanced TNM stage.[15] Enforced expression of miR-154 in NSCLC A549 cells was able to inhibit cell proliferation, colony formation, invasion and migration, and induce apoptosis and G0/G1 cell cycle arrest *in vitro*, and suppress the growth of cancer cell xenografts *in vivo*.[15] In hepatocellular carcinoma, low levels of miR-154 correlated with poor tumour differentiation, lymph node metastasis, and later clinical stage.[16] Restoration of miR-154 expression in HepG2 cells reduced cell proliferation and invasion, as well as suppressed tumour growth in a nude mouse model.[16] In addition, miR-154 inhibited EMT by targeting high-mobility group AT-hook 2 in prostate cancer cells,[23] and suppressed cell growth and motility by targeting toll-like receptor 2 in colorectal cancer.[17] Taken together, miR-154 might act as a tumour suppressor in human malignancies, and would be a potential therapeutic target.

This present study had several limitations. First, it was a retrospective study and the sample size was relatively small. Secondly, although the results demonstrated low miR-154 levels in human gliomas and their clinical significance, the underlying mechanisms have not been well characterized. It is now clear that miRNAs exhibit oncogenic or tumour suppressive properties by regulation of target gene expression.[24] Therefore, identification of miR-154 function and its downstream genes in glioma

cells would be an important goal in future investigations.

In summary, this present study demonstrated the downregulation of miR-154 in human glioma and this correlated with shorter overall survival. Decreased miR-154 levels might be an independent biomarker for a poor prognosis. Large scale prospective studies are needed to confirm these preliminary findings and to clarify the molecular mechanisms involved.

Declaration of conflicting interests

The authors declare that there are no conflicts of interest.

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