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Reduced β-catenin Expression is Associated with Good Prognosis in Astrocytoma

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Abstract The aim of this study was to evaluate the expression of β-catenin in astrocytoma, and the clinical relevance and prognostic significance of the expression of β-catenin was also analyzed. Immunohistochemistry was performed on 63 resected astrocytoma tumor specimens to detect the expression of β-catenin. The correlation between the results of immuoexpression and the clinicopathologic parameters and patient survival was processed statistically. In 63 samples of astrocytoma, 36 cases were immunoreactive for β-catenin at cytoplasm, ten cases of astrocytoma were immunoreactive at cytomembrane, and four cases of astrocytoma were stained for β-catenin at nucleus. Spearman analysis showed that the distribution of β-catenin was not correlated with the grades of astrocytoma. However, the expression profiles were correlated with the patient's 2-year survival, but not correlated with the grades, tumor size, sex, age, or tumor location. Patients with low β-catenin expression levels tended to be associated with a better prognosis than those who with high levels (p=0.042). Our results suggest that β-catenin is useful for the prognosis evaluation of astrocytoma.

Keywords Astrocytoma · β-catenin · Prognosis · Immunohistochemistry

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Introduction

Astrocytoma is the most common primary brain tumors. Modern molecular genetic analyses have revealed that the progression of astrocytic tumors results from accumulating inactivation of different tumor suppressor genes and/or amplification of certain oncogenes [1–3]. A significant number of patients who undergo apparently curative operation unfortunately develop local recurrences leading to shorter survival. Identification of factors that affect tumor aggressiveness and allow a more accurate prognosis is required.

The WNT signaling pathway regulates cellular proliferation and differentiation in vertebrates and invertebrates and has an important role in tumor progression. As a key molecule in WNT signaling pathway, β-catenin is a multifunctional protein which plays crucial roles in the maintenance of cell-cell adhesion [4, 5].When it accumulates in the nucleus, β-catenin loses its function as a cell-adhesion molecule, which activates the WNT signaling pathway and switches on a series of transcriptions of target genes such as c-myc or cyclinD1, resultin in proliferation and metastasis of tumor cells [6, 7]. Recently, β-catenin has drawn great attention because its role in tumorigenesis has been reported in many types of tumors, including colon carcinoma, gastric carcinoma, esophageal cancer [8], hepatocellular carcinoma [9], prostate carcinoma [10], and endometrial carcinoma [11]. However, knowledge of β-catenin expression in the astrocytoma is not clear. In this study, we detected the expression of β-catenin in 63 samples of astrocytoma, explored the relationship between the expression of β-catenin and the clinicopathologic

factors, and further evaluated the patient survival with low or high β-catenin expression.

Materials and Methods

Patients and Tumors

We obtained 63 surgically removed astrocytoma tissues from the Xijing Hospital, the affiliated hospital of the Fourth Military Medical University, China. All samples were pathologically confirmed astrocytoma. Patients (39 males and 24 females) were 6–75 years of age, and the mean age was 49 years (Table 1). These tumors were classified according to the classification system of CNS (2007) of World Health Organization (WHO), including 26 cases of Grade II, 25 cases of Grade III, and 12 cases of Grade IV. Tissue specimens had previously been fixed in 10% buffered formalin and embedded in paraffin for routine histological examination.

β-catenin Immunostaining

Immunohistochemistry was carried out as previously described [12]. Briefly, the deparaffinized and rehydrated slides were blocked with 20 mL/L fetal calf serum for 30 min to reduce nonspecific binding. Then the anti-β-catenin antibody (1:2000; Sigma, USA) was applied to the sections and incubated at 4°C overnight. The colon carcinoma was used the positive control of β-catenin. Negative control was obtained by replacing the primary antibody with PBS or rabbit serum. The sections were subsequently incubated with the second antibody (Dako, Denmark) at 37°C for 40 min, and stained with DAB-H_2O_2 for 5 min and counterstained with hematoxylin.

Evaluation of β-catenin Staining

Evaluation of the immunohistochemical staining was conducted by two pathologists who had no knowledge of the clinical characteristies of the patients. The expression was scored as "++++" if more than 75% of tumor cells were immunostained positive, as "+++" if 50% to 75% of cells were positive, as "++" if 25% to 50% of cells were positive, as "+" if 5% to 25% of cells were positive, and as "–" if less than 5% were positive.

Table 1 Biopsy specimens

Histopathological diagnosis	Total case number	Male	Female	Age	
				Median	Range
WHO grade II	26	15	11	41.9	6–67
WHO grade III	25	13	12	43.8	12–75
WHO grade IV	12	11	1	54.2	40–70

Statistical Analysis

Analyses were carried out using SPSS11.5 software. The Spearman's correlation test was used to access the relationship of the expression levels of β-catenin with the clinicopathologic alterations of astrocytoma. Survival curves were estimated by the Kaplan-Meier method and compared by the log-rank test. p values less than 0.05 were considered statistically significant.

Results

The Localization of β-catenin is Cytoplasm, Cytomembrane and Nucleus in Astrocytoma

The expression of β-catenin was detected in cytoplasm, cytomembrane and nucleus (Fig. 1). In 63 samples of astrocytom, 36 cases were immunoreactive for β-catenin at cytoplasm, ten cases of astrocytoma were immunoreactive at cytomembrane, and only four cases of astrocytoma were stained for β-catenin at nucleus. Spearman analysis showed that the distribution of β-catenin (cytoplasm, cytomembrane and nucleus) was not correlated with the grades of astrocytoma (Table 2).

The Relationship Between the Level of β-catenin Expression and Clinicopathologic Characteristics of Astrocytoma

The expression of β-catenin was classified as five ranks: − , + , ++, +++ and ++++. Fifty of 63 astrocytomas showed β-catenin positive expression. The correlative analysis between β-catenin expression and the clinicopathologic characteristics of patients with astrocytoma is summarized in Table 3. There were significant correlation between the level of β-catenin expression and 2-year suvival of patients ($R=-0.490$, $p<0.05$). However, the expression of β-catenin was not correlated with the grades, tumor size, age, sex or tumor location ($p>0.05$).

Reduced Expression of β-catenin with a Good Prognosis

To further clarify the relationship between expression of β-catenin and prognosis of patients, we carried out on 42 patients followed-up 50 months. The survival curves for patients with astrocytoma showing low (− and +) expression of β-catenin tend to be associated with good prognosis, but high (++, +++ and ++++) expression of β-catenin tend to be associated with poor prognosis (Fig. 2).

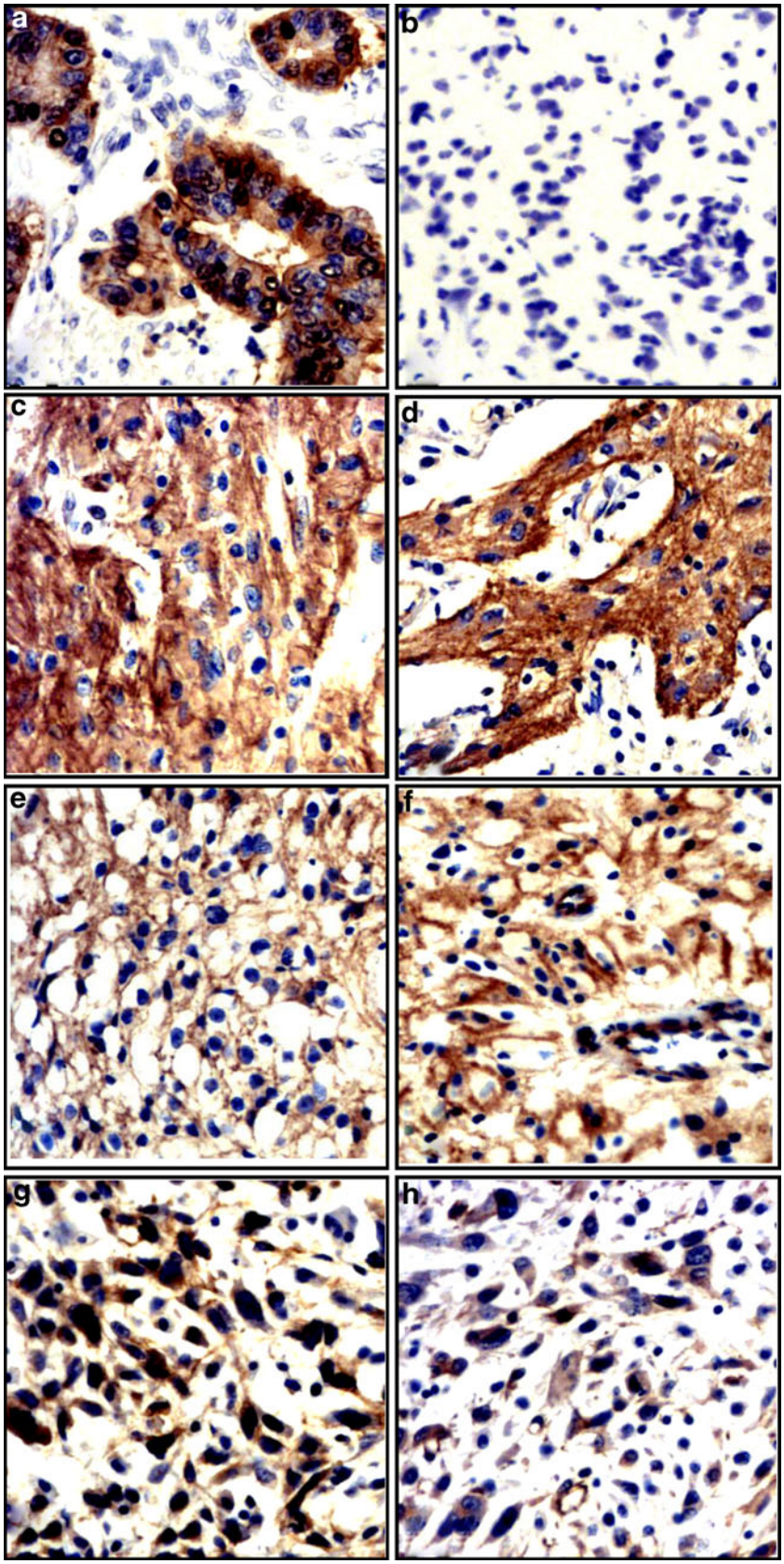

Fig. 1 Immunohistochemical staining of β-catenin in astrocytoma. The localization of β-catenin was detected in cytoplasm, cytomembrane and nucleus. **a** The colon carcinoma was used as the positive control. **b** As a negative control, the primary antibody was replaced by PBS. **c, d** The localization of β-catenin was preserved in cytoplasm. **e, f** The localization of β-catenin was preserved in cytomembrane. **g, h** The localization of β-catenin was preserved in nucleus

Table 2 The expression of β-catenin in cytoplasmic, cytomembrane and nucleus

Grade	N	Cytoplasm				Cytomembrane				Nucleus			
		$R=-0.160$ $p>0.05$											
		++++	+++	++	+	++++	+++	++	+	++++	+++	++	+
		$R=-0.068$ $p>0.05$				$R=-0.223$ $p>0.05$				$R=-0.577$ $p>0.05$			
Grade II	21	3	7	2	2	0	1	2	2	0	0	2	0
Grade III	18	2	4	3	6	0	1	0	2	0	0	0	0
Grade IV	11	2	3	1	1	0	1	1	0	1	0	1	0

N refer to the number of positive staining cases

Discussion

The localization of β-catenin gene is at 3p2.1. β-catenin was first identified as a member of Wnt signaling pathway. In the cells, β-catenin has two positioning pool: one is located in the cytomembrane and involved in cell adhesion; the other in the cytoplasm and/or nucleus [13] mainly involved in the regulation of Wnt signaling pathway. The complex of β-catenin-TCF/LEF is the core of Wnt signaling pathway. Wnt signaling pathway could be activated throght the translocation of β-catenin. Once β-catenin is located in the nucleus with TCF/LEF binding, it can activate Wnt signaling pathway [14–16].

Table 3 The expression of β-catenin in 63 cases of astrocytoma

	β-catenin expression					*p* Value
	++++	+++	++	+	−	
Grades						
II	3	8	6	4	5	$R=-0.061$
III	2	5	3	8	7	$p>0.05$
IV	3	4	3	1	1	
Tumor size						
<3 cm	5	8	5	7	5	$R=0.091$
≥3 cm	3	9	7	6	8	$p>0.05$
Sex						
Male	4	10	7	10	8	$R=-0.104$
Female	4	7	5	3	5	$p>0.05$
Age						
<30	2	8	5	3	4	$R=0.058$
≥30	6	9	7	10	9	$p>0.05$
Two-year survival						
>2 years	1	7	6	11	11	$R=-0.490^{**}$
≤2 years	7	10	6	2	2	$p<0.05$
Tumor location						
Frontoparietal	3	3	3	7	6	$R=-0.161$
Temproparietal	2	6	4	2	2	$p>0.05$
Occipital	0	1	1	1	0	
Cerebellum	1	2	1	1	3	
Canalis spinalis	0	1	2	1	0	
Others	2	4	1	1	2	

Some research showed that various β-catenin abnormal changes have been found in colon, liver, stomach, endometrial cancer and malignant melanoma, mainly to the cytoplasm and/or nucleus storage of β-catenin and cytomembrane deletion of β-catenin [16, 17].The abnormal changes play an important role on the incidence and development of tumor. However, studies about the expression of β-catenin in astrocytomas and the relationship between β-catenin distribution and the progress of astrocytoma rarely reported. Moreover, there is rare research about the relationship between the expression of β-catenin and the prognosis of astrocytoma patients.

In this study, 63cases of astrocytoma samples were used to detect the expression of β-catenin. Thirty-six of 63 cases showed cytoplasm positive expression, and ten or four of 63 cases showed cytomembrane or nucleus positive expression respectively. Spearman correlation analysis didn't show a significant correlation between the distribution of β-catenin (cytoplasm, cytomembrane and nucleus) and the grades of astrocytoma, suggesting that the cytoplasmic to nuclear translocation of β-catenin in Wnt

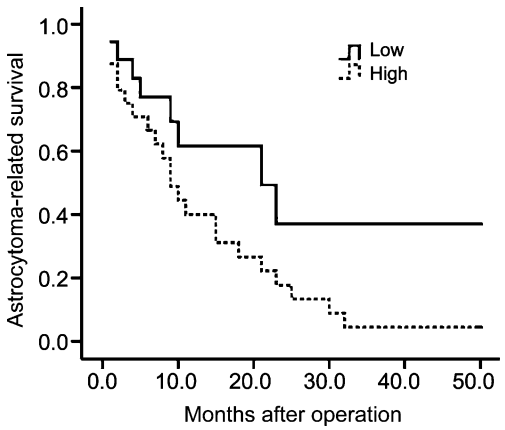

Fig. 2 Kaplan-Meier estimates of survival for astrocytoma patients with different levels of β-catenin exprssion. Low expression of β-catenin includes − and + staining, high expression of β-catenin includes ++, +++ and ++++ staining. The result indicated that patients with low expression of β-catenin tended to be associated with a better prognosis than those with high expression of β-catenin. (Log-rank test, $p=0.042$)

signaling pathway could not play a key role in malignant astrocytomas progress.

Several studies indicated that β-catenin cytoplasmic to nuclear translocation was related to tumor progression [18, 19]. On the other side, Grabsch et al. pointed out that β-catenin abnormal had no correlation with tumor progression [20]. Similarly, there are controversial studies on the correlation between β-catenin expression levels and histopathological grades. Utsuki et al. found that β-catenin expression levels increased with higher grade astrocytoma [12], whereas another study believed that β-catenin expression levels was negatively related with histopathological grades[21]. Interestingly, we did find that β-catenin expression had no correlation with the grade of astrocytoma in this study. Meanwhile, β-catenin expression levels were not related to tumor size, location, patients' age and gender. More importantly, we revealed that β-catenin expression levels were negatively correlated with the 2-year survival rate of patients. To further clarify the relationship between expression of β-catenin and prognosis of patients, we carried out on 42 patients followed-up 50 months. The results showed that patients with low expression of β-catenin tended to be associated with a better prognosis than those with high expression of β-catenin.

Collectively, this study shows that, the localization of β-catenin is not correlated to the grade of astrocytoma, whereas β-catenin expression level is closely related to the prognosis of astrocytoma patients. These results suggested that β-catenin could serve as one of the factors to evaluate the prognosis of astrocytoma patients.

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