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Research Article

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High expression levels of fascin-1 protein in human gliomas and its clinical relevance

Abstract: Introduction: The fascin-1 protein is a cytoskeleton-like protein, which can prompt structural changes in cell membranes and affect the integrity of intercellular relations to promote invasion and metastasis of tumor cells. In this study, we researched the expression of fascin-1 in glioma.

Material and methods: The fascin-1 protein and mRNA were detected by immunohistochemistry and reverse transcription polymerase chain reaction (RT-PCR). Then, we analyzed the relationship between the expression of fascin-1 protein and the clinical pathological characteristics of patients with glioma. Finally, the fascin-1 protein expression status and prognosis of glioma patients were investigated.

Results: The fascin-1 protein was mainly located in the cytoplasm of cells from glioma. The high expression rate of fascin-1 protein in glioma tissue was higher than that of normal brain tissue. At same time, we found that high fascin-1 protein expression was significantly correlated with World Health Organization (WHO) grading of glioma patients. The results survival analysis suggested high expression of fascin-1 protein in glioma patients with a shorter survival time. Multivariate analysis showed that high expression of fascin-1 protein was an independent predictor of the prognosis of patients with glioma.

Conclusions: High expression of the fascin-1 protein indicates poor prognosis for glioma patients.

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1 Introduction

Glioma is a gliacyte tumor originating from the brain neuroderm. It is the most common malignant tumor of the nervous system and accounts for about 45% of all intracranial tumors detected [1]. Characteristics of brain gliomas include high rates of morbidity, mortality, and recurrences. In China, the annual incidence of glioma is 3-6/100,000 people with 30,000 related deaths reported per year [2]. Although excision, chemoradiotherapy, and biotherapy are employed as interventions against glioma, recurrence of the tumor may occur in a short period. The current research focus includes a search for pathogenic factors of glioma that could be effectively translated into therapeutic targets for brain tumors. According to the World Health Organization (WHO) Pathological Grading Standard (2007 version), gliomas are divided into four levels. Grade I has the lowest degree of malignancy and patients have the best prognosis. However, grade IV has the highest degree of malignancy and patients have the worst prognosis.

Cell migration and invasion play a crucial role in the occurrence, development, and metastasis of tumors. These biological responses of tumor cells are closely linked to the structural abnormalities and expression levels of cytoskeletons [3-4]. Fascin-1 protein is a cytoskeleton-organizing protein with a relative molecular mass of 55 kDa [5]. It can prompt structural changes in cell membranes and affect the integrity of intercellular interactions to promote invasion and metastasis of tumor cells [6-7]. Fascin-1 mainly exists in interstitial tissue with low or no expression in epithelial tissues [5, 6]. Previous studies have shown that fascin-1 is highly expressed in many malignancies including gastric cancer, esophageal cancer, ovarian cancer, and lung cancer [8-10]. Often, high expression of fascin-1 is closely associated with certain markers of tumor invasion. This study discusses the expression of fascin-1 in gliomas and its probable role in the clinic.

2 Methods

2.1 Tissues specimens

Samples of glioma and normal brain tissues from 120 patients, who underwent surgery at the Longnan Hospital from January 2006 to December 2012, were collected for this study; the samples consisted mainly of glioma tissue and the adjacent normal brain tissue. Normal brain tissue adjacent to the brain tumors was used as the negative control. Diagnoses of all patients were confirmed by pathology and immunohistochemistry aided by complete clinical data. There were 72 men and 48 women among the 120 patients with glioma, aged 24–61 years, with an average age of 43.2 ± 10.1 years. Tumor classification was carried out according to the WHO Pathological Grading Standard (2007 version) [11]. As per the staging criteria, there were 27 cases of Grade I, 33 cases of Grade II, 25 cases of Grade III, and 35 cases of Grade IV disease. These 120 patients with glioma received no anti-cancer treatment such as chemotherapy, radiotherapy, or biotherapy before surgery. Patients or their relatives submitted a signed informed consent form. The ethics committee of the Longnan Hospital approved the research protocol.

2.2 Immunohistochemistry and evaluation of fascin-1 protein expression

Immunohistochemical assays were performed using the streptavidin-peroxidase (S-P) method according to the manufacturer's protocol. The staining procedures carried out included conventional deparaffinizing of paraffin sections, antigen retrieval, blocking of endogenous peroxidase with 3% hydrogen peroxide, blocking non-specific binding with goat serum, and incubation with human anti-fascin-1 monoclonal antibody (1:400; Santa Cruz Biotechnologies, Santa Cruz, CA, USA). After washing, the sections were incubated with secondary antibodies and a streptavidin–peroxidase complex, followed by color development with diaminobenzidine (DAB), contrast staining, and mounting of sections for microscopic evaluation. Sections of known normal tissue were used as the positive controls and PBS was used to replace the primary antibody in the negative controls. A single-blind method (where pathologists were unaware of the clinical data) was used to evaluate the sections.

Five moderate magnification (×200) fields were selected randomly, and 200 tumor cells were counted in each field, with 1000 cells counted in total. Staining intensity was classified into four levels according to the intensity of color [10]. 0: no staining; 1: light yellow staining; 2: light brown staining; and 3: brown staining. Staining intensity and percentage of fascin-1-positive tumor cells were assessed. The final scores were calculated by multiplying the scores of the intensity with those of the extent. In this study, 0 and 1 were defined as low fascin-1 expression while scores of 2 and 3 were denoted to high expression of the protein.

2.3 Reverse transcription polymerase chain reaction (RT-PCR)

Total RNA was extracted from 50 mg tissue with Trizol reagents and the purification was conducted according to the kit instructions (Cat: 12183555, Invitrogen, Carlsbad, USA). The 500 ng total RNA was reversely transcribed into cDNA (Cat: 6210A, TaKaRa, Dalian, China) by oligo dT primer (reaction volume: 10 ul) and then PCR amplification (Biometra, Tadvanced 96 SG, Jena, German) was carried out with PCR amplification kit (Cat: R011, TaKaRa, Dalian, China) according to the manufacturer instructions. Briefly, PCR mixture: 1 ul cDNA; 2 ul 10×PCR Buffer; 1.6 ul dNTP; 1 ul primers, (10 uM); 0.1 ul Taq enzyme; 14.3 ul ddH2O. Reaction conditions were as follows: pre-denaturation at 94°C for 5 min, denaturation at 94°C for 30 s, annealing at 56.2 □ for 30 s (the annealing temperature of the internal-reference GAPDH was 65°C), extension at 72°C for 45 s with a total of 35 cycles, and extension at 72°C for 2 min. After that, 1.5% agarose gel was prepared and 6 μL of PCR products were set to electrophoresis. The JS-780 Gel Imaging System (CHINCAN, ZHEJIANG, China) was used to carry out analysis of the bands. The fascin-1 gene-specific primers for PCR amplification were as follows: 5′-CTGGCTACACGCTGGAGTTC-3′ (forward primer) and 5′-CTG AGTCCCCTGC TGTCTCC-3′ (reverse primer). The GAPDH primers as follows: 5′-TCAACTTTCGATG-GTAGTCG-3′ (forward primer), and reverse primer, 5′-TCCTCGTTAAAGGATTTAAA-3′ (reverse primer).

2.4 Statistical methods

By using χ^2 test, the relationship between fascin-1 expression and clinicopathological parameters of the patients with glioma was analyzed. Fascin-1 mRNA expression in glioma tissues was compared with expression in normal adjacent tissues using the Mann-Whitney test (for two groups), and the results were analyzed by 2-dCt method. One-way analysis of variance (ANOVA) was used to

compare the relative expression of fascin-1 mRNA in different grades of gliomas. The Kaplan-Meier and log-rank testing methods were applied to perform survival analysis in the 120 patients with glioma. Overall survival (OS) and progression-free survival (PFS) were the primary outcomes of this study. The Cox proportional hazard model was used to analyze the combined outcomes for various variables. SPSS 17.0 statistical software (SPSS Inc., Chicago, USA) was used for statistical analysis. If P value was < 0.05, the difference was considered statistically significant.

3 Results

3.1 Fascin-1 expression in glioma

According to the results of the immunohistochemical evaluation, fascin-1 was mainly located in the cytoplasm of cells obtained from glioma tissue (Figure 1). As expected, the expression rate of fascin-1 protein in glioma tissue was higher (46.7%, 56/120) than that in normal brain tissue (28.3%, 34/120), and this difference was significant (χ^2 = 8.604, P = 0.003). In order to validate these results, we randomly selected 60 glioma tissues and normal tissues, and performed a quantitative estimation of fascin-1 mRNA levels by using RT-PCR. We found that the content of fascin-1 mRNA in glioma cells was higher than that in normal brain tissue (P < 0.05, Figure 2). According to the

WHO grades of gliomas, we found that the levels of fascin-1 mRNA differed significantly in tissue samples obtained from glioma of various pathological grades (Figure 3).

3.2 Expression of fascin-1 protein in glioma and its correlation with clinicopathologic factors

Based on the immunohistochemical results, we divided patients into two groups: low-expression fascin-1 protein group and high-expression fascin-1 protein group. Concurrently, we analyzed the relationship between the expression of fascin-1 protein and the clinicopathological characteristics of patients with glioma. We found that high fascin-1 protein expression was significantly correlated with the WHO tumor grading in patients with glioma (*P* = 0.001). No significant correlation was observed between fascin-1 protein expression levels and other clinicopathologic variables, such as age, gender, Karnofsky performance score, tumor size, and extent of resection (P > 0.05, Table 1).

3.3 Survival analysis of patients with glioma

In order to investigate the correlation between expression of fascin-1 protein and prognosis in patients with glioma, we carried out long-term follow-up of all patients. After
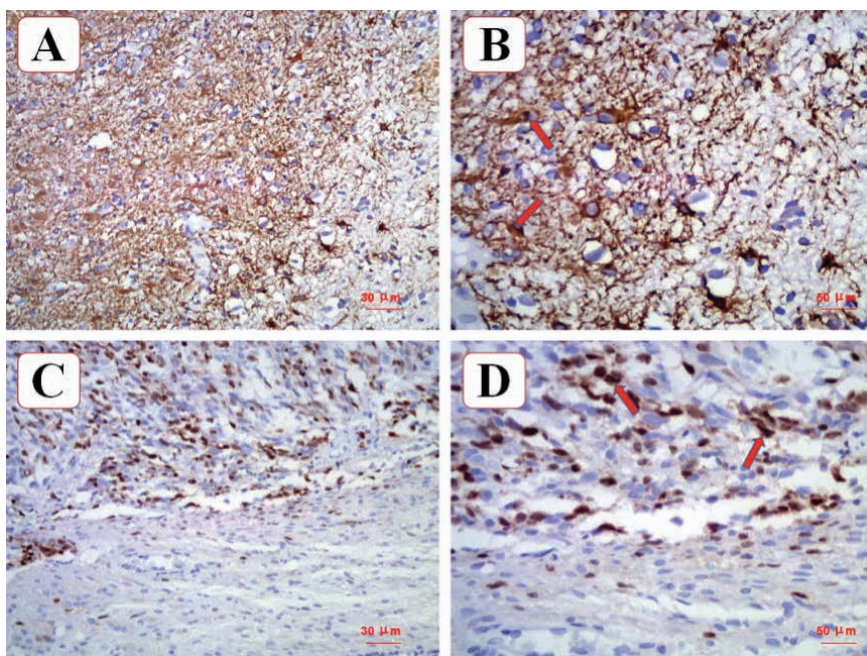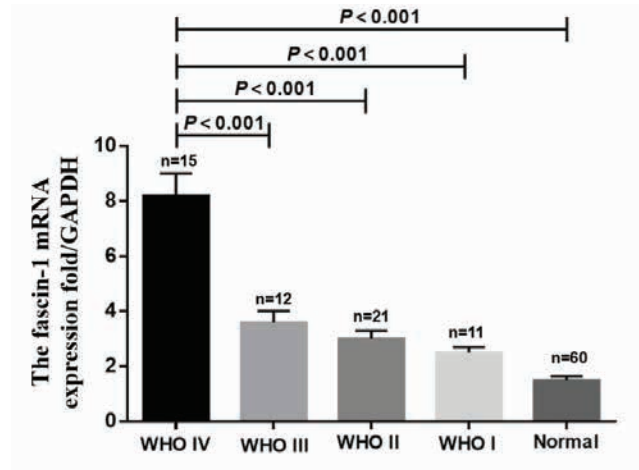

Figure 1: The fascin-1 protein expression in glioma tissues (A and B) and normal brain tissue (C and D). (A and C) ×20; (B and D) ×50. (A and B) Male, 57 years old; (C and D) Female, 49 years old.

follow-up, we obtained survival curves of patients with glioma. The Kaplan-Meier survival model showed that for OS and PFS, the survival duration of patients with glioma in the low-expression group was higher than that in the high-expression group (Figures 4 and 5). Using a Cox pro-portional hazard model, we analyzed each variable with
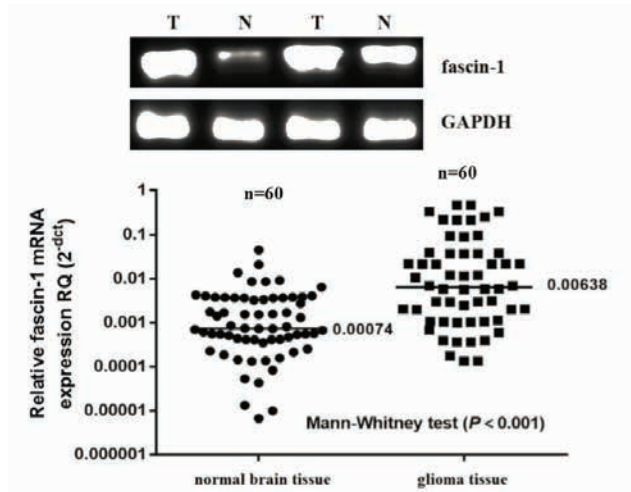
Figure 2: The fascin-1 mRNA was detected by RT-PCR. T: glioma tissue; N: normal brain tissue.

Figure 3: The relationship of fascin-1 mRNA expression level and pathological grades of patients with glioma. ANOVA was the statistical test used.

Table 1: Clinicopathological features and fascin-1 protein expression in glioma

| Variables	Cases (n=120)	Fascin-1 protein expression		P-value
		Low expression	High expression	
Age (years)				0.883
<55	63	34	29	
≥55	57	30	27	
Gender				0.550
Male	72	40	32	
Female	48	24	24	
WHO grade				0.001
I-II	60	41	19	
III-IV	60	23	37	
KPS score				0.136
< 80	58	35	23	
≥ 80	62	29	33	
Tumor size (cm)				0.243
< 5	71	41	30	
≥ 5	49	23	26	
Extent of resection				0.300
< 98%	51	30	21	
≥ 98%	69	34	35	

Note: WHO means World Health Organization; KPS score means Karnofsky score

OS of patients with glioma. Univariate analysis showed that the prognosis of patients with glioma was related to the WHO tumor grades. The data mentioned above indicate that a higher WHO tumor grade is associated with a worse prognosis in patients with glioma. Multivariate analysis showed that high expression of fascin-1 protein was an independent predictor of prognosis in patients with glioma (Table 2).

4 Discussion

The gene coding for fascin-1 is situated on chromosome 7q22. Fascin-1 is primarily distributed in the microvilli and rugae, on the edges of cytomembranes that have movement abilities or have undergone mitosis [12]. Serine-39 of fascin-1 is the major phosphorylation target site for protein kinase C, and the phosphorylation of this site has been shown to regulate fascin binding to actin filaments [12-13]. By altering the functions of β-catenin and cadherin, fascin-1 is known to reduce intercellular adhesion activity [14]. Direct specificity assembly and actin filament decomposition change the movement of tumor cells and reduce adhesion between cells and stromata. Fascin-1 not only takes part in the formation of cell migration structures, but also participates in adhesion between cells, indicating that fascin-1 might be playing a definite role in the invasion and metastasis of cells. Some researchers have explored the relationship between fascin protein and various biological responses of tumor cells using in vitro models. Xu et al. [15] have found that fascin-1 promotes pancreatic cancer cell migration, invasion, and scatter-
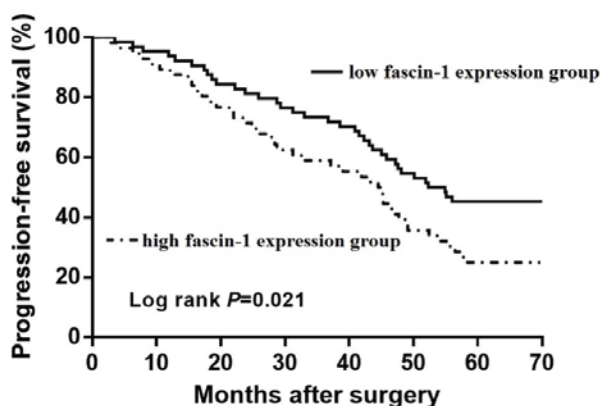
Figure 4: The fascin-1 protein expression status and progression-free survival of glioma patients.
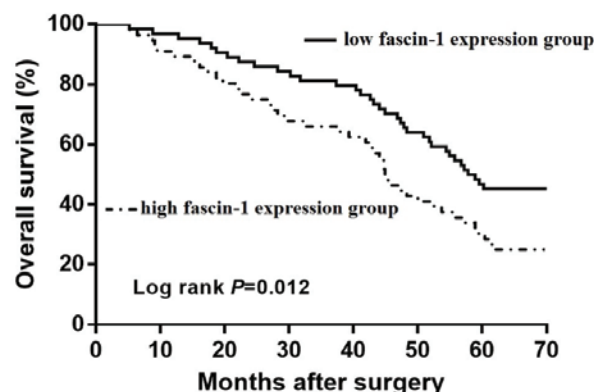
Figure 5: The fascin-1 protein expression status and overall survival of glioma patients.

Table 2: Univariate and multivariate analyses for overall survival

Variable	Univariate analysis			Multivariate analysis		
	HR	95% CI	P-value	HR	95% CI	P-value
Age	1.347	0.862-2.107	0.191			NR
Gender	0.726	0.456-1.157	0.178			NR
WHO grade	3.311	2.059-5.324	0.000	3.084	1.896-5.015	< 0.001
KPS score	1.296	0.828-2.028	0.257			NR
Tumor size	1.325	0.845-2.075	0.220			NR
Extent of resection	1.490	0.939-2.364	0.091			NR
Fascin-1 protein expression	1.768	1.128-2.772	0.013	1.400	1.083-2.220	0.032

Note: HR means Hazard ratio; CI means Confidence interval; NR means No statistical significance

ing, thus contributing to the aggressive behavior of pancreatic cancer cells. A report by Hayashi [16] suggests that fascin-1 acts primarily as a migration factor associated with epithelial-mesenchymal transition in hepatocellular carcinoma cells, and on combination with matrix metalloproteinases, facilitates invasiveness. In a study of brain gliosarcoma, the investigators found that over-expression of cortactin, fascin, and survivin is associated with malignant transformation of brain gliosarcomas [17]. Regarding the relationship between fascin protein expression and glioblastomas, Gunal et al. evaluated fascin expression in glial tumors and its association with histological grading. The results showed that fascin expression levels correlated with histologic grade and that fascin over-expression may play an important role not only in the biological responses of glial astrocytic tumors but also in the prognosis of glioblastomas [18]. In contrast to the our study, they examined the expression of fascin-1 in glioblastoma. In our study, we have explored the expression of fascin-1 in fibrous astrocytoma, low grade astrocytoma, degenerative astrocytoma and glioblastoma multiforme. Whether fascin-1 has a similar effect in human glioma is not yet clearly understood. In addition, we found no reports on the clinical significance of fascin-1 expression in diagnosis or prognosis of patients with glioma.

In this study, first, we established the expression pattern of the fascin-1 protein in glioma tissue using immunohistochemistry. We found that the fascin-1 protein was expressed in the cytoplasm of cells obtained from glioma tissue. On statistical analysis, the fascin-1 protein showed high expression in glioma tissues. Second, RT-PCR results also indicated that the levels of fascin-1 mRNA were higher in glioma tissue than in normal brain tissue. We found that the expression levels of the fascin-1 protein correlated with the WHO grading of patients with glioma. It is essential to analyze the relationship between fascin-1 protein expression status and prognosis in patients with glioma. Using long-term follow-up data of 120 patients with glioma, we found that patients with high expression of fascin-1 protein had shorter survival duration compared to patients with low expression of the protein. On multi-factor regression analysis, high expression of fascin-1 protein was found to be one of the important independent factors for predicting prognosis in patients with glioma. This also endorses the relationship between fascin-1 protein expression and WHO tumor grading of patients with glioma, as higher WHO tumor grades signifies worse clinical outcomes in patients with glioma.

Combined with previous findings, we hypothesize the mechanism of fascin-1 in tumorigenesis and progression. The mechanism of up-regulation of fascin-1 is different across different tumors. In breast cancer, fascin-1 maybe an inducer protein for c-erbB-2 that in turn could initiate transcription of fascin-1 genes by activating nuclear factor-κB and TATA core factors [14, 19]. In esophageal cancer, it has been found that the transcription factor, specificity protein 1(Sp1), could directly regulate expression of fascin-1 by directly binding to the promoter region of fascin-1 [20]. Up-regulation of the transcription factor Sp1 corresponds to up-regulation of fascin-1 expression and vice versa. A specific inhibitor of extracellular signal–regulated kinases 1/2 (ERK1/2) can decrease the phosphorylation level of transcription factor Sp1, leading to inhibition of the fascin-1 gene transcription, resulting in down-regulation of protein expression [21-22]. Stimulation activation of epidermal growth factors can increase the phosphorylation level of transcription factor Sp1 by activating ERK1/2 pathways to enhance the expression of fascin-1. In addition, signaling in the tumor necrosis factor-related pathways such as -α, Wnt, and interleukin-6 can up-regulate expression of fascin-1 [14, 23-24]. Therefore, additional studies on the regulatory mechanism of fascin-1 are necessary.

5 Conclusion

In conclusion, high expression of the fascin-1 protein indicates poor prognosis for patients with glioma. Therefore, fascin-1 could be an important molecular marker for prediction of prognosis in patients with glioma.

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