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Quantitative analysis and biological significance of HOXD10 and BFGF in glioma patients

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Abstract

Objective To investigate the expression and biological significance of gomeotic genes-A13(HOXD10) and Basic fibroblast growth factor (BFGF) in gliomas. **Methods** 60 patients diagnosed with glioma were diagnosed in our hospital from Jan 2016 to December 2019 were chosen. The serum expression levels of S100, HOXD10 and Plexin -B2 in the experimental group (glioma patients) and the control group were measured by qRT-PCR. And the expression of HOXD10 and BFGF in different stages of glioma patients was analyzed, the relationship with the degree of tumor malignancy was analyzed. **Results** Compared with those of the control group, the expression levels of HOXD10 and BFGF in cerebrospinal fluid and plasma were significantly higher[blood: HOXA,13,(1.21 ± 0.14)vs 0; BFGF, (1.57 ± 0.33)vs 0 and cerebrospinal fluid: BFGF,(4.87 ± 1.13)vs 0; BFGF,(5.88 ± 0.21)vs 0].The larger the tumor volume, the lower the degree of differentiation and the higher the expression level of HOXD10 and BFGF in the blood. The level of HOXD10 and BFGF protein in cerebrospinal fluid was significantly higher than that in plasma. **Conclusion** The expression of HOXD10 and BFGF in brain glioma patients is up-regulated and is significantly correlated with the degree of malignancy. The expression of HOXD10 and BFGF in cerebrospinal fluid is significantly increased and can be used as a marker of malignant degree of glioma.

Keywords: Glioma; HOXD10; BFGF; qRT-PCR

Glioma is one of the most common malignant tumors in brain tumors, and it is one of the malignant tumors that seriously endanger human health. According to the recent statistics published by the National Cancer Control Office, the mortality rate of brain tumor adjustment is ranked as the tenth leading malignant tumor. The incidence rate of male brain in big city is tenth [1-2].crucial. In this study, we detected the expression of HOXD10 and BFGF in plasma and cerebrospinal fluid (CSF) to explore the biological significance of HOXD10 and BFGF in glioma, It is reported as follows.

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1. Materials and methods

1.1 general information

All patients in the control group were treated by pathology from January 2016 to December 2019. Inclusion criteria: the pathological diagnosis was clear and the patients agreed. Exclusion criteria: severe complications affecting the experimental process; severe liver and kidney damage; pregnant and lactating women. According to the 2014 Chinese guidelines for molecular diagnosis and treatment of gliomas, there were 30 cases of grade I-II glioma and 30 cases of grade III-IV glioma. There was no significant difference in general condition of patients ($P > 0.05$). See Table 1. Patients were informed of the content and purpose of the experiment, and informed consent was signed.

Table 1 general clinical data of two groups

Group n	Average age (years)	Male/female	Average body mass index(kg/m^2)
Experimental group 60	62(69-79)	28⁄32	23.5(18.8-26.8)
Control group 30	65(43-76)	13⁄17	20.1(16.7-27.5)

1.2 Total RNA extraction

PCR primers were designed with primer 5.2: Forward Primer: AAGGATGCCGTGGATAAATTGC; Reverse Primer: ACACGATGAACTCACTGAAGTC; HOXD10: Forward Primer: CTCCCGCGCTAAGGAGTTC; Reverse Primer: CCGGCACCACTGGCATATC; BFGF: Forward Primer: ATGCCAACTCCCTCTCCTG; Reverse Primer: CTAGGCGTATT TCCACGAT- GC。All primers were synthesized by Shanghai bioengineering company. Collect 3-5 ml of non anticoagulant blood and store it in refrigerator at 4 ℃. Centrifuge at 4 ℃ for 10 min at 3500 R. collect the upper serum and freeze it in - 80 ℃ refrigerator with 200 UL / tube branch number. The total RNA extraction kit was provided by BTEC. RT-PCR kit was produced by Takara company in Japan. The total RNA was extracted from plasma and cerebrospinal fluid of all patients according to the total RNA extraction kit, and the cDNA was synthesized with oligo according to the instructions of RT kit.

1.3 qRT-PCR

The gene expression was detected by SYBR Green I fluorescence quantitative PCR on iqtm5, and the results were analyzed by the instrument. The results were repeated three times. The reverse transcription conditions were 95 ℃ 30 s, 95 ℃ 5 s, 60 ℃ 30 S-40 cycles.

1.4 observation index

The expression of S100, HOXD10 and BFGF in plasma and cerebrospinal fluid of experimental group and control group were different; the expression of S100, HOXD10 and BFGF protein in different grades of glioma were different. If S100, HOXD10 and BFGF protein can be detected respectively, the expression of S100, HOXD10 and BFGF protein is positive; otherwise, it is negative.

1.5 statistical methods

SPSS13.0 software was used to analyze the experimental data, The measurement data are as follows($\overline{X}$ ±*s*)express, The difference was statistically significant by t test and χ^2 test in the counting data.

2. Results

2.1 expression of S100, HOXD10 and BFGF in plasma and cerebrospinal fluid of patients with glioma in two groups

The expression of S100 in healthy patients was higher than that in glioma patients [(6.98 ± 0.23) vs (1.01 ± 0.19), t = 1.908, P < 0.05], but HOXD10 and BFGF were not detected. However, the expression levels of HOXD10 and BFGF were significantly increased in both plasma [HOXD10: (1.21 ± 0.14); BFGF: (1.57 ± 0.33)] or cerebrospinal fluid [BFGF: (4.87 ± 1.13); BFGF: (5.88 ± 0.21)]. The expression levels in CSF were higher than those in plasma (t = 2.571, P < 0.01). Only one patient did not detect the expression of HOXD10 and BFGF, the positive rate was 96.76%. See Figure 1.

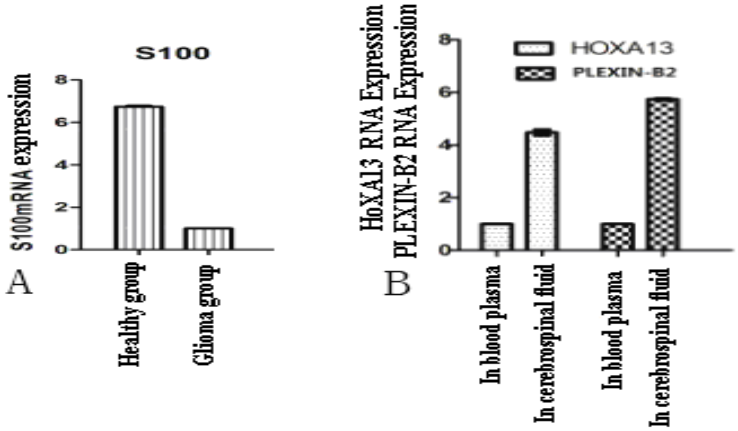

Figure 1 expression of S100, HOXD10 and BFGF in plasma and cerebrospinal fluid of the two groups(Figure A: the expression of S100 protein in healthy patients was significantly higher than that in glioma patients; in Figure B, the expression of HOXD10 and BFGF in glioma patients was significantly up-regulated, and the increase of this protein in cerebrospinal fluid was more significant

2.2 expression of S100 protein in different grade gliomas

S100 protein can be detected in serum and cerebrospinal fluid of healthy patients. The positive rate of S100 protein in glioma patients decreased gradually with the increase of malignant degree (x2 = 1.773, P < 0.05), as shown in Table 2.

Table 2 expression of S100 protein in different grade gliomas [n (%)]

group		serum	cerebrospinal fluid
control group		30(100)	30(100)
experience group	Glioma grade I-II	26(13.33)*	25(83.33)*
	Glioma grade III-IV	4(13.33)	5(16.67)

Note: compared with grade III-IV glioma, (x2 = 1.773, * P < 0.05)

2.3 expression of HOXD10 protein in different grades of gliomas in experimental group

HOXD10 protein was not expressed in serum and cerebrospinal fluid of healthy patients. The positive rate of HOXD10 protein was gradually increased with the increase of malignant degree (x2 = 1.878, P < 0.05). See Table 3.

Table 3 expression of HOXD10 protein in different grade gliomas of experimental group [n (%)]

experience group	serum	cerebrospinal fluid
Glioma grade I-II	9(30.00)	8(26.67)
Glioma grade III-IV	21(70.00)	22(73.33)

Note: compared with grade I-II glioma, (x2 = 1.878, * P < 0.05)

2.4 expression of BFGF protein in different grades of gliomas in experimental group

There was no expression of BFGF protein in serum and cerebrospinal fluid of healthy patients. The expression of BFGF protein was significantly increased in glioma patients, and the positive rate of BFGF protein increased gradually with the increase of malignant degree (x2 = 3.112, P < 0.05). See Table 4.

Table 4 expression of BFGF protein in different grades of gliomas in experimental group [n (%)]

experience group	serum	cerebrospinal fluid
Glioma grade I-II	3(10.00)	28(93.33)
Glioma grade III-IV	27(90.00)*	2(6.67)*

Note: compared with grade I-II glioma, X2 = 3.112, * P < 0.05

3. Discussion

Glioma is the most common malignant brain tumor. The incidence rate remains high, so early detection and early diagnosis of glioma are of great importance. Glioma is a heterogeneous group of tumor formation, which accounts for the majority of primary tumors in the central nervous system; despite the progress made in the combined treatment of radiation and chemotherapy, the curative effect of patients with good prognosis is still frustrating, and the median survival after diagnosis is about 15 months [3-4]. Therefore, it is necessary to identify new biomarkers specific to glioma stage. Although in recent years, with the development of imaging, the diagnosis and treatment of glioma have made great progress, but the overall curative effect has not been significantly improved, and the 5-year survival rate is still around 6% [5-6].

S100 protein is an acidic Ca2 + binding protein family, which participates in the regulation of intracellular and extracellular factors. Calcium ion plays an important role in the biological effect of S100 protein [7]. S100 protein is involved in many intracellular and extracellular functions, such as regulating intracellular Ca2 + homeostasis, cell prolife-

ration and apoptosis, cell invasion, protein phosphorylation, cell scaffold construction, autoimmunity and inflammation [8]. Studies have shown that S100 protein is negatively correlated with tumor proliferation [9]. The results showed that the more severe the lesion, the higher the pathological grade, the less S100 protein, suggesting that the less S100 protein or silencing the expression of S100 protein may provide the basis for the treatment of glioma.

HOXD10 was first found in Drosophila melanogaster with 183 base pairs, which encodes 61 highly conserved amino acid peptides, known as homologous domains [10-11]. HOXD10 is located at the 5 'end of HOXA. Recent studies have shown that HOXD10 is involved in carcinogenesis and promoting tumor growth. Some reports have shown that abnormal expression of HOXD10 in [12-13] is associated with hepatocellular carcinoma, and overexpression of HOXD10 suggests that HCC patients are prone to metastasis and have poor prognosis. The experimental data showed that HOXD10 gene was related to the degree of malignancy, and the higher the malignant degree, the higher the expression of HOXD10 gene. Studies have shown that Hox family genes are associated with tumor grade and predict poor prognosis in glioma [14]. HOXD10 inhibits the growth of glioma cells in glioma cell lines and xenotransplantation models [15].

Basic fibroblast growth factor (BFGF) is a transmembrane receptor, which participates in axonal guidance and cell migration in response to signal elements, and participates in tumor cell invasion and angiogenesis. Studies have shown that glioma patients have a median survival period of less than 2 years, with poor prognosis and poor quality of life [16-17]. BFGF plays a key role in glioma growth and invasion [18]. In addition, recent analysis of samples from patients with gliomas has identified BFGF as a potential biomarker for advanced gliomas [19]. In addition, previous studies have established plexin B2 as an important regulator of migrating embryonic and adult neural precursor cells [20]. Studies have shown that BFGF is up-regulated in human glioma, and its expression level is related to glioma grade and poor survival. The mechanism may be that plexin B2 activation changes actin cytoskeleton and promotes glioma cell migration [21].

In conclusion, HOXD10 and BFGF may be novel markers for the diagnosis and prognosis of patients with glioma. As a potential prognostic biomarker and a new drug target for advanced gliomas.

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