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The Expression of PI3K, AKT, β-Catenin and VEGFR2 in Medulloblastoma

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Abstract

Medulloblastoma (MB) is common tumor of the central nervous system in children. It's reported that PI3K/AKT and Wnt signal pathway have important roles in MB. This study aims to investigate the expression of PI3K, AKT, *B*-catenin and VEGFR-2 in MB to find a new pathway for MB. A total of 33 MB and 17 control brain cases were retrospectively evaluate d for PI3K, AKT, β-catenin and VEGFR-2 expression by immunohistochemical staining, and the relationship with clinical feature were analyzed. The positive rate of PI3K, AKT, β-catenin and VEGFR-2 in 33 MB were significantly greater than those in control group ($P < 0.05$), and significant positive correlation was found between PI3K, AKT, β-catenin and VEGFR-2 each other in MB ($P < 0.05$). Moreover, the clinical data, such as age, gender, tumor size was no significant correlation between the expression of PI3K, AKT, β-catenin and VEGFR-2 was found ($P < 0.05$).

Keywords

PI3K, AKT, β-Catenin, VEGFR2, Medulloblastoma

1. Introduction

VEGF family and their receptor VEGFR are affected especially in develop and matastasis of tumor. VEGFR2 Which could adjust reaction of VEGF in intercellular [1], and was considered that it was major medium for promoting angiogenesis and enhancing rasopermeability, which participating in regulation of multi-signal pathway each other. It was reported that VEGF and receptor VEGFR2 are formed to dimmer in extracellular, which make its intracellular phosphorylation, which further phosphorylate PI3K, activate a series of signal pathway. Recent years, it has been become the hotspot that PI3K/AKT and Wnt signal path-

way are affected in genesis, development, therapy and prognosis in tumors. The published studies focused on breast cancer [2], ovarian cancer [2], endometrial cancer [3], nasopharyngeal cancer [4], human gliblastomas [5], etc.

Furthermore, present studies were responsible for the occurrence and development of tumor by adjusting Wnt signal pathway. MB is the most common maliganat brain tumor in children. The relevance of PI3K/AKT and Wnt signal pathway have not yet been sufficiently elucidated in MB, and further investigations are required. The aim of this study is to investigate the expression and correlation of PI3K, AKT, β-catenin and VEGFR-2 in 33 MB and 17 control brain tussle by immunohistochemical staining.

2. Materials and Methods

2.1. Patients and Tissues

MB tissue samples from 33 patients were diagnosed between 2005 and 2009 at Department of Pathology in Chongqing Medical University. Clinical data were obtained from retrospective review.

2.2. Histopathology

The diagnoses of MB were based on a combination of clinical information, morphologic examination and immunohistochemical results. All cases were classified according to World Health Organization (WHO) 2007 as classical [6].

2.3. Immunohistochemistry

PI3K, AKT, β-catenin and VEGFR-2 were analyzed by immunohistochemistry. Antigen retrieval was used to enhance PI3K, AKT, β-catenin and VEGFR-2 expression by immunohistochemical staining by high pressure in citrate buffer(ph 6.0) for 3 min. Then the sections incubated with rabbit monoclonal anti-PI3K (1:100 SANTA CRUZ company), anti-AKT(1:100 SANTA CRUZ company), mouse monoclonal anti-β-catenin (1:100 SANTA CRUZ company), anti-VEGFR2 (1:100 SANTA CRUZ company) over-night at 4˚C. Slides were stained with DAB until desired stain intensity developed and mounted before observation by light microscopy.

2.4. Assessment of Immunoreactivity

The positive reaction is defined as discrete localization of the chromogen in the cytoplasm/nucleus of all slides. The intensity of cytoplasm/nucleus reaction is graded as negative (− positive cells percentage bellow 10%), mild positive (+ positive cells percentage is 11% - 30%), moderate positive (++ positive cells percentage is 31% - 70%), and strong positive (+++ positive cells are above 70%) [7].

2.5. Data Analysis

X^2 fisher's exact test was applied using the SPSS 10.0 to determine the significance of difference in expression of PI3K, AKT, β-catenin and VEGFR-2. Spearman correlation coefficients were determined for comparisons between immu-

nohistochemical expression data of PI3K, AKT, β-catenin and VEGFR-2. The results were considered as statistically significant difference when $P < 0.05$.

3. Result

1) The immunohistochemical expression of PI3K, AKT, β-catenin, VEGFR2 in MB and control brain tissues group:

We employed immunohistochemistry to evaluate the expression of PI3K, AKT, β-catenin, VEGFR2 in MB and control group. The positive staining of PI3K was found in 31/33 (93.9%) cases of human MB (**Figure 1**), and 8/17 (47.1%) cases in control brain tissues group. The positive staining of AKT was found in 27/33 (81.8%) cases of human MB (**Figure 2**), and 8/17 (47.1%) in control group. Moreover, the positive staining of β-catenin was found in 22/33 (66.7%) cases of human MB (**Figure 3**), and 2/17 (11.8%) in control group. The positive staining of VEGFR2 was found in 29/33 (87.9) cases of MB (**Figure 4**), and 3/17 (17.6%) in control group. On all accounts, the prevalence of PI3K, AKT, β-catenin and VEGFR2 positive expression in MB were significantly greater than those in control brain group ($P < 0.05$) (**Table 1**).

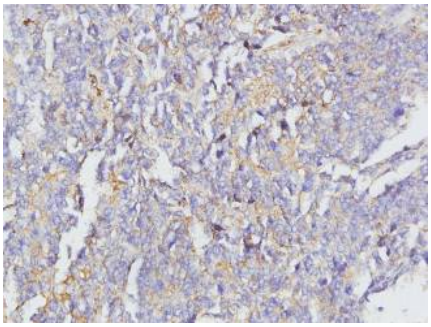

Figure 1. The positive staining of PI3K in MB (×200).

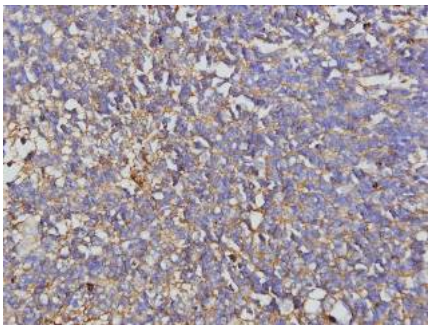

Figure 2. The positive staining of AKT in MB (×200).

Table 1. Expression of PI3K, AKT, β-catenin, VEGFR2 in medulloblastoma and control group.

Group	Case	PI3K				AKT				β-catenin				VEGFR2			
		+++	++	+	−	+++	++	+	−	+++	++	+	−	+++	++	+	−
Medulloblastoma	33	15	10	6	2	6	12	9	6	7	5	10	11	17	7	5	4
Control Group	17	1	2	5	9	1	2	5	9	0	0	2	15	0	1	2	14
P value		<0.05				<0.05				<0.05				<0.05			

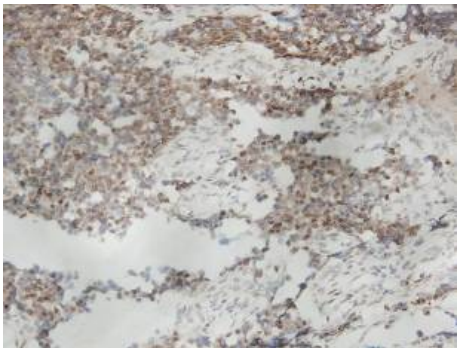

Figure 3. The positive staining of β-catenin in MB (×200).

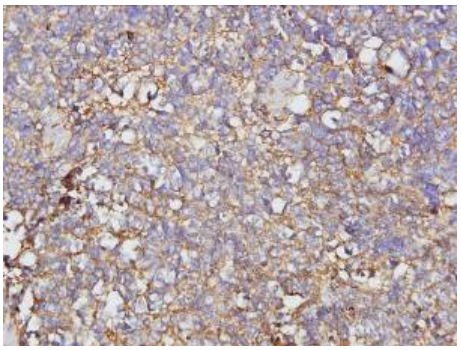

Figure 4. The positive staining of VEGFR2 in MB (×200).

2) The relation between protein expression of PI3K, AKT, β-catenin, and VEGFR2 in MB with each other:

Significant positive correlations were found between PI3K and AKT (r = 0.375, $P = 0.032$); PI3K and β-catenin (r = 0.544, $P = 0.001$); meanwhile, significant positive correlation were found between PI3K and VEGFR2(r = 0.604, $P = 0.000$), AKT and β-catenin (r = 0.405, $P = 0.019$); AKT and VEGFR2 (r = 0.488, $P = 0.004$) in MB by spearman's correlation test.

3) The relation between protein expression of PI3K, AKT, β-catenin, and VEGFR2 in MB with clinical characteristics:

No significant correlations were found between expression of PI3K, AKT, β-catenin and VEGFR2 with clinical characteristics of pathological type ($P > 0.05$) (**Table 2**).

4) The relation between protein expression of PI3K, AKT, β-catenin, and VEGFR2 in MB with different kinds of MB (classic, desmoplastic, MBEN/large cell and anaplastic MB):

No significant difference was found in the expression of PI3K, AKT, β-catenin and VEGFR2 in different pathological type MB ($P > 0.05$) (**Table 3**)

4. Discussion

The recent study shows that multi-signal pathway and VEGF family have important effect in occurrence and development of tumor, furthermore, VEGFR2 participate in regulating multi-signal pathway. PI3K/AKT signal pathway and Wnt/β-catenin signal pathway were considered to be important roles in it, which were

Table 2. Relation between expression of PI3K, AKT, *β*-catenin, VEGFR2 with clinical characteristics in MB.

Group		PI3K		AKT		*β*-catenin		VEGFR2	
		+	−	+	−	+	−	+	−
Gender	Male	18	1	16	4	13	6	17	2
	Female	13	1	11	2	9	5	12	2
Age	≤3 y	5	0	4	1	4	1	4	1
	>3 y	26	2	23	5	18	10	25	3
Tumor	≤1.5 cm^3	11	1	9	3	8	4	11	1
	>1.5 cm^3	20	1	18	3	14	7	18	3
P value		>0.05		>0.05		>0.05		>0.05	

Table 3. Relation between expression of PI3K, AKT, *β*-catenin, VEGFR2 with type of MB.

Group	PI3K					AKT					*β*-catenin					VEGFR2				
	+++	++	+	−	P	+++	++	+	−	P	+++	++	+	−	P	+++	++	+	−	P
Classic	8	5	6	1		2	8	7	3		4	4	7	7		6	5	6	3	
Desmoplastic	3	2	3	1	>0.05	2	3	3	1	>0.05	3	1	3	2	>0.05	8	0	0	1	>0.05
MBEN/large cell	1	1	0	0		1	0	0	1		0	1	0	1		0	1	0	1	
Anaplastic	1	1	0	0		1	0	0	1		0	0	1	1		1	1	0	0	

found in nasopharyngeal cancer [8], colon cancer [9] and glioblastoma [10].

PI3K is a kind of kinase with differential phosphory lation of phosphatidylinositol 3-hydroxy and frequently activated in human cancer, which playing a crucial role in controlling cell proliferation, survival, and metastasis downstream of many growth factor receptors, it has been activated by the stimulation of phosphorylated 3-hydroxy on the inositol ring, and then producing secondary messengers PI-3, 4-P2, PI3, 4, 5-P3 to phosphorylated AKT on ser 473, ser308 to downstream target protein, involved in cell proliferation and an angiogenesis [11]. The current study presents that the signal pathway of PI3K/AKT plays important role as anti-apoptosis in appearance and development of several kinds of tumors. However, apoptosis is the major mode of inducing the malignancy cells to be dead and it is the major mechanism of the generation of resistance and antiradiation in the malignancy cells to both chemotherapy and radiotherapy. Therefore, it is so important that study the effect of signal pathway of PI3K/AKT. We have chosen the 33 MB samples which had been clearly treated and 17 brain tissues samples as control group to study the effect of PI3K/AKT signal pathway in MB by immunohistochemical methods. Our results showed that the positive rate of PI3K and AKT in these 33 MB 93.2%, 81.8%, respectively, while the positive rates are 47.1%, 47.1% in control group. Overexpression of PI3K and AKT protein are detected in MB compared with control group ($P < 0.05$). Our results suggested thatPI3K/AKT signal pathway would affect important impact in MB, which are consistent to the experiment result of Wolfgang Hartmann [12].

B-catenin is a significant upstream factor in Wnt signal pathway, which forms

multi-protein degradation compounds. After Wnt signal pathway was activated multi-protein degradation is degraded, to entrance a great quantity of β-catenin into cell nucleus, and form to complex with transcription factor Tcf/Lef, Which activated downstream target gene, lead to proliferation and metastasis of tumor [13]. Meanwhile, abnormal cumulation of β-catenin causes E-cadherin inactivation, which participates in inter-cellular conglutination and infiltrative growth of tumor cells.

Ellison [14] found that nucleus abnormal expression of β-catenin was closely related with no desmoplastic MB. Our results showed that the positive rate of β-catenin in 33 MB is 66.7%, but 11.8% in 17 control group, which has significant difference ($P < 0.05$).

Recent researches indicate that it caused angiogenesis and increased vascular permeability after VEGF combined to VEGFR which promoted infiltration and metastasis.

Dejana [15] indicated that VEGF would combine with VEGFR2, E-cadherin and β-catenin to promote, cell survivel. In our study, we also analyzed correlation of PI3K, AKT, β-catenin and VEGFR2 in MB. Our result showed the protein expression of PI3K, AKT, β-catenin and VEGFR2 pressents positive statistical significance correlation each other in MB ($P < 0.05$), which confirmed the cooperation of PI3K, AKT, β-catenin and VEGFR2 in MB. The mechanism may be that the transmembrane complex formed with VEGFR2, E-cadherin and β-catenin actived PI3K, which phosphorylate AKT, which is due to GSK3β inavtiveted by phosphorylated at ser 9 locus in serine, as a result that reduce the degradation of β-catenin made by complex with APC, Axin and GSK3β, which made β-catenin abnormal gather into nucleus, which stimulate to downstream gene, result in proliferation and metastasis. So, it's considered that Wnt/β-catenin signal pathway play a direct important role in occur and develop of tumor. Meanwhile, PI3K/AKT signal pathway regulates it.

At the same time, we analyzed the correlation of expressions of PI3K, AKT, β-catenin and VEGFR2 between different kinds of MB (classic, desmoplastic, MBEN/large cell and anaplastic MB). The result illustrates that there is no significant difference in the expression of PI3K, AKT, β-catenin and VEGFR2 in different pathological type MB ($P > 0.05$). Our results demonstrate there is no significant correlation between the PI3K, AKT, β-catenin, VEGFR2 with the difference of types of tumor cells, it may be the common pathway of tumor appearance.

In addition, we analyzed the correlation between expression of PI3K, AKT, β-catenin and clinic data such as grender, ages and tumor size. Our results show that there is no significant correlation among the expression of PI3K, AKT, β-catenin, VEGFR2, and the clinical data in MB ($P > 0.05$).

In summary, PI3K, AKT, β-catenin, VEGFR2 play synergy in the occurring and development of MB in order to promote the hyperplasia, metastasis and apoptosis of tumor cells, which may benefit the future target spot in therapy of MB.

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