Relationship between Expression of beta-catenin and VEGFs (VEGFA, VEGF-C), VEGF Receptors-2 (VEGFR-2) in Medulloblastoma

ZHANG Hong-mei (张红梅)**, ZHANG Xiong (张雄), LI Yu (李昱)*, MI Can (米粲)

Department of Pathology, Chongqing University of Medical science, Chongqing 400016

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ABSTRACT

Objective: To investigate the expression of beta-catenin and VEGFs (VEGF-A, VEGF-C) and VEGF receptor-2 (VEGFR-2) protein in medulloblastoma. **Methods**: Immunohistochemical staining with SP method was conducted to determine the expression of beta-catenin and VEGFs (VEGF-A, VEGF-C) and VEGFR-2 in 33 cases of medulloblastoma and 10 normal cerebellar tissues. **Results**: The expression rate of beta-catenin, and VEGFs (VEGF-A, VEGF-C) and VEGFR-2 in medulloblastoma were significantly higher than that in normal tissue. A significant positive correlation was found between beta-catenin and VEGFs (VEGF-A, VEGF-C) and VEGFR-2 protein in medulloblastoma. **Conclusion**: There was a correlation between beta-catenin and VEGFs (VEGF-A, VEGF-C) and VEGFR-2 in medulloblastoma, which may play a role in the pathogenesis and development of medulloblastoma.

Key words: **beta-catenin; VEGFs (VEGF-A, VEGF-C) and VEGFR-2; Medulloblastoma**

Medulloblastoma (MB) occurs most frequently in the cerebellum of children. It is a kind of high malignant neuroepithelium tumor in central nervous system. The tumor cells grow outward, requiring large numbers of microvessels to warrant the nutritional supply. In this process, VEGFs, as the main regulator in angiopoiesis, have chemotaxic effects and promote the division of cells. Many studies have identified that expression levels of VEGFs in malignant tumor were tightly correlated with the degree malignancy, microvessel density and prognosis.

The canonical Wnt/beta-catenin signaling pathway plays critical roles in both embryonic development and tumorigenesis. Central to the pathway is the turnover of beta-catenin, a protein that functions in both cell adhesion and transcription[1]. In the absence of a Wnt signal, free cytosolic beta-catenin is phosphorylated and degraded by a ubiquitin ligase/proteasome system. In the presence of a Wnt signal, the binding of Wnt to its receptor leads to inhibition of beta-catenin phosphorylation, resulting in accumulation of beta-catenin. It enters into nucleus and activates the transcription of the downstream target genes, such as c-myc, cyclinD1. Thereby, it plays important roles in the development and metastasis of tumors. Recently, many studies revealed that the members of VEGFs, such as VEGF-A, VEGF-C and VEGFR-2 were also the downstream target genes of the Wnt pathway[2].

In addition, studies have demonstrated that co-expression of VEGFs and VEGFR-2 in MB, and their expression are closely related to MB initiation, progression and prognosis[3]. Also, there were some studies of Wnt-beta-catenin signaling in MB[4]. However, there are few reports concerning the relationship between the co-expression of VEGFs and beta-catenin in malignant tumors, especially in MB in Chinese patients.

The aim of this study was to investigate the expression of beta-catenin and two members of VEGF family VEGF-A, BEGF-C and one of their receptors VEGFR-2 in MB and explore their roles in the pathogenesis and development of MB, so to

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*Author to whom correspondence should be addressed. E-mail: liyu100@163.com

**E-mail: zhmje@126.com

provide a new target for clinical treatment of MB patients.

MATERIALS AND METHODS

Samples

Surgically resected tumor specimens of 33 patients accurately diagnosed as MB and 10 normal cerebellar tissues from the pathologic Department of Chongqing Medical University, Chongqing between 1996 and 2006 were selected for the study.

Immunohistochemical Staining

The formalin-fixed, paraffin-embedded tissues were sectioned at 5 μm thickness for standard immunohistochemical staining. Slides were de-waxed in xylene for 15 min twice, in absolute alcohol for 5 min, in 95% ethanol for 2 min, in 80% ethanol for 2 min, and in distilled water for 5 min, and then rehydrated in distilled water through graded alcohols. Antigen retrieval was performed to enhance beta-catenin immunohistochemistry by microwaving of the slides in citrate buffer (pH 6.0) for 5 min. Then the sections were washed and blocked with normal horse serum for 30 min at room temperature. Sections were than incubated with a mouse monoclonal anti-beta-catenin antibody (1:100; SANTA CRUZ, USA) or a rabbit monoclonal anti-VEGF-A (1:50; SANTA CRUZ, USA) or a rabbit monoclonal anti-VEGFR-2 (1:100; SANTA CRUZ, USA) antibody over-night at 4°C. Slides were washed in phosphate-buffered saline, and then incubated with a biotinylated horse anti mouse secondary antibody for 30 min at room temperature. Antigen-antibody complexes were detected with the avidin-biotin peroxidase for 30 min at room temperature. Slides were stained with DAB until desired stain intensity developed and observed by light microscopy.

Statistical Analysis

All data were analyzed by a SPSS 10.0 software package. Wilcoxon tests were used to determine the significance of differences in expressions of beta-catenin, VEGF-A and VEGF-C. Spearman correlation coefficients were determined for comparisons between expression of beta-catenin, VEGF-A, VEGF-C and VEGFR-2.

RESULTS

Expression of beta-catenin, VEGF-A, C and VEGFR-2

In 10 samples of normal cerebellar tissue, 1 presented positive membrane and cytoplasmic staining of beta-catenin and cytoplasmic staining of VEGF-C, VEGFR-2 and none presented positive staining of VEGF-a. In 33 MB cases, 2 presented positive nuclear and cytoplasmic expression of beta-catenin, and 20 presented positive cytoplasmic staining of beta-catenin. The overall positive rate was 66.7% (22/33). 29 cases presented positive cytoplasmic staining of VEGFR-2. The positive rate was 87.9% (29/33). 25 cases showed positive cytoplasmic staining of VEGF-A. The positive rate was 75.8 (25/33). 22 cases showed positive cytoplasmic staining of VEGF-C. Positive rate was 66.7% (22/33). These were all significantly higher than those in the control. The positive staining was mainly located in the MB tumor cells, and a few were expressed by vascular endothelial cells (Fig. 1–8 and Tab. 1, 2).

Tab. 1. Expression of beta-catenin, VEGF-A, C and VEGFR-2 in control

Protein type	+++	++	+	–
beta-catenin	0	0	1	9
VEGF-A	0	0	0	10
VEGFR-2	0	1	0	9
VEGF-C	0	0	1	9

Tab. 2. Expression of beta-catenin, VEGF-A, C and VEGFR-2 in MB

Protein type	+++	++	+	–	*P*
beta-catenin	7	5	10	11	<0.001*
VEGF-A	12	6	7	8	<0.001**
VEGFR-2	17	7	5	4	<0.001***
VEGF-C	2	10	10	11	<0.001****

*Comparison with the control group (Uc=3.33, $P<0.05$)

**Comparison with the control group (Uc=5.62, $P<0.001$)

***Comparison with the control group (Uc=4.40, $P<0.001$)

****Comparison with the control group (Uc=4.58, $P<0.001$)