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Clinical Study

Overexpression of tissue microRNA10b may help predict glioma prognosis

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ABSTRACT

We investigated the relationship between microRNA-10b (miR-10b) expression and prognosis in human glioma patients. Quantitative real-time polymerase chain reaction (qRT-PCR) analysis was used to characterize the expression patterns of miR-10b in 128 glioma and 20 normal brain tissues. Clinical information – age, sex, Karnofsky Performance Status (KPS) and World Health Organization (WHO) grade – were also collected. The associations between miR-10b expression and the clinicopathological factors and outcome of glioma patients were statistically analyzed. Expression levels of miR-10b in glioma tissue were significantly higher than in normal brain tissue ($P < 0.001$). High-grade glioma (WHO grade III and IV) had much higher miR-10b expression levels than low-grade tumors (WHO grade I and II). Additionally, the increased miR-10b expression in the glioma tissues was significantly associated with a low KPS ($P = 0.03$). Kaplan–Meier survival curves and Cox regression analyses showed that overexpression of miR-10b ($P = 0.01$) and high grade ($P = 0.02$) were independent factors predicting poor outcome for glioma patients. Furthermore, subgroup analyses showed that the miR-10b expression level was significantly associated with poor overall survival in glioma patients with high grades ($P < 0.001$). Up-regulation of miR-10b may have value in predicting clinical outcome in glioma patients, particularly for those with high pathological grades.

1. Introduction

Glioma is the most common intracranial tumor worldwide [1]. According to the World Health Organization (WHO) classification, gliomas are classified as pilocytic astrocytoma (WHO grade I), diffuse astrocytoma (WHO grade II), anaplastic astrocytoma (WHO grade III) and glioblastoma (GBM, WHO grade IV), in order of increasing malignancy [2]. Despite progress in surgical techniques, radiotherapy, chemotherapy and target therapy, patient prognosis remains poor [3]. The mean survival time for GBM patients is only 12 to 14 months [4]. Therefore, there is an urgent need to further explore the molecular mechanisms of treatment-resistant GBM to improve therapeutic effects in these patients.

MicroRNA (miRNA or miR) are a class of small non-coding RNA measuring approximately 21 nucleotides in length; they regulate approximately 30% of human genes at the post-transcription level and, subsequently, affect the biological processes of cells [5]. Currently, there are more than a hundred ongoing trials incorporating miRNA, including miR-21, miR-210 and miR-10b, as biomarkers. The miR-10b gene is located in the middle of the HOXD cluster on chromosome 2q31, near HOXD4. miR-10b inhibits the translation of mRNA encoding HOXD10, which modulates many genes that promote invasion, migration, extracellular matrix remodeling and tumor progression [6]. Inhibition of miR-10b reduces glioma cell growth through cell cycle arrest and apoptosis [7]. In addition, several studies have reported that miR-10b expression is associated with glioma grade and that its expression is significantly lower in low-grade gliomas compared to high-grade astrocytic tumors [8,9]. However, the association between miR-10b expression in gliomas and prognosis has not been reported to our knowledge. Therefore, the aim of this study was to investigate the clinical significance of miR-10b expression in human gliomas.

2. Materials and methods

2.1. Patients and tissue specimens

The study was approved by the Ethics Committee at Shandong Cancer Hospital, China. Written informed consent was obtained

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from all patients. A total of 128 glioma patients, including 40 low-grade tumors and 88 high-grade tumors, were collected from the Department of Neurosurgery, Shandong Cancer Hospital, China, from January 2005 to December 2010. Resected tissue samples were immediately cut and snap-frozen in liquid nitrogen before being stored at −80°C until RNA extraction. None of the patients had undergone radiotherapy or chemotherapy prior to surgery. Twenty normal brain tissues samples taken from patients who underwent surgery for reasons such as cerebral trauma served as the control group. All tissue samples were re-evaluated by two pathologists using the WHO classifications. In the follow-up period, overall survival was measured from diagnosis to death or last follow-up. Clinical information of age, sex, Karnofsky Performance Status (KPS) and WHO grade were also collected.

Clinical follow-up was available for all patients (median, 27 months; range, 2–105 months). By the end of follow-up, 22 patients were still alive, and 106 had died. Overall survival was calculated from the date of the initial surgery until death. All patients who died from other diseases or unexpected causes were excluded from the evaluation.

2.2. Real-time quantitative reverse transcription-polymerase chain reaction for miRNA

Total RNA was purified from the 128 glioma tissue and 20 control brain tissues using Trizol reagent (Invitrogen, Carlsbad, CA, USA). Real-time absolute quantification was utilized to insure the sample quality. miR-10b and RNU6B (as an internal control) specific cDNA were synthesized from total RNA using gene-specific primers, according to the TaqMan MicroRNA assays protocol (Applied Biosystems, Foster City, CA, USA). The reverse transcription products were then amplified and detected through real-time polymerase chain reaction (PCR) using the Taqman MicroRNA Assay (Applied Biosystems) specific for miR-10b. Quantitative mRNA expression data were acquired and analyzed using the ΔΔ-Ct method with an Applied Biosystems 7500 real-time PCR system.

2.3. Statistical analysis

All statistical analyses were performed using the Statistical Package for the Social Sciences version 18.0 software (IBM, Armonk, NY, USA). The measurement data were analyzed using one-way analysis of variance. The correlation between miR-10b and the clinicopathological index was assessed using Spearman's rank tests. Survival curves were plotted using the Kaplan–Meier method, and differences between the survival curves were tested using the log-rank test. Cox's proportional hazards model was used to identify the factors with an independent influence on survival, and $P < 0.05$ was considered to be significant.

3. Results

3.1. Quantitative analysis of miR-10b expression levels in glioma using quantitative reverse transcription-PCR

The expression levels of miR-10b in glioma tissues and normal brain tissues were analyzed using real-time quantitative reverse transcription-PCR. miR-10b exhibited a higher expression level in glioma tissues compared with normal brain tissues ($P < 0.01$, Fig. 1). In addition, the expression of miR-10b increased with WHO tumor grade ($P < 0.01$, Fig. 1). miR-10b was up-regulated in all gliomas, particularly high-grade tumors, with a mean value of 39.74 for grade III gliomas and 120.42 for grade IV gliomas. The median value of miR-10b was used as the boundary for grouping high and low expression levels of miR-10b; thus, the cutoff value for distinguishing high and low expression was defined as 40.265.

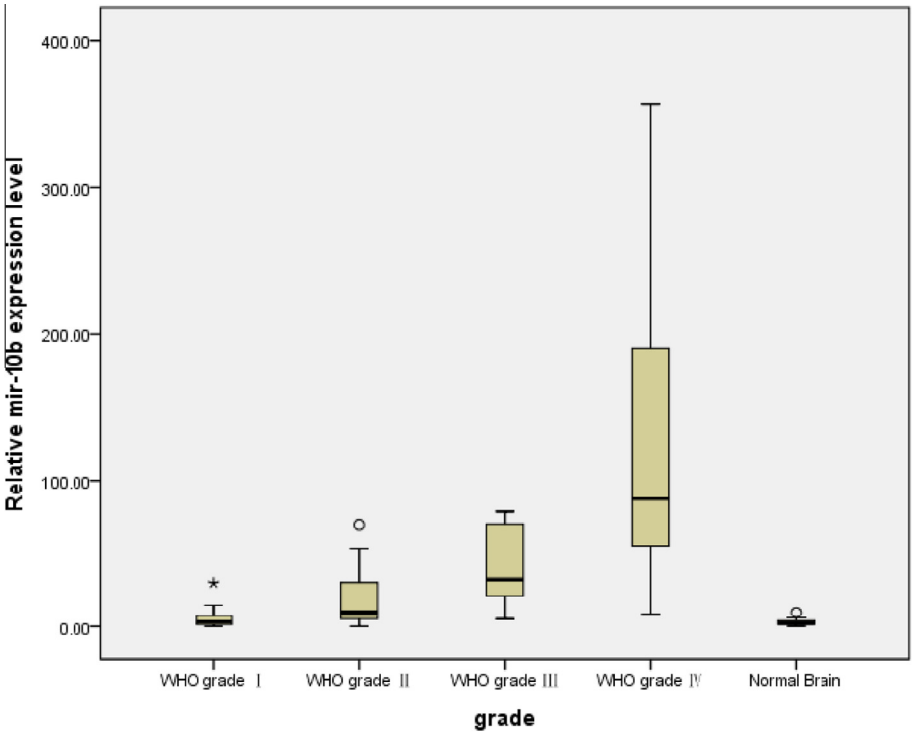

Fig. 1. Relative miR-10b expression in human gliomas. All tumor specimens were obtained from the center of the tumor. Quantitative miRNA expression data were analyzed using the ΔΔ-Ct method. The expression level of miR-10b in non-neoplastic brain tissues served as the control. The expression of miR-10b was normalized to that of the U6B small nuclear RNA gene (RNU6B) control. The cutoff value for distinguishing between high and low miR-10b expression levels was defined as the median value, 40.265. miR-10b = microRNA-10b, WHO = World Health Organization.