

Original Article

MiR-184 has prognostic implication and is down-regulated during the malignant progression in human astrocytoma

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Abstract: Recent studies have indicated that miR-184 is dysregulated and function both as oncogene and tumor suppressor in several types of cancer. However, the role of miR-184 in astrocytoma is still not clearly understood. Therefore, we in the present study aimed to investigate the clinical significance of miR-184 expression in human astrocytomas. We examined the expression level of miR-184 in 76 astrocytoma tissues and 5 cell lines by qRT-PCR and found that miR-184 expression was markedly reduced in the tumor tissues and cell lines, compared with that in non-neoplastic brain tissues and human astrocyte, respectively. In addition, low expression of miR-184 was significantly associated with the aggressive clinicopathological features (advanced tumor degree, advanced patient age, low Karnofsky performance score and high ki-67 index of tumor tissue) in astrocytoma patients. Furthermore, the correlations of miR-184 expression with prognosis of astrocytoma patients were also modeled by Kaplan-Meier method and multivariate analysis. Our results showed that patients with low miR-184 expression had significantly poor survival ($P < 0.001$, Kaplan-Meier method) and miR-184 was an independent prognostic indicator for astrocytoma patients ($P < 0.001$; risk ratio = 5.7, Cox regression analysis). Moreover, we examined miR-184 expression in paired tumor tissues from seven patients with primary lower-grade astrocytomas and the spontaneously recurrent higher-grade astrocytomas. MiR-184 showed an absolute down-regulation in recurrent astrocytomas as compared with the corresponding primary tumors. In conclusion, our data suggest that down-regulation of miR-184 may have potential value for predicting clinical outcomes in astrocytoma patients, and miR-184 is an important candidate tumor suppressor, and its down-regulation may contribute to malignant progression of human astrocytoma.

Keywords: microRNA, miR-184, astrocytoma, down-regulation, prognosis, malignant progression

Introduction

Astrocytomas are the most frequent tumors of central nervous system, accounting for more than 60% of all primary brain tumors of human adults [1]. They are aggressive, highly invasive and neurological destructive tumors considered being among the deadliest of human cancers. Based on histomorphological criteria, diffuse infiltrating astrocytomas are classified into three ascending grades of malignancy including well-differentiated diffuse astrocytoma (DA, grade II), anaplastic astrocytoma (AA, grade III), and glioblastoma (GBM, grade IV), according to the World Health Organization (WHO) grading system [1]. In addition, GBMs can be further divided into two subtypes that have distinct dif-

ferent clinical and molecular profiles with each other: primary GBM (pGBM) with no pathological precursor lesion, which have a clinical progression of less than 3 months, and, secondary GBM (sGBM) which may develop through progression from lower-grade astrocytomas, DA or AA [2]. The clinical outcome is distinctly different among patients with different grade of astrocytoma. Comparing to DA (grade II) and AA (grade III) patients with median survivals of about 6-8 years and 2-5 years, respectively, patients with GBM, grade IV glioma, have a mean survival time as short as only 15 months, despite being treated with current therapies including surgical resection and chemoradiotherapy [3]. As the currently used histology-based grading is subjective, it is necessary to

identify new biomarkers for prognostic predictions and treatment options and explore novel therapeutic targets for astrocytoma.

MicroRNAs (miRNAs) are recently discovered small, non-coding endogenous RNA molecules of about 18-25 nucleotides. They are considered to play important roles in a variety of biological processes including cell proliferation, apoptosis, migration and differentiation, through post-translationally regulating expression of their target genes [4, 5]. These short RNA molecules have been reported to be aberrantly expressed in various human cancers and act as important regulators of tumor biologic behaviors in tumorigenesis and aggressive progression, by targeting oncogenes or tumor suppressors [6, 7]. Accumulating evidences indicate that identification of specific miRNAs in cancer cells has substantial value for diagnostic and prognostic determinations as well as for eventual therapeutic interventions [8, 9].

In the last decade, a number of specific miRNAs have been identified to be abnormally in human astrocytomas. They are involved in tumorigenesis and malignant progression of astrocytoma by functioning as oncogenes or tumor suppressors [10-13]. Most of these miRNAs have also been demonstrated to significantly correlate with patients' survival and could function as prognostic and predictive indicators in human cancers, such as miR-21 [14], miR-155 [15], miR-196 [16] and miR-326 [17]. MiR-184 is located in region 25.1 on the long arm of chromosome 15 and is particularly enriched in human brain. This miRNA has been reported to be dysregulated and function both as oncogene and tumor suppressor in numerous human cancers [18-21]. However, the functions and the exact mechanisms of miR-184 in tumorigenesis and progression of human astrocytoma remain controversial. Several recent studies showed that miR-184 was significantly down-regulated in astrocytoma tissues compared with normal brains and acted as a potential tumor suppressor by inhibiting cell proliferation and invasion [22-24]. In contrary, other groups found that miR-184 could enhance the aggressive biological behaviors of glioma cell lines [25, 26]. In addition, the clinical significances of miR-184 in astrocytomas are still poorly understood.

We in the present study examined expression level of miR-184 in a large panel of astrocytomas and cell lines and statistically evaluated the correlations between miR-184 expression and clinical pathological factors in these patients. As our results, miR-184 was remarkably down-regulated in astrocytoma tissues and tumor cell lines as compared with non-neoplastic brain tissues and low miR-184 expression was significantly associated with aggressive clinical pathological features of astrocytoma. In addition, miR-184 expression statistically correlated with patients' survival and was an independent prognostic indicator. Furthermore, significant lower expression of miR-184 was observed in recurrent higher-grade tumor compared with corresponding primary lower-grade tumor in a series of paired astrocytomas. Our observations suggest that miR-184 might function as a tumor suppressor and could be a potential biomarker for prognosis and aggressive progression in human astrocytoma.

Materials and methods

Astrocytoma specimens and patients

Astrocytoma specimens were obtained from patients during surgery at First Affiliated Hospital of China Medical University. A portion of the tumor tissue was saved and made into paraffin sections for histopathological diagnosis in strict accordance with World Health Organization (WHO) criteria by two established neuropathologists, with differences resolved by careful discussion. And the remaining tissue was snap-frozen in liquid nitrogen then stored at -80°C for RNA extraction and other biological molecular experiments. To analyze the association between miR-184 expression and clinicopathological features of astrocytomas, a panel of 76 tumor specimens were collected, including 50 primary GBMs (grade IV), 13 AAs (grade III) and 13 DAs (grade II) (see **Table 1** for patients' information in detail). Subsequently, expression level of miR-184 was examined on all of the 76 tumors and 10 non-neoplastic brain tissues by real-time PCR. These non-neoplastic brain tissues used as controls were obtained by collecting donations with consents from individuals who died in traffic accidents and were confirmed to be free of any prior pathological lesions. On the other hand, to observe

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Table 1. Correlation of miR-184 relative expression level with clinicopathological factors of astrocytoma patients

Clinicopathological features	No. of cases	miR-184 expression		P values
		High (n, %)	Low (n, %)	
WHO grade				≤ 0.001
II	13	13 (0.0%)	0 (100.0%)	
III	13	10 (76.9%)	3 (23.1%)	
IV	50	15 (30.0%)	35 (70.0%)	
Age				0.031
> 50	48	19 (39.6%)	29 (60.4%)	
≤ 50	28	19 (67.9%)	9 (32.1%)	
Gender				1.000
Male	41	20 (48.8%)	21 (51.2%)	
Female	35	18 (51.4%)	17 (48.6%)	
KPS				0.011
< 90	42	15 (35.7%)	27 (64.3%)	
≥ 90	34	23 (67.6%)	11 (32.4%)	

Abbreviations: KPS, Karnofsky performance scale.

the alteration of miR-184 expression during the malignant progression of astrocytoma, expression level of miR-184 was determined in primary lower-grade (grade II or III) and recurrent higher-grade (grade III or IV) tumor pairs derived from seven independent astrocytoma patients. In addition, a validation step involved analysis of 9 DAs, 8 AAs and 8 secondary GBM from 25 independent patients. All astrocytoma patients were well followed up, and overall survival time was calculated from the date of the initial surgical operation to death. Patients, who died of diseases not directly related to their astrocytomas or due to unexpected events, were excluded from this study. The present study was approved by the Ethics Committee of China Medical University.

Glioma cell lines and human astrocyte

The astrocytoma cell lines U87, U251, U373, T98G and SF295 were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA) and maintained in Dulbecco's modified eagle's medium (DMEM) (Gibco, USA) supplemented with 10% fetal bovine serum (FBS) (Gibco) and penicillin/streptomycin (100 U/mL). The human astrocyte was a gift from Dr. T. Sasaki (Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan) and maintained in DMEM supplemented with 2% FBS and 1% N-2 supplement (Gibco).

RNA extraction, reverse transcription and real-time PCR quantification for miRNA detection

Total RNA was extracted from frozen tissues of astrocytoma and non-neoplastic brain using a mirVana miRNA Isolation Kit (Ambion, Austin, TX, USA) according to the manufacturer's instruction. RNA concentration was determined using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA), and RNA quality was measured using a denaturing 15% polyacrylamide gel. To examine the expression levels of miR-184 in astrocytoma tissues and cell lines, cDNA synthesis and subsequent quantitative real-time PCR were performed using a TaqMan MiRNA Reverse Transcription Kit (Applied Biosystems) and individual TaqMan miRNA assay (Applied Biosystems), and Applied Biosystems 7500HT Fast Real-Time PCR System (Applied Biosystems), as previously described [27]. RNU6B were used as endogenous controls, and non-neoplastic brain tissues and human astrocyte were used for calibrations. Relative quantification of miR-184 expression was calculated with the $2^{-\Delta\Delta Ct}$ method.

Statistical analysis

All computations were carried out using the software of SPSS version 19.0 for Windows (SPSS Inc., Chicago, IL, USA). Data were expressed as mean ± standard deviation (SD). Student's t-test was used to compare the expression levels of miRNAs between different subtypes of astrocytoma, as well as between astrocytomas and non-neoplastic brains. Associations of miR-184 expression with clinicopathological features and the ki-67 index were estimated using a Pearson's chi-square test and Pearson's correlation analysis, respectively. A life table was calculated according to the Kaplan-Meier method. Risk ratios for the time-to-event endpoint were estimated using the multivariate Cox regression analysis in a forward stepwise method to evaluate the effect of multiple independent prognostic factors on overall survival outcome. Differences were con-

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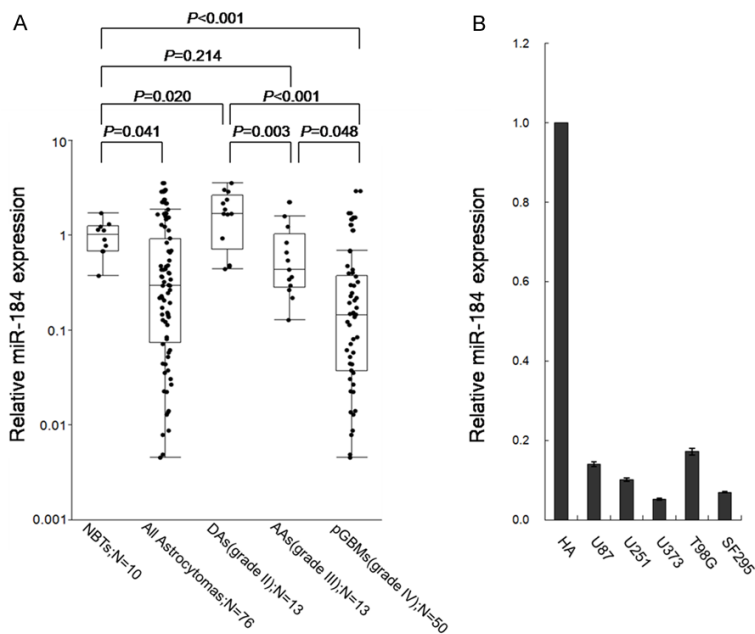


Figure 1. MiR-184 expression in 76 astrocytoma tissues, 5 cell lines compared with 10 non-neoplastic brain tissues and human astrocyte, respectively, detected by qRT-PCR analysis. A. MiR-184 was significantly down-regulated in astrocytoma tissues compared with non-neoplastic brains tissues ($P = 0.041$). Its expression level was decreased with the increasing malignancy degree of the tumor. B. Astrocytoma cell lines showed remarkably lower expression of miR-184 in comparison with human astrocyte (HA).

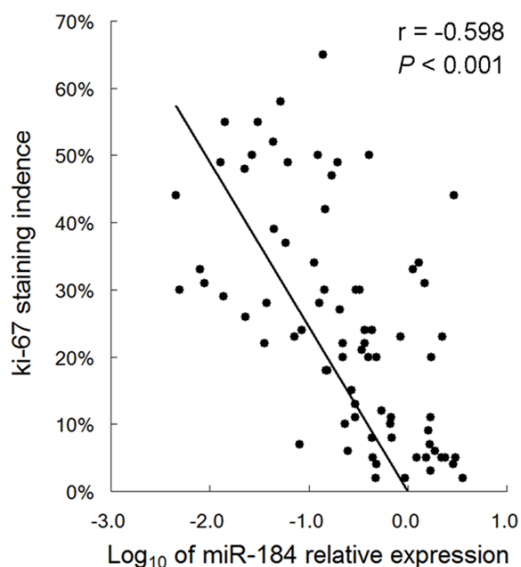


Figure 2. Pearson's correlation analysis to evaluate the correlation of miR-184 expression with the ki-67 labeling index in 76 astrocytoma tissues.

considered statistically significant when P was less than 0.05.

Results

MiR-184 was down-regulated in astrocytoma tissues and cell lines

To detect the aberrant expression of miR-184 in astrocytoma tissues and cell lines, we compared its expression level between tumor tissues and non-neoplastic brains, as well as between tumor cell lines and a human astrocyte. First, miR-184 expression was examined and statistically compared between a panel of 76 gliomas including 50 pGBMs (grade IV), 13 AAs (grade III), 13 DAs (grade II), and 10 non-neoplastic brain tissues, by qRT-PCR. As shown in **Figure 1A**, miR-184 was significantly decreased in astrocytoma tissues as compared with non-neoplastic brains (fold change = 0.66; $P = 0.041$, Student's t -test). In addition, we also statistically compared miR-

184 expression of each malignancy grade of astrocytomas with that of non-neoplastic brains. We found that grade II DAs had an about 1.80-fold higher expression of miR-184 relative to brain tissues ($P = 0.020$, **Figure 1A**). However, no significant difference was observed between miR-184 expression of grade III AAs and normal brain tissues ($P = 0.214$, **Figure 1A**). In contrast, expression level of miR-184 was remarkably reduced in grade IV pGBMs as compared with that in brain tissues (fold change = 0.35; $P \leq 0.001$, **Figure 1A**). Furthermore, miR-184 showed a decreased expression with the increasing degree of malignancy of astrocytomas (P values in grade II vs. III, grade II vs. IV and grade III vs. IV were 0.003, ≤ 0.001 and 0.048, respectively, **Figure 1A**). Moreover, miR-184 expression was examined in five commonly used model cell lines (U87, U251, U373, T98G, and SF295, **Figure 1B**) derived from human malignant astrocytomas. As demonstrated in **Figure 1B**, we found a profoundly decreased expression of miR-184 in these tumor cells. The expression level of miR-184 was about 0.05- to 0.17-fold lower in tumor cell

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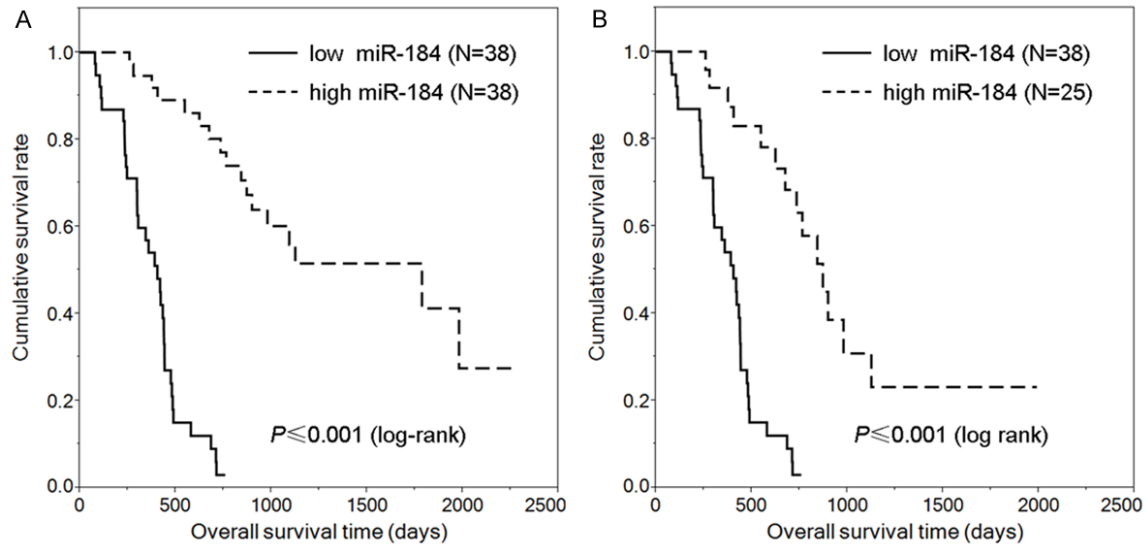


Figure 3. Prognostic performance of miR-184 for astrocytoma patients. A. Astrocytoma patients with low miR-184 expression (left, solid line, $n = 38$) had significantly shorter overall survival time than did patients with high miR-184 expression (right, dotted line, $n = 38$; $P \leq 0.001$, log-rank test). B. Among the 63 high-grade astrocytoma patients (grade III AAs and grade IV pGBMs), those with low miR-184 expression (left, solid line, $n = 38$) had significantly shorter survival periods than did patients with high miR-184 expression (right, dotted line, $n = 25$; $P \leq 0.001$).

lines relative to a human astrocyte. These results suggested that miR-184 might act as a tumor suppressor in astrocytoma tumorigenesis and its down-regulation might be involved in aggressive progression of astrocytoma.

Low miR-184 expression was associated with aggressive clinicalpathological features of astrocytoma

Subsequently, correlations of miR-184 expression with several clinicopathological features (tumor grade, patients' age at diagnosis, gender and pre-operative Karnofsky performance scale (KPS)) of these 76 patients mentioned above were statistically evaluated by χ^2 test as demonstrated in **Table 1**. Patients were assigned to high-miR-184 group ($n = 38$) and low-miR-184 group ($n = 38$) that were tumors with miR-184 expression above and under the median value of miR-184 expression in all of the 76 astrocytomas, respectively. As summarized in **Table 1**, low miR-184 expression was significantly associated with advanced malignancy degree of tumor ($P \leq 0.001$, χ^2 test), advanced patient's age ($P = 0.031$) and low KPS ($P = 0.011$). However, there was no statistically significant correlation between miR-184 expression and patient's gender. In addition, we statistically analyzed the correlation

between miR-504 expression and ki-67 labeling index in these astrocytoma tissues. As shown in **Figure 2**, the ki-67 index was negatively associated with \log_{10} of the relative miR-504 expression ($P < 0.001$, $r = -0.598$, Pearson's correlation analysis).

Expression level of miR-184 had prognostic implication in astrocytoma patients

We furthermore evaluated the potential prognostic performance of miR-184 expression in astrocytoma patients. As the result shown in **Figure 3A**, miR-184 expression displayed a significant correlation with astrocytoma patients' overall survival. We observed that patients with low-miR-184 expression ($n = 38$) had significantly poorer survival compared to patients with high-miR-184 group ($n = 38$) (median overall survival times for patients with low- and high-miR-184 expression were 404 and 1786 days, respectively, $P \leq 0.001$, log-rank test). In addition, univariate and multivariate analysis using Cox proportional hazard regression model were performed to statistically evaluate the possibilities for miR-184 expression and other clinicopathological features as independent prognostic indicators in astrocytoma patients. As summarized in **Table 2**, our result showed that both low-miR-184 in

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Table 2. Univariate and multivariate Cox regression analysis for overall survival in astrocytoma patients

Univariate analysis					Multivariate analysis			
Variant	No. of case (%)	Median OS	95% CI	<i>P</i> (log-rank)	Variant	RR	95% CI	<i>P</i>
WHO grade				≤ 0.001	WHO grade			≤ 0.001
II	13 (17.1%)	1980	1687-2273		IV vs. III vs. II	8.6	3.0-24.4	
III	13 (17.1%)	1124	765-1483					
IV	50 (65.8%)	418	367-469					
Age				≤ 0.001	Age			0.401
> 50	48 (63.2%)	442	368-516		> 50 vs. ≤ 50	0.7	0.3-1.7	
≤ 50	28 (36.8%)	1786	756-2816					
Gender				0.888	Gender			0.266
Male	41 (53.9%)	579	263-895		Male vs. Female	1.4	0.8-2.6	
Female	35 (46.1%)	547	291-803					
KPS				≤ 0.001	KPS			0.602
< 90	42 (55.3%)	439	396-482		< 90 vs. ≥ 90	1.2	0.6-2.5	
≥ 90	34 (44.7%)	1786	532-3040					
Surgical resection				0.182	Surgery			0.066
GTR	38 (50.0%)	710	293-1127		PR vs. GTR	1.8	1.0-3.4	
PR	38 (50.0%)	441	172-710					
miR-184 expression				≤ 0.001	miR-184 expression			≤ 0.001
Low	38 (50.0%)	404	300-508		Low vs. High	5.7	2.4-13.8	
High	38 (50.0%)	1786	681-2891					

Abbreviations: KPS, Karnofsky performance scale; GTR, gross total resection; PR, partial resection; OS, overall survival; RR, risk ratio.

tumor tissue ($P \leq 0.001$; risk ratio 5.7) and advanced malignancy degree ($P \leq 0.001$; risk ratio 8.6) were independent predictors of poor prognosis in glioma patients (**Table 2**). Moreover, we also performed Kaplan-Meier analysis to evaluate the prognostic performance of miR-184 expression in high-pathological grade astrocytoma patients (grade III AAs and grade IV pGBMs). Similarly, we observed that low-miR-184 expression showed a statistically significant correlation with poor clinical outcome in patients with these malignant astrocytomas ($P \leq 0.001$, **Figure 3B**).

MiR-184 was down-regulated in malignant progression of astrocytomas

Moreover, we discovered the dynamic alteration of miR-184 expression during the malignant progression of astrocytoma. Paired tumor tissues from seven patients with primary lower-grade astrocytomas (grade II DAs or grade III AAs) and the spontaneously recurrent higher-grade astrocytomas (grade III AAs or grade IV sGBMs) were collected for detection of miR-

184 expression by qRT-PCR. These seven astrocytoma patients displayed three different progression patterns of this tumor: two of them were with primary grade II DA that spontaneously progressed to grade III AA; three of them suffered from primary grade II DA that recurred as grade IV sGBM; whereas, other two had primary grade III AA and recurrent grade IV sGBM. As shown in **Figure 4A**, we observed that miR-184 was absolutely down-regulated during each of these three malignant progression patterns. It showed an about 1/2- to 1/20-fold significantly lower expression in the recurrent higher-grade astrocytomas relative to the corresponding primary lower-grade tumors (**Figure 4A**). To validate the progression-associated down-regulation of miR-184, we analyzed expression of miR-184 in an independent series of 9 grade II DAs, 8 grade III AAs and 8 grade IV sGBMs from another panel of 25 patients. As shown in **Figure 3B**, miR-184 showed an obvious decrease along with the increasing malignant grade of the tumor (mean \pm SD of relative miR-184 expression for grade II, III and IV astrocytomas were 2.70 ± 1.40 ,

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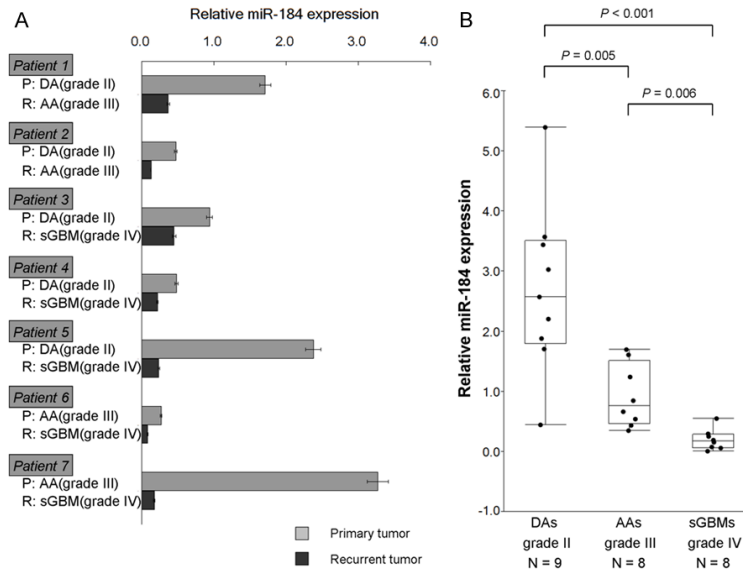


Figure 4. Alteration of miR-184 expression during the malignant progression of astrocytoma. A. Expression levels of miR-184 in seven astrocytoma patients with primary lower-grade tumors that recurred as higher-grade tumors. miR-184 showed an about 1/2- to 1/20-fold significantly lower expression in the recurrent higher-grade astrocytomas relative to the corresponding primary lower-grade tumors. Patient numbers 1-7 encode the individual patient; Gray and black columns indicate expression levels of miR-184 in primary and recurrent tumors, respectively. P, primary tumor; R, recurrent tumor. B. Validation experiment for miR-184 expression in an independent series of 9 DAs, 8 AAs and 8 secondary-GBMs. miR-184 showed a significant progression-associated down-regulation in gliomas with different WHO grade ($P = 0.005$, 0.006 and ≤ 0.001 for grade III vs. II, grade IV vs. III and grade IV vs. II, respectively).

0.92 ± 0.53 and 0.20 ± 0.17 , respectively). The differences were statistically significant between grade II and III ($P = 0.015$), grade III and IV ($P = 0.016$), as well as grade II and IV ($P \leq 0.001$) tumors (Figure 4B).

Discussion

In recent years, accumulating evidences have indicated that miRNAs play important roles in tumorigenesis and aggressive progression by regulating multiple oncogenes and tumor suppressors, in various human cancers. Previous studies have identified a number of dysregulated miRNAs including miR-21, miR-155, miR-196 and miR-326 in astrocytomas, the most frequent and aggressive tumors of human central nervous system. These miRNAs were demonstrated to play important roles in crucial biological processes such as cell proliferation, apoptosis and invasion, in tumorigenesis of astrocytoma [10-13]. In addition, their expression signatures have also been proved to have

substantial value of diagnostic and prognostic determinations for patients with these malignant brain tumors [14-17].

However, the biological functions of miR-184 in astrocytoma tumorigenesis still remain controversial and the clinical significances of miR-184 expression in astrocytoma patients are poorly understood. We in the present study showed that miR-184 was remarkably decreased in human astrocytoma tissues and cell lines as compared with non-neoplastic brain tissues and normal astrocyte, respectively. In addition, expression level of miR-184 in astrocytoma tissues negatively correlated with ki-67 staining index of the tumors and low miR-184 expression was significantly associated with aggressive clinicopathological features in astrocytoma patients. Furthermore, patients with low expression level of miR-184 in tumor tissues

had significantly poorer overall survival and low miR-184 expression was a statistically significant risk factor of poor survival for astrocytoma patients. Moreover, miR-184 was significantly down-regulated in the recurrent higher-grade astrocytomas compared with the corresponding primary lower-grade tumors. To our knowledge, this is the first study to analyze the expression profile and clinical significance of miR-184 in large panel of astrocytomas.

miR-184, which is located in region 25.1 of chromosome 15q, is particularly enriched in human brain and testes. Its corresponding transcript is comparatively small (84 bp) and is not encoded other clustered miRNAs [28, 29]. Previous studies have indicated that miR-184 is dysregulated in a variety of human cancers and acts as a tumor promoter or suppressor in an organ-specific fashion [18-21]. Functioning as a tumor suppressor, miR-184 was originally reported to be aberrantly expressed in neuroblastomas and involved in tumorige-

nesis of these aggressive pediatric tumors, in a global miRNA expression profiling study, by Chen et al. [18]. They found that miR-184 was significantly down-regulated in MYCN-amplified tumors that have poor prognosis as compared with other types of neuroblastoma, and their function analyses clearly showed that miR-184 overexpression induces apoptosis and cell cycle arrest in neuroblastoma cells [18]. The investigation by Foley et al. confirmed the tumor suppressive role of miR-184 and revealed that this miRNA inhibits cell survival by targeting AKT2 kinase in neuroblastoma [19]. Conversely, other researchers have revealed the potential oncogenic role of this miRNA. Wong et al. found that the plasma expression levels of miR-184 were significantly associated with the presence of primary tumors and might be used as a novel cancer marker in tongue squamous cell carcinoma [20]. In addition, Wu et al. demonstrated that miR-184 promotes cell proliferation in human hepatocellular carcinoma by post-transcriptionally regulating SOX7 expression [21].

However, the biological functions of miR-184 in astrocytoma tumorigenesis still remain controversial, and miR-184's clinical significance in astrocytoma patients is not clearly understood. Actually, miR-184 was first reported to function as a negative regulator in malignant progression of astrocytoma by Malzkorn et al. [22]. They found that miR-184 showed significantly decreased expression upon the progression from low-grade to high-grade astrocytomas. And their function analysis revealed that overexpression of miR-184 inhibits cell proliferation and invasion in glioma cell lines, A172 and T98G [22]. At almost the same time, Guan et al. reported that miR-184 was significantly down-regulated in grade IV astrocytomas as compared with grade III astrocytomas, by investigating the expression profiles of 365 miRNAs in 12 high-grade astrocytomas [16]. In addition, a recent study by Cheng et al. showed that miR-184 was down-regulated in astrocytoma tissues and decreased with the increasing degree of malignancy, although the number of cases used was limited [23]. These observations suggested the possibility that miR-184 might act as a tumor suppressor in tumorigenesis of astrocytoma. We in the present study increased the tumor cases to further confirm the expression and clinical implication of miR-184 in astrocytomas. In accordance with the results from these previous investigations, we

found that down-regulation of miR-184 significantly correlated with aggressive progression and poor survival in astrocytoma patients. Furthermore, recent studies have revealed the biological mechanisms by which miR-184 modulates astrocytoma tumorigenesis through functioning as a tumor suppressive miRNA. The study by Chen et al. mentioned above demonstrated that miR-184 inhibits cell proliferation and invasion by specifically targeting TNF- α in astrocytoma [23]. Meanwhile, Emdad et al. indicated that suppression of miR-184 in malignant astrocytomas down-regulates its direct target, SND1, and promotes tumor aggressiveness both *in vitro* and *in vivo* [24]. These collective data provided sufficient evidence that miR-184 functions as a negative regulator in astrocytoma tumorigenesis. Conversely, several other investigations have demonstrated that miR-184 acts as a tumor promotor in astrocytoma cells. Yuan et al. found that up-regulation of miR-184 enhances the malignant biological behavior of human astrocytoma cell line A172 by targeting FH1 [25]. Similarly, Cui et al. showed that miR-184 promotes proliferation ability of glioma cells by regulating FOXO3 [26]. Taken together, the detailed biological mechanism(s) through which miR-184 modulates tumorigenesis of astrocytoma still remains unclear, and thus needs further investigation.

On the other hand, miRNAs are directly involved in the progression in various cancers. For astrocytoma, DA of WHO grade II inherently tends to locally recur and spontaneously progress to AA of WHO grade III and eventually sGBM of WHO grade IV [1]. In previous studies, the molecular basis of astrocytoma progression has been investigated by analyzing chromosomal and genetic aberrations, and dysregulation of mRNA [30]. However, the involvement of miRNAs in astrocytoma progression is still not clearly understood. Malzkorn et al. originally identified and functionally characterized several candidate miRNAs that might contribute to the malignant progression of human astrocytoma, by analyzing the expression profiles of 157 miRNAs in 4 patients with primary WHO grade II DAs that spontaneously progressed to WHO IV sGBMs [22]. In addition, by analyzing data of Chinese Glioma Genome Atlas (CGGA), Yan et al. recently demonstrated that miRNA may play a critical role during progression from low grade gliomas to anaplastic gliomas or secondary

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glioblastomas and not contribute to the malignant progression from anaplastic gliomas to secondary glioblastomas [31]. In consistent with our results, Malzkorn et al. showed that expression of miR-184 was reduced upon the progression from grade II to grade IV astrocytomas [22]. We in the present study clearly showed that miR-184 was absolutely down-regulated during each of the three patterns (from grade II to grade IV, from grade II to grade III and from grade III to grade IV tumors) of astrocytoma progression from low-grade to high-grade tumors (**Figure 4A**). Our results confirm the progression-associated down-regulation of miR-184 and suggest miR-184 as an important candidate contributes to malignant progression of human astrocytoma.

In summary, we in the present study showed that expression of miR-184 was markedly reduced in both astrocytoma tissues and cell lines. In addition, our results demonstrated that miR-184 down-regulation correlated with aggressive clinicopathological features and poor survival in astrocytoma patients. Furthermore, we found that miR-184 was absolutely down-regulated during the malignant progression from low-grade to high-grade astrocytomas. Our data confirm the tumor suppressive role of miR-184 in astrocytoma tumorigenesis and suggest that miR-184 might serve as a prognostic and predictive biomarker, as well as a novel therapeutic target for these malignant brain tumors.

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Disclosure of conflict of interest

None.

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