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Concomitant microRNA-31 downregulation and radixin upregulation predicts advanced tumor progression and unfavorable prognosis in patients with gliomas

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ABSTRACT

Purpose: To clarify the clinical significance of microRNA-31 (miR-31) and radixin (RDX) in human glioma.
Methods: Quantitative real-time polymerase chain reaction (qRT-PCR) analysis was used to characterize the expression patterns of miR-31 and RDX mRNA in 108 glioma and 20 normal brain tissues. The associations of miR-31 and RDX mRNA expressions with clinicopathologic factors and prognosis of glioma patients were also statistically analyzed.
Results: The expression levels of miR-31 in glioma tissues were significantly lower than those in normal brain tissues ($P < 0.001$), while RDX mRNA was significantly overexpressed in glioma tissues compared with normal brain tissues ($P < 0.001$). There was a negative correlation between miR-31 and RDX mRNA expression in glioma tissues ($r = -0.69$, $P = 0.01$). Additionally, concomitant miR-31 downregulation and RDX upregulation (miR-31-low/RDX-high) was significantly associated with advanced pathological grade ($P = 0.001$) and low Karnofsky performance score ($P = 0.01$). Moreover, Kaplan–Meier survival and Cox regression analyses showed that the glioma patients with miR-31-low/RDX-high expression had poorest overall survival ($P < 0.001$) and conjoined expression of miR-31-low/RDX-high was an independent prognostic indicator of glioma ($P = 0.01$). Furthermore, subgroup analyses showed that miR-31-low/RDX-high expression was significantly associated with poor overall survival in glioma patients with high pathological grades (for grade III–IV: $P < 0.001$).
Conclusions: Our findings have implications concerning the importance of concomitant miR-31 downregulation and RDX upregulation in tumor progression and poor prognosis of patients with gliomas. A combined detection of miR-31/RDX expression may benefit us in predicting clinical outcomes of glioma patients with high pathological grades.

1. Introduction

Human gliomas, tumors of astrocytic glial cells, are the most frequently occurring primary central nervous system (CNS) malignancies [1]. Glioma cells are characterized by high proliferation and invasion potentials, which are the reasons for their aggressive and malignant phenotypes leading to unfavorable clinical outcomes [2]. According to the World Health Organization (WHO) classification, gliomas are divided into pilocytic astrocytoma (PA, WHO grade I), diffuse astrocytoma (DA, WHO grade II), anaplastic astrocytoma (AA, WHO grade III), and glioblastoma (GBM, WHO grade IV) in the order of increasing malignancy [3]. Among these, GBMs account for the vast majority of human gliomas, and are highly proliferative and invasive tumors characterized by remarkable biological heterogeneity and poor response to present treatments [4,5]. The original cell types of gliomas with different grades are still uncertain and the molecular determinants of their aggressiveness have been the subject of numerous investigations. However, few molecular signatures have been validated and widely accepted as prognostic indicators in clinical practice. Therefore, it is extremely necessary to screen more precise prognostic indicators and more effective therapeutic strategies for gliomas.

MicroRNAs (miRNAs) are short, endogenous non-coding small RNAs with 22–25 nucleotides long [6,7]. Functionally, miRNAs negatively regulate gene expression either by degrading specific mRNA or inhibiting translation. According to the roles of their target genes, miRNAs are implicated into the regulation of processes such as development, differentiation, cell proliferation and apoptosis [8]. Since many of these processes are often altered in carcinogenesis, it is not surprising to find that miRNAs are linked to variety of cancers, in which miRNAs were shown to act like either oncogenes or tumor suppressors. Moreover, miRNAs may be transferred between glioma cells and adjacent cells through gap junctions and induce targeted inhibition of protein expression in the acceptor cells [9] and they also participate in cell to cell signaling via an exosomal-mediated transfer between cells [10]. A number of studies have measured the miRNA expression profiles in human glioma tissues and several specific miRNA patterns for this tumor have been evidenced. miR-31, located

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at 9p21.3, has been found to be aberrantly expressed in cancer cells [11–18]. miR-31 can either elicit promoting or inhibitive effects on cancers depending on cancer types. Especially in human gliomas, Hua et al. [19] demonstrated that miR-31 may be down-regulated in GBM compared with normal brain tissues. They also found that the ectopic expression of miR-31 can inhibit migration and invasion ability of glioma cells by regulating migration and invasion related gene radixin (RDX) which belongs to the ezrin–RDX–moesin family [20–22]. Accumulating studies have indicated the importance of the interaction between miR-31 and RDX involved in tumor progression [23,24]. However, the clinical significance of miR-31 and RDX in human glioma has not been fully elucidated.

In the current study, we examined the expression levels of miR-31 and RDX mRNA in glioma tissues of various WHO grades, and explored the association between miR-31 and RDX mRNA expressions, and the clinicopathologic parameters of gliomas. In addition, we also determined the prognostic value of the miR-31 and RDX mRNA expressions in patients with gliomas.

2. Materials and methods

2.1. Patients and tissue samples

This study was approved by the Research Ethics Committee of Second Hospital of Hebei Medical University, P.R. China. Written informed consent was obtained from all of the patients. All specimens were handled and made anonymous according to the ethical and legal standards.

One hundred and eight human glioma tissue samples for qRT-PCR were obtained from the Department of Neurosurgery, Second Hospital of Hebei Medical University. Samples were quickly removed at surgery and immediately divided into two parts: one part was fixed in 4% paraformaldehyde for 24 h, paraffin embedded and used for histopathological diagnosis, and the remaining part was snap frozen in liquid nitrogen and maintained at −80 °C until used for RNA isolation. All the slides were re-evaluated according to WHO classifications [3] by two pathologists, with differences resolved by careful discussion. A total of 64 males and 44 females (1.45:1) were enrolled in this study, and the median age was 43 years (range, 13–72). Thirty of the 108 gliomas were classified as low-grade [18 pilocytic astrocytomas (WHO I) and 12 diffuse astrocytomas (WHO II)], and 78 were classified as high-grade gliomas [32 anaplastic astrocytomas (WHO III), and 46 primary glioblastomas (WHO IV)]. None of the patients had received chemotherapy or radiotherapy prior to surgery. Patients with WHO grade I and II gliomas received stereotactic fractionated radiotherapy to a total dose of 45 Gy postoperatively. Patients with WHO grade III gliomas received stereotactic fractionated radiotherapy to a total dose of 60 Gy postoperatively. Patients with WHO grade IV gliomas received stereotactic fractionated radiotherapy to a total dose of 60 Gy and three courses of carmustine given at 4-week intervals postoperatively. The clinicopathologic features of all patients were indicated in Table 1. Twenty normal brain tissue samples 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. The controls contained 12 males and 8 females (1.5:1), and the median age was 42 years (range, 21–56). These normal control samples were collected by partial resections of normal brain tissue required as decompression treatment for severe head injury to reduce increased intracranial pressure.

All patients had complete five-year follow-up until death. 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 gliomas or due to unexpected events, were excluded from this study.

2.2. RNA extraction and qRT-PCR for miRNA detection

Total RNA and small RNA from fresh glioma and normal brain tissues were respectively extracted using RNeasy Mini Kit (Qiagen, Valencia, CA, USA) and mirVana miRNA Isolation Kit (Ambion, Austin, TX, USA) according to the manufacture's instruction. Total RNA (1 mg) and small RNA (10 ng) were respectively synthesized with the ReverTra Ace qRT Kit (Toyobo, Osaka, Japan) and TaqMan MicroRNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA). To generate cDNA of miRNA, 10 ng of small RNA was first denatured at 70 °C with 50 nM stem-loop RT primer (miR-31 5′-GTC GTA TCC AGT GCT GGG TCC GAG TGA TTC GCA CTG GAT ACG ACC AGC TA-3′) for 5 min before quenching on ice, and then 1 mM final of each of the four deoxynucleotide triphosphates, 1 U/μl ribonuclease inhibitor, 10 U/μl M-MLV reverse transcriptase and 1 × M-MLV RT buffer (TaKaRa Biotechnology Co., Ltd, Dalian, China) were added together to make up a final volume of 10 μl reaction mix. qRT-PCR was performed on

Table 1
Association of miR-31 or RDX mRNA expression in human glioma tissues with different clinicopathologic features.

Clinicopathologic features	No. of cases	miR-31-low (n, %)	P	RDX-high (n, %)	P	miR-31-low/RDX-high (n, %)	P
WHO grade							
I	18	0 (0)	0.001	0 (0)	0.001	0 (0)	<0.001
II	12	4 (33.33)		4 (33.33)		1 (8.33)	
III	32	15 (46.88)		17 (53.13)		8 (25.00)	
IV	46	39 (84.78)		39 (84.78)		39 (84.78)	
Age							
<50	42	23 (54.76)	NS	23 (54.76)	NS	18 (42.86)	NS
≥50	66	35 (53.03)		37 (56.06)		30 (45.45)	
Gender							
Male	64	36 (56.25)	NS	36 (56.25)	NS	28 (43.75)	NS
Female	44	22 (50.00)		24 (54.55)		20 (45.45)	
Tumor size							
≥6 cm	75	40 (53.33)	NS	42 (56.00)	NS	33 (44.00)	NS
<6 cm	33	18 (54.55)		18 (54.55)		15 (45.45)	
KPS							
<90	78	46 (58.97)	0.01	46 (58.97)	0.02	38 (48.72)	0.01
≥90	30	12 (40.00)		14 (46.67)		10 (33.33)	

Note: 'NS' refers to the difference without statistic significance.