

## THE PROLIFERATIVE ACTIVITY OF MEDULLOBLASTOMA AND ITS RELATIONSHIP WITH PROGNOSIS

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**Proliferating cell nuclear antigen (PCNA) is a cell-cycle-related nuclear protein, which is essential for DNA synthesis. It could reflect cell proliferating activity. And there're reports that it has got relationship with prognosis of many tumors. Immunohistochemistry was used to detect the expression of PCNA in medulloblastoma and to study its relationship with prognosis, differentiation, histological classification, size and mitotic index. The results showed that the expression of PCNA is related significantly only with the prognosis, i.e. the lower the expression, the better the prognosis. But no relationship of the expression of PCNA with differentiation, histological classification, size and mitotic index of the tumor could be found.**

**Key words: Proliferating cell nuclear antigen (PCNA), Prognosis, Medulloblastoma, Immunohistochemistry**

Proliferating cell nuclear antigen (PCNA) is the polymerase & accessory protein in DNA synthesis. So the level of PCNA is directly correlated with DNA synthesis and the proliferative activity of cells, and PCNA plays an important role in it.<sup>1</sup> Hence PCNA expression demonstrates the proliferative activity of cells.<sup>2</sup> The level of PCNA expression has got some relationship with prognosis, differentiation, etc. of tumors.<sup>3-5</sup> Medulloblastoma is a common malignant

tumor in childhood. But there're only a few reports about PCNA expression on medulloblastoma until now. We observed PCNA expression in medulloblastoma and studied its relationship with histological classification, differentiation, size, mitotic index and prognosis of medulloblastoma.

### MATERIALS AND METHODS

A total of 63 cases of resected medulloblastoma from 1974-1995 were selected from the Dept. of Pathology, Affiliated Hospital, Shandong Medical University, which has got detailed data including follow-up. All the specimen were routinely formalin-fixed, paraffin-embedded and HE stained.

Routine LSAB method was applied following microwave procession. The monoclonal antibody of PCNA (PC-10) is the product of Sigma Corp with working solution 1: 100. LSAB Kit was bought from Boster Corp.

### RESULTS

#### General Analysis

According to WHO histological typing of tumors of central nervous system (1993),<sup>6</sup> 63 cases of medulloblastoma include 46 classic, 7 desmoplastic and 10 classic with partial desmoplastic. Measuring the longest diameter of the tumors, the maximal is 7

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cm while the mean is 4.3 cm. Mitosis could be found in each of the cases more or less,  $\leq 10$  mitosis per 5 high fields was taken as weak(+),  $>10$  as strong (++) . Then 31 cases was measured as (+), 32 as (++) . And differentiation could be observed in 44 cases, which is Homer-Wright rosette, pale island and glial differentiation. In some cases only one form of differentiation could be observed while in the others two or all the three ones.

### Immunohistochemistry

The positive granules of PCNA are brownish yellow localized in the nuclei of the tumor cells. The intensity was scored according to the percentage of positive cells as the following:  $\leq 25\%$  as very weak (+), 26%— 50% as weak (++) , 51%— 75% as strong (+++) and  $>75\%$  as very strong (++++). Thus 21 out of 63 is (+), 22 is (++) , 15 is (+++) and 5 is (++++). The distribution and intensity of the positive cells are not even. They are diffusely scattered in one region while densely packed in the others, and strongly positive in one region while weakly positive in the other (Figure 1,2). In the regions showing differentiation only a few positive cells exist, esp. in pale island there've only a few positive cells at the peripheral region (Figure 3), which is in keeping with the observation of Schiffer and Katsetos<sup>4,7</sup> and also shows that the differentiation of the tumor cells has got certain impact on the expression of PCNA. The relationship of PCNA expression with differentiation was listed in Table 1, with size in Table 2, with histological classification in Table 3, with mitotic index in Table 4. Statistical test shows no relationships of PCNA expression with these four indices could be found (all Ps  $>0.05$ ).

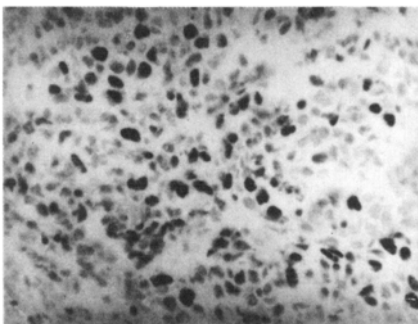


Fig 1. PCNA tumor cells are diffusely spread and weakly positive (LSAB  $\times 40$ )

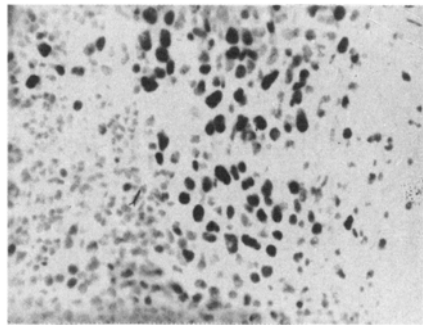


Fig 2. PCNA tumor cells are densely packed and strongly positive (LSAB  $\times 40$ )

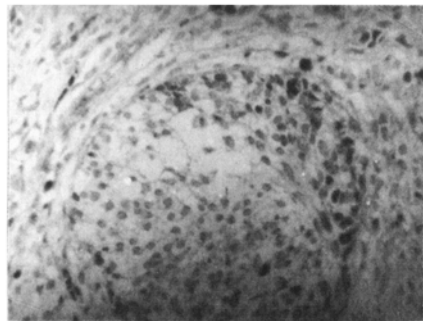


Fig 3. PCNA only a few positive cells existed in the peripheral region of pale island (LSAB  $\times 40$ )

### Prognosis

The follow-up was completed in all the cases. 34 cases had got survival period of  $<12$  months while 29 had one of  $\geq 12$  months, among which 5 had got much longer survival period of  $>100$  months and the longest is 190 months. The mean one is 24 months. Table 5 shows the relationship of PCNA expression with prognosis, from which we can see that the stronger the PCNA expression, the worse the prognosis. And statistics confirmed the significant relationship ( $P < 0.05$ ).

Table 1. Relationship of PCNA expression with differentiation

	PCNA Intensity				Total
	+	++	+++	++++	
Differentiation+	15	16	12	1	44
Differentiation -	6	6	3	4	19
Total	21	22	15	5	63

$P > 0.05$

Table 2. Relationship of PCNA expression with size

	PCNA Intensity				Total
	+	++	+++	++++	
≤ 4 cm	11	10	5	3	29
> 4 cm	10	12	10	2	34
Total	21	22	15	5	63

$P > 0.05$

Table 3. Relationship of PCNA expression with histological classification

	PCNA Intensity				Total
	+	++	+++	++++	
Classic	13	18	12	3	46
Desmoplastic	3	1	2	1	7
Classic with partial Desmoplastic	5	3	1	1	10
Total	21	22	15	5	63

$P > 0.05$

Table 4. Relationship of PCNA expression with mitotic index

	PCNA Intensity				Total
	+	++	+++	++++	
Mitotic index+	3	10	5	3	31
Mitotic index++	8	12	10	2	32
Total	21	22	15	5	63

$P > 0.05$

Table 5. Relationship of PCNA expression with prognosis

	PCNA Intensity				Total
	+	++	+++	++++	
<12 Months	8	10	12	4	34
≥12 Months	13	12	3	1	29
Total	21	22	15	5	63

$P < 0.05$

## DISCUSSION

PCNA is a 36kD acidic nucleoprotein only synthesized and expressed in the proliferating cells. Its synthesis and expression are closely related with cell cycle of proliferation. It begins to increase at G1 stage, and reach a peak at S stage so that it could effectively reflect the proliferative activity of cells.<sup>1,8,9</sup> The amino acid sequence, gene localization, structural characteristics and growth regulatory mechanism of

PCNA have been interpreted clearly.<sup>2</sup> There're reports PCNA expression is closely related with the prognosis of tumors such as leukemia, lymphoma and astrocytoma, etc.<sup>1,5</sup>

Medulloblastoma is originated from primitive neuroectoderm,<sup>10</sup> characterized by high malignancy and poor prognosis. Most of the tumor cells is highly undifferentiated, but some could show signs of differentiation in certain degree.<sup>10,11</sup> Schiffer and Katsetos<sup>4,7</sup> found that relatively less positive cells existed in the regions differentiation occurred, which is in accordance with our observation. But statistics couldn't found significant relation between them. At the same time statistics also found no significant relations between PCNA expression and histological classification or size or mitotic index of the tumor (all  $P > 0.05$ ). This is probably because most of tumor cells of medulloblastoma is very primitive and differentiation is only local or in small region, which could not manifest the differentiation of medulloblastoma as a whole.

Our study also showed that PCNA expression was related with the prognosis of medulloblastoma. In the 29 cases with longer survival period, only 4 (13.8%) is strongly positive and the 5 cases whose survival period is >100 months, 4 is + and only 1 is ++, which indicates that PCNA expression is closely related with the prognosis, i.e. the better the prognosis of medulloblastoma, the weaker the PCNA expression. Statistics also confirmed the significant relationship of PCNA expression with the prognosis. This demonstrates PCNA could be an independent parameter for the prognosis of medulloblastoma.

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