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Nuclear Factor Erythroid 2-Related Factor 2 and Heme Oxygenase-1 Protein Expression and Clinicopathological Features in Glioblastoma

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Abstract

Glioblastoma is one of the most common primary brain tumors, and local recurrence and distant metastasis are common posttreatment manifestations in patients. The overall five-year survival still remains poor. Nuclear factor erythroid2-related factor2 (Nrf-2) and heme oxygenase-1 (HO-1) have been considered to play major roles in the pathogenesis of many tumors. In this study, the expressions of Nrf-2 and HO-1 in glioblastoma were investigated to explore the possible impacts of them on the growth of glioblastoma. 49 cases of glioblastoma patients were analyzed to summarize their gender, age, tumor recurrence, size of tumor, postoperation radiotherapy and chemotherapy. Immunohistochemistry (SP) was applied to evaluate the expression of Nrf-2 and HO-l in pathological sections of 49 cases of giloblastoma and 23 cases of adjacent control tissues. Results showed that the positive rates of Nrf-2 and HO-1 in the 49 glioblastoma tissue sections were 85.7% and 89.8%, respectively, and that of Nrf-2 and HO-1 were 34.8% and 26.1%, respectively in 23 cases of adjacent control sections. There were significant differences between the two groups ($P < 0.001$). Furthermore, positive correlations were confirmed between the expression of Nrf-2 and HO-1 ($r = 0.440$, $P < 0.05$). There were no correlations among gender, age, tumor recurrence, size of tumor, postoperation radiotherapy and chemotherapy ($P > 0.05$). Nrf-2 and HO-1 may play an important role in the pathogenesis of glioblastoma, and might be a potential therapeutic target for glioblastoma.

Keywords

Glioblastoma, rf-2, HO-1, Immunohistochemistry

1. Introduction

Glioblastoma is one of the most common primary brain tumors, and the stan-

dard treatment for this is safe surgical resection with combination radiotherapy and adjuvant temozolomide chemotherapy. However, local recurrence and distant metastasis are common posttreatment manifestations in patients. The overall five-year survival still remains poor with an average survival of 14 months after diagnosis [1] [2]. Moreover, various side effects are produced that can greatly influence a patient's quality of life. Efficient methods of treatment for giloblastoma are still lacking. Oxidative stress and reactive species-induced damage to molecules and organelles play important and interactive roles in cancer initiation and progression. Genes in apoptotic signaling pathways related to oxidative stress is likely to become potential targets for cancer treatment. Nrf-2 is a member of transcription factor and plays a critical coordinator as regulating the redox balance and protecting cells against oxidative and inflammatory lesions. The expression of Nrf-2 altered in many oxidative stress related diseases, such as asthma, pulmonary fibrosis, renal fibrosis, Parkinson's disease, *et al.* HO-1 is one of the rate-limiting enzymes of the heme oxygenase, HO-1 expression increased in stress and also constitutively active in many tumor types [3] [4]. The Keap1-Nrf2 pathway regulates the expression of numerous cytoprotective genes including antioxidant ones such as HO-1. Our previous study showed that the expression of Nrf-2 and HO-1 increased in vitro model of Alzheimer disease and neuroblastoma. In this study, the expression of Nrf-2 and HO-1 in glioblastoma was studied by immunohistochemistry to explore the role of Nrf-2 and HO-1 in the formation and development of glioblastoma.

2. Materials and Methods

2.1. Patients and Tissues

Glioblastoma tissue samples from 49 patients and control brain tissue samples from 23 tissues adjacent to the tumor were diagnosed between September 2005 and December 2010 at the Department of Pathology in Chongqing Medical University. The diagnoses of glioblastoma were based on a combination of clinical information, morphologic examination and immunohistochemical results.

2.2. Immunohistochemistry

Nrf-2 and HO-1 expression were analyzed by immunohistochemistry.

Antigen retrieval was carried out by steaming (20 minutes at 80˚C) in citrate buffer at pH 6.0. The following primary antibodies were used: a polyclonal anti-Nrf-2 antibody (Santa Cruz Biotechnology, CA, USA), diluted 1:200; a polyclonal anti-HO-1 antibody (Santa Cruz Biotechnology, CA, USA), diluted 1:100. Antigen visualisation was achieved by applying a standard streptavidin-perosidase (S-P) method, with diaminobenzidine as the chromogen. Sections treated without primary antibodies served as negative controls.

2.3. Assessment of Immunoreactivity

The positive reaction was defined as discrete localization of the chromogen in the cytoplasm and nuclear of all slices. The intensity of cytoplasmic and nucleic

reaction were graded as negative (-, positive cells percentage bellow 5%), mild positive (+, positive cells percentage is 6% - 25%), moderate positive (++, positive cells percentage is 26% - 50%) and strong positive (+++, positive cells are above 51%).

2.4. Statistical Analysis

The statistical analyses were performed using SPSS 16.0 (SPSS Inc., Chicago, IL, USA). The parametric variables were analyzed by using spearman rank correlation analysis. $P < 0.05$ was regarded statistically significant.

3. Results

3.1. Immunohistochemical Expression of Nrf-2 and HO-1 in Glioblastoma and Control Brain Tissue

We employed immunohistochemistry to evaluate the expression of Nrf-2 and HO-1 in glioblastoma and normal brain tissues. The results of the immunohistochemical staining of Nrf-2 and HO-1 are summarized in **Figure 1** and illustrated in **Figure 2**.

3.2. Relationship between the Expression of Nrf-2 and HO-1 in Glioblastoma and Control Brain Tissue

Positive expression of both Nrf-2 and HO-l was seen in 40 cases in giloblastoma tissues. Negative expression of both Nrf-2 and HO-l was seen in 7 cases. 2 cases only expressed Nrf-2 protein and 3 cases only expressed HO-l protein. The expression of Nrf-2 is positively correlated to the expression of HO-1 ($r = 0.440$, $p < 0.05$).

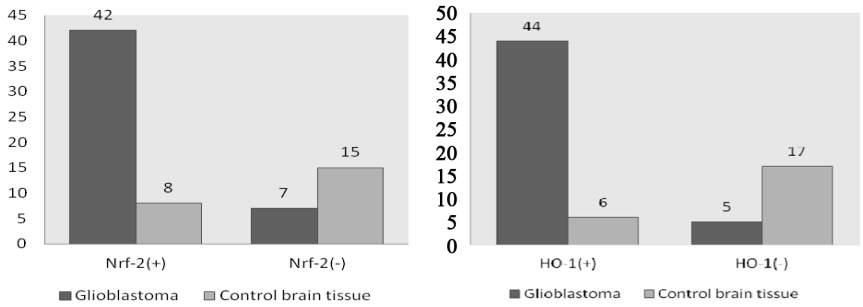

Figure 1. Expression of Nrf-2 in glioblastoma and control brain tissue.

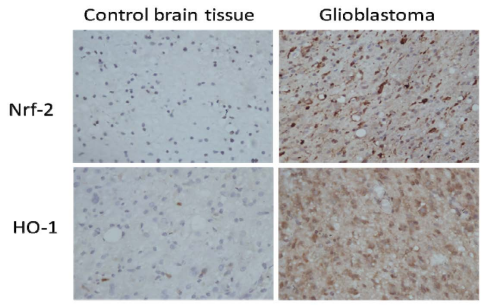

Figure 2. Expression of Nrf-2 and HO-1 in glioblastoma and control brain tissue (×400).

3.3. Relationship of Nrf-2 and HO-1 Expression with Clinicopathological Features of Glioblastoma

There were 28 males and 21 females ranging in age from 13 to 74 years (mean 48.8 years). 71.4% patients (35/49) were younger than 60 years old. Glioblastoma recurred in 34.7% patients (17/49). The tumor sizes were less than 1.5c m in 79.6% (39/49) patients. 18.4% (9/49) patients were taken radiotherapy and chemotherapy. There was also no significant association of Nrf-2 and HO-1 expression with the clinical features of glioblastoma. Clinicopathological findings and classification are summarized in **Table 1**.

4. Discussion

Glioblastoma is the most common primary malignant brain tumor, comprising 16% of all primary brain and central nervous system neoplasms [5]. Although glioblastomas occur almost exclusively in the brain, they can also appear in the brain stem, cerebellum, and spinal cord. Current standard therapy includes maximal safe surgical resection, followed by concurrent radiation with temozolomide (TMZ), an oral alkylating chemotherapy agent, and then adjuvant chemotherapy with TMZ. Extensive and complete surgical resection of glioblastoma is difficult because these tumors are frequently invasive and are often in eloquent areas of the brain, including areas that control speech, motor function, and the senses. Because of the high degree of invasiveness, radical resection of the primary tumor mass is not curative, and infiltrating tumor cells invariably remain within the surrounding brain, leading to later disease progression or recurrence. Despite maximal initial resection and multimodality therapy, about 70% of glioblastoma patients will experience disease progression within one year of diagnosis, with less than 5% of patients surviving five years after diagnosis [6]. Improving prognosis is still the hotspot of glioblastoma research. Living organisms are frequently exposed to oxidative stress and toxic insults and may damage DNA and proteins, as a consequence the cellular processes are disturbed. Nrf-2 is the master regulator of antioxidant defenses, regulating more than 200 cytoprotective genes in response to oxidative stress. Nrf-2 is repressed through

Table 1. Relationship of Nrf-2 and HO-1 expression with clinicopathological features in glioblastoma.

		Gender		Age		Tumor size		Recurrence		Radiotherapy and chemotherapy	
		Male	Female	≤60years	>60years	≤5cm	>5cm	Yes	No	Yes	No
Nrf-2	+	15	11	21	5	19	7	9	17	1	25
	++	6	5	6	5	10	1	3	8	4	7
	+++	3	2	2	3	5	0	1	4	2	3
	Number(%)	28(57.1)	21(42.9)	35(71.4)	14(28.6)	39(79.6)	10(20.4)	17(34.7)	32(65.3)	9(18.4)	40(81.6)
	$\chi 2$	0.00		0.20		0.01		1.82		0.05	
	P	>0.05		>0.05		>0.05		>0.05		>0.05	
HO-1	+	8	8	13	3	11	5	5	11	5	11
	++	17	8	16	9	23	2	9	16	3	22
	+++	1	2	2	1	2	1	1	2	0	3
	Number(%)	28(57.1)	21(42.9)	35(71.4)	14(28.6)	39(79.6)	10(20.4)	17(34.7)	32(65.3)	9(18.4)	40(81.6)
	$\chi 2$	0.67		0.20		1.32		0.07		0.01	
	P	>0.05		>0.05		>0.05		>0.05		>0.05	

binding to the homodimeric protein Kelch-like erythroid cell-derived protein with CNC homology associated protein 1 (Keap1) in the cytosol under unstressed conditions. Studies of Nrf-2 have suggested that its induction can ameliorate neurodegeneration, whereas its deficiency can exacerbate neurodegeneration. Nrf-2 activated at late stages of all neurodegenerative diseases, expression of Nrf-2 decreased in the brains of Alzheimer's (AD), and an increased nuclear staining of Nr-f2 in surviving neurons of postmortem Parkinson's disease (PD) patients. Pre-treatment of pharmacological activators of Nrf-2 has been shown to be protective in animal models of numerous neurological conditions such as Huntington's disease, amyotrophic lateral sclerosis, PD, AD, and cerebral ischemia. These protective effects have also been seen in breast cancer, colorectal cancer and adenocarcinoma of lung. When oxidative or electrophilic agents cause a conformational change in Keap1, it will regulate the expression of antioxidant and detoxifying genes such as heme oxygenase 1 (HO-1). Nrf-2 and HO-1 are frequently upregulated in different types of tumours and correlate with tumour progression, aggressiveness, resistance to therapy, and poor prognosis [7] [8] [9] [10]. However, the role of HO-1 in cancer biology is not completely understood and some disputes in literature remain about its role in tumour progression, especially with regard to different types of tumours. Several studies have reported that HO-1 activation prevents breast cancer proliferation and prostate cancer angiogenesis and mediates the anticancer activity of some drugs. In our study, although there was no significant difference in Nrf-2 and HO-1 staining according to the clinical features of the patients, but there was an increased expression of Nrf-2 and HO-1 (85.7% and 89.8%) in giloblastoma, obviously higher than that(34.8% and 26.1%) in control tissues. It indicated the positive expression of Nrf-2 in GBM and might play an important role in the pathogenesis of glioblastoma. Furthermore, the results showed that the expression of Nrf-2 is positively correlated to the expression of HO-1 ($r = 0.440$, $p < 0.05$) in these cases, it seemed the upregulation of Nrf-2 and the activation of HO-1 in tumour progression which correlates with cancer aggressiveness and malignancy. When HO-1 activation is dependent on Nrf-2 activity, it leads to highly aggressive cancer phenotypes. The mechanisms and regulators for the expression of Nrf-2 and HO-1 need to be further studied to provide new directions and targets for developing therapeutic intervention.

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