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Original Article

Overexpression of HLA-DR is associated with prognosis of glioma patients

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Abstract: Aims: Since the poor prognosis of glioma, our study was aimed to find out the role of *HLA-DR* in the prognosis of glioma patients that may contribute to the timely post-operative treatment on the glioma patients. Methods: 60 glioma patients were enrolled in the prospective cohort study. Western blotting was used to detect the content of *HLA-DR*. Kaplan-Meier curve was adopted to evaluate the effects of *HLA-DR* on the survival time of glioma patients. Cox regression analysis was used to evaluate the roles of clinical features and *HLA-DR* in the pathogenesis of glioma. Results: The expression level of *HLA-DR* was higher in tumor tissue, compared with normal tissues ($P < 0.05$). Moreover, expression levels of *HLA-DR* were correlated with the factors of pathological degree, Enneking staging and KPS score. The survival rate of patients with high content of *HLA-DR* was lower than those of patients with low content of *HLA-DR*. Cox regression analysis indicated that Enneking staging and *HLA-DR* were all associated with the prognosis of glioma (HR=14.43, 95% CI=1.05-199.16; HR=21.39, 95% CI=2.07-220.76). Conclusion: *HLA-DR* may serve as a biomarker for the prognosis of glioma patients.

Keywords: *HLA-DR*, glioma, prognosis

Introduction

Glioma, mainly deriving from neuroepithlial tissue, accounts for 40%-50% of intracranial tumors and 1.5% of malignant tumors of whole body and among 100,000 population, there are 3.5-4.5 individual suffering glioma [1, 2]. It is well known that glioma is characterized with high malignant degree, poor prognosis and life-threat, which make it rank the 11th in various tumors with high mortality rate [3]. So it is urgent to find out a biomarker for glioma prognosis that contributes to comprehensive grasp on the state of patients and timely treatment. Some studies have investigated several potential indicators for the outcome of glioma [4-7]. However, the relationship of *HLA-DR* and glioma prognosis has not been identified.

HLA-DR belongs to type II cell-surface antigen encoded by major histocompatibility complex (MHC), which is exclusively distributed in immune cells with the function of stimulating allogenic immune reactions and regulating the intercellular immune response [8]. Recently, HLA class II antigen has been reported to serve as a favorable prognostic marker of colorectal carcinoma [9], which indicated that *HLA-DR* might be a predictive marker for prognosis of glioma.

So our study was conducted to detect the level of *HLA-DR* and then investigate whether there was significant association of *HLA-DR* and glioma prognosis.

Materials and methods

Subjects

From January 2006 until January 2008, relative data were obtained from 60 glioma patients with postoperative pathology confirmation in Neurosurgery Department of First Affiliated Hospital of Chongqing Medical University. Meanwhile, 30 samples of normal brain tissues re-

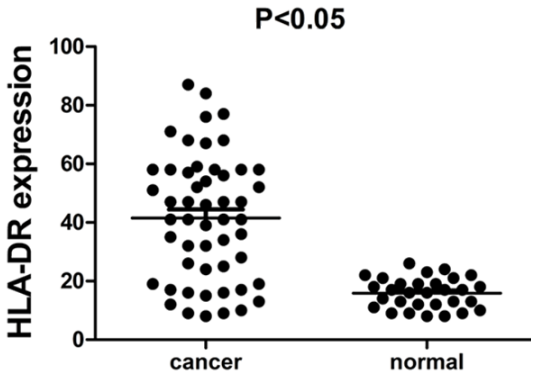

Figure 1. Comparison of HLA-DR expression level between cancer tissues and normal tissues.

Table 1. Clinical characteristics

Variables	Glioma patients (n=60)
Tumor site	
Supratentorial	27
Subtentorial	33
Tumor size	
>5 cm	38
<5 cm	22
Pathological degree	
Low	26
High	34
Enneking staging	
IB	18
IIA	26
IIB	16
KPS score	
>70	18
<70	42

sected in the surgery of internal decompression. The subjects in the survival analysis must meet the following demand: (1) operation on the first episode of supratentorial tumor without anti-tumor therapy; (2) treated with conventional radiotherapy after surgery; (3) fine follow-up compliance; (4) surgery and after-surgery review with magnetic resonance imaging (MRI) were operated by same group of senior physicians and tumors were confirmed as total or subtotal resection; (5) patients were all adult without obesity and diabetes. And the patients were excluded if they were incompliant in the observation, lost to follow-up, dying from other causes or receiving other anti-tumor therapies (e.g. operation) besides conventional radiotherapy.

Methods

Western blotting: Tumor tissues and normal tissues were completely homogenated, then were qualified by the method of Coomassie brilliant blue (CBB). Same amount of protein were tested by SDS-PAGE and transfered with semi-dry film method. After transfered on the nitrocellulose membrane, the samples were sealed by 5% skim milk, followed by warming process overnight with primary antibody, rinsing by PBS, reaction with second antibody labeled by HRP and color development. Based on the content of β-actin, the level of *HLA-DR* was measured.

Statistical methods: Mann-Whitney U test was applied to evaluate differences on *HLA-DR* expression between groups. χ^2 test was used to analyze the correlation between *HLA-DR* expression levels and clinical pathologic features. Kaplan-Meier curve was used to evaluate survival rate between high content and low content of *HLA-DR*. Cox regression analysis was used to evaluate the roles of clinical features and *HLA-DR* in the pathogenesis of glioma. All the statistical analysis were performed with SPSS 18.0 software and P value < 0.05 indicates statistical significance.

Results

Expression level of HLA-DR

In contrast to normal tissues, content of *HLA-DR* in tumor tissue of giloma was much higher (P < 0.05) (**Figure 1**).

Association of HLA-DR with clinical pathologic features

Clinical features of subjects were collected and listed in (**Table 1**).Then we analyzed the association of *HLA-DR* with clinical pathologic features, the results indicated that the expression level of *HLA-DR* was not correlated with factors of tumor site and tumor size (**Figure 2A**, **2B**), however, there was a close relationship of expression of *HLA-DR* with factors of pathological degree, Enneking staging and KPS score (**Figure 2C-E**).

Association of HLA-DR with outcome of glioma patients

In the follow-up observation, 13 patients were survived, while 41 were died and 6 were cen-

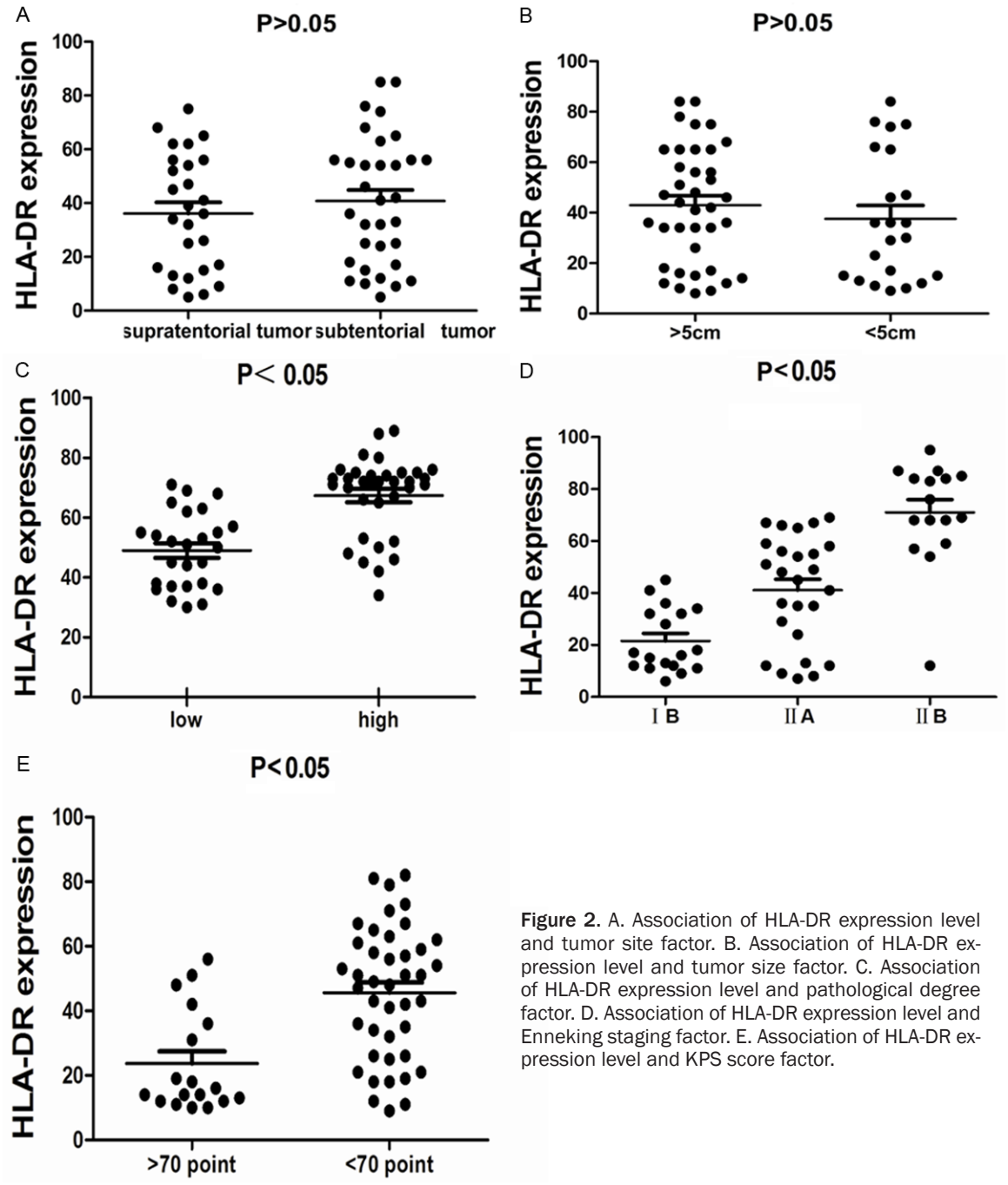

Figure 2. A. Association of HLA-DR expression level and tumor site factor. B. Association of HLA-DR expression level and tumor size factor. C. Association of HLA-DR expression level and pathological degree factor. D. Association of HLA-DR expression level and Enneking staging factor. E. Association of HLA-DR expression level and KPS score factor.

sored. Patients with high content of *HLA-DR* were found with survival rate of 16.7% (6/36) and patients with low content of *HLA-DR* was 38.9% (7/18) (**Figure 3**).

Cox regression analysis

Cox regression suggested that Enneking staging appeared to be a predictive index for glioma prognosis (HR=14.43, 95% CI=1.05-199.16). Meanwhile, we also found that *HLA-DR* could be a biomarker for glioma prognosis (HR=21.39, 95% CI=2.07-220.76) (**Table 2**).

Discussion

Glioma, a common type of intracranial tumors with high malignancy, is characterized by rapid growth, power invasiveness, easy to relapse and poor prognosis [10, 11]. At present, there

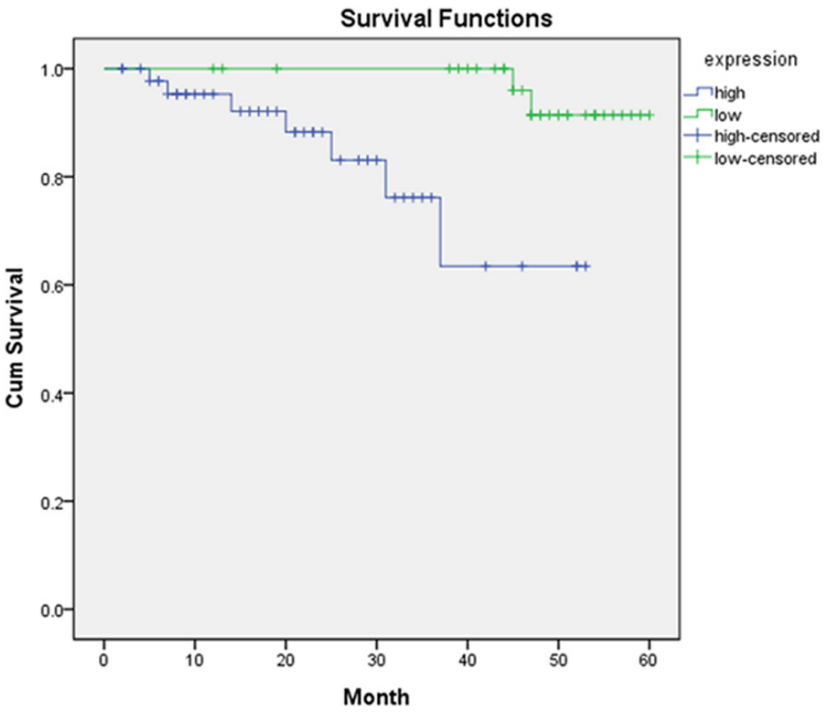

Figure 3. Kaplan-Meier survival curve.

Table 2. Cox regression analysis

	B	SE	Wald	df	Sig.	HR	95.0% CI	
							Lower	Upper
HLA-DR	3.063	1.191	6.614	1	0.010	21.389	2.072	220.762
Enneking staging	2.670	1.339	3.974	1	0.046	14.433	1.046	199.159

exist two huge problems in treatment for glioma. On the one hand, due to high malignancy, rapid development, short disease course and 3-5d tumor cell cycles, the postoperative recurrence of glioma cannot be avoided even after the total section. On the other hand, the tumor cells are adapt to strongly invade adjacent normal tissues, several lobes and even deep structures and may approach the contra hemisphere via corpus callosum [12, 13]. So it is urgent to avoid metastasis and improve the outcome of glioma patients. Our study was aimed to explore the role of *HLA-DR* in glioma prognosis and provide a predictive index for glioma prognosis.

HLA gene, located on the short arm of chromosome 6, is a 4000 kb gene complex composed of hundreds of tightly connected gene groups, which is known for maximum allele polymorphisms so far and is closely related to the functions of immune system in humans [8, 14-17]. At present, approximately 224 genes within *HLA* have been identified, whereas specific functions of majority of genes remains unknown [18]. Based on sequence, classic *HLA* gene is generally divided into three regions: HLA-I, HLA-II and HLA-III [19]. Antigen encoded by HLA-II gene mainly appears on the cytomembrane of macrophage and B lymphocyte. The whole cytomembrane is crossed over by α and β chains of HLA-II antigen. HLA-II was divided into HLA-DP, HLA-DQ, *HLA-DR*, HLA-DN and HLA-DO [20].

Nowadays, the research on *HLA-DR* primarily concentrates on diseases of immune system [21-24]. However, there was no report on the function of *HLA-DR* in the glioma prognosis. Our result suggested that the expression level of *HLA-DR* was closely associated with the malignant degree of glioma, which indicates that *HLA-DR* could serve as a biomarker for the malignant degree of glioma.

The prognosis of glioma patients is influenced by many factors, so our study took the factors of tumor site, tumor size, pathological degree, Enneking staging, KPS score and *HLA-DR* expression into consideration. Cox regression result indicated that Enneking staging and *HLA-DR* expression exhibited prognostic values for glioma. For Enneking staging, we labeled the staging of benign tumors with Arabic numerals and malignant tumors with roman numerals. IA and IB stood for low malignant, and IIA and IIB for high malignant. The higher the degree was, the poor prognosis the glioma patients would get.

Our study found that expression level of *HLA-DR* was high in tumor tissue of glioma compared with that of normal tissues and *HLA-DR* expression was significantly associated with

outcome of glioma patients, high content of *HLA-DR* corresponding to increased mortality. In summary, up-regulated expression of *HLA-DR* involves in the pathogenesis of glioma, which can serve as a biomarker for glioma prognosis.

Disclosure of conflict of interest

None.

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