EXPRESSION OF PROLIFERATING CELL NUCLEAR ANTIGEN (PCNA) IN HUMAN PARATHYROID ADENOMA

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Paraffin-embedded sections of parathyroid adenoma from 27 patients with primary hyperparathyroidism were examined for the expression of proliferating cell nuclear antigen (PCNA). PCNA was expressed on the nuclei of the adenoma cells. The positive rate for PCNA ranged from 0.1% to 2.8% (mean 0.9%). The size of adenomas was not related to the positive rate for PCNA, while the preoperative serum calcium level and positive rate for PCNA revealed statistically the linear regression ($y=0.42x-3.96$, $p<0.05$). Mean level of serum calcium level in the group in which positive rate of PCNA is more than 1.0% is statistically higher than that in the group in which positive rate of PCNA is less than 1.0% ($p<0.05$).

Parathyroid adenoma is an established clinical entity causing primary hyperparathyroidism, however, its pathogenesis is still unknown. Moreover, its histological appearance varies in each case. There are no histological criteria of parathyroid adenoma reflecting clinical appearances and making differential diagnosis from adenocarcinoma (2). Our previous studies showed possibility of further classifying parathyroid adenomas using some lectins or oncogene analyses (4, 12).

Proliferating cell nuclear antigen (PCNA) is positive at phase S and G1, and available in paraffin-embedded sections. Recently PCNA has been evaluated in many human materials and reported to be a marker of cell proliferation (5, 6). This study, using paraffin-embedded materials with monoclonal antibodies for proliferating cell nuclear antigen, demonstrated that the cell turn-over could be one of indicators reflecting clinical appearances.

MATERIALS AND METHODS

Materials

All specimens were surgically obtained and pathologically diagnosed as parathyroid adenomas. Twenty-seven specimens were weighed and immediately

fixed in 10% formalin and embedded in paraffin. In 5 cases, frozen materials were also evaluated. These specimens were processed for the following steps. A part of tumors resected was embedded in OCT compound and immediately frozen in liquid nitrogen, then stored at -80 degree until they were used. The remaining 2 specimens were available for frozen sections alone.

Methods

Paraffin blocks were cut into $5\text{ }\mu\text{m}$ sections, deparaffinized and rehydrated, then the slides were preincubated with phosphate-buffered saline (PBS; pH 7.4) at room temperature. The endogenous peroxidase was blocked with 0.3% H_2O_2 -methanol, and normal goat serum was used for blocking non-specific binding. Primary monoclonal antibodies against PCNA (19A2, IgM, Coulter Immunology, Hialeah, Florida, USA) were then applied without dilution for 1 hour at room temperature. After washing with PBS, the slides were reacted with secondary antibodies, processed for avidin-biotin peroxidase complex staining and visualized with diaminobenzidine. Nuclei were counter-stained with 1% methylgreen. Frozen materials were sectioned into $5\text{ }\mu\text{m}$ with cryostat, then the same methods were applied as those for paraffin-embedded sections. The rates of positive cells for PCNA were calculated as follows: Computed image analysis system (FDB98-RGB, PHOTRON, Tokyo, Japan) was applied and high power fields were chosen for counting. About 1,000 cells were counted, then

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the rate of positive cells were calculated.

Before parathyroidectomy, the fasting serum calcium levels were measured. When measured more than once, the maximum values were selected.

The results were evaluated statistically using linear regression methods with p value of 0.05, then classified into two groups according to the values of PCNA and evaluated with Welch's test.

FIG. 1. Case 14. Immunohistochemical staining of parathyroid adenoma for PCNA on paraffin-embedded section. The staining is diffusely recognized in the nuclei of the two cells. $\times 260$

FIG. 2. Case 9. In this field positive cells are intensively collected. $\times 260$

RESULTS

The diffusely stained nuclei of the adenoma cells were defined positive. No staining in the cytoplasm was seen (Figs. 1 and 2). On paraffin sections, 19/27 cases (70.4%) were positive for PCNA and the positive rates ranged from 0.1% to 2.8% (mean 0.9%), while on frozen sections all 5 cases were positive for PCNA, and the positive rates range from 1.0% to 8.9% (mean 4.3%).

The clinical data available are shown in Table 1., the mean age of the patients was 46.8 (range: 19-77) and the ratio between male and female was 1 : 1.14. The weights of adenomas ranged from 0.05 g to 9.50 g (mean 1.80 g). The preoperative serum calcium

levels ranged from 10.0 to 13.4 mg/dl (mean 11.5 mg/dl).

The weights of adenomas and the preoperative serum calcium levels were compared with the positive rates for PCNA. The correlations were recognized between the positive rates for PCNA and the preoperative serum calcium levels on paraffin sections ($p < 0.05$) (Table 2). No correlation was seen between positive rates for PCNA and the weights of adenomas.

Cases were classified into two groups whether the value of PCNA is more or less than 1.0%. The mean value of preoperative serum calcium level was significantly higher in the former groups than in the latter groups (11.9 mg/dl vs 11.1 mg/dl, $p < 0.05$).

TABLE 1. *Reviews of cases examined*

Case No	sex	age	preoperative serum level (mg/dl)	weight of adenoma (g)	positive rate for PCNA on paraffin sections (%)	positive rate for PCNA on frozen sections (%)
1.	F	24	12.0	1.20	1.0	—
2.	M	45	10.7	6.00	0.0	—
3.	M	60	10.7	0.30	0.0	—
4.	M	60	11.1	0.50	2.7	—
5.	M	38	11.0	0.30	0.0	—
6.	F	35	10.9	1.00	0.0	—
7.	M	43	11.0	0.30	1.5	—
8.	F	37	11.7	0.22	0.0	—
9.	M	45	13.1	2.70	2.8	—
10.	M	50	11.3	5.00	0.1	—
11.	F	37	11.7	0.82	1.4	—
12.	M	46	11.4	0.90	0.2	—
13.	F	25	10.5	0.20	1.2	—
14.	M	30	13.0	1.40	2.7	—
15.	F	75	11.0	0.50	0.3	—
16.	F	43	13.4	1.12	1.5	—
17.	F	61	10.0	0.83	1.4	—
18.	M	52	10.2	1.70	0.7	—
19.	F	19	11.7	0.40	0.6	—
20.	F	70	10.6	4.70	0.4	—
21.	M	44	13.4	1.12	1.1	6.2
22.	F	46	10.8	0.40	0.6	3.8
23.	F	48	11.6	0.65	1.3	6.7
24.	F	77	10.6	3.10	0.0	—
25.	F	52	13.5	9.50	1.2	1.0
26.	M	57	11.2	0.77	0.9	4.5
27.	F	41	11.7	2.00	0.0	—
28.	M	60	11.0	0.05	—	8.9
29.	M	38	12.2	4.50	—	1.4

TABLE 2. Correlation between preoperative serum Ca and positive rate for PCNA on paraffin-embedded specimen

DISCUSSION

The cell proliferation has been difficult to be evaluated because the use of radioisotopes or bromodeoxyuridine (BrdU) has been inevitable. However, the development of monoclonal antibodies for proliferating related antigens, such as Ki-67, enabled to evaluate the cell cycles without using isotopes or a cancer inducing drug injection such as BrdU. Moreover, in recent years, the expressions of proliferating cell nuclear antigen (PCNA) have been more often applied as a marker for cell proliferations because of the usefulness of its antibodies for both paraffin sections and frozen sections (5, 6, 8). This antigen is a 36 kD protein associated with the cell cycle and an auxiliary protein of DNA polymerase delta, appearing only in late G1 and S stages (1, 14).

There have been several reports for the clinical evaluations of PCNA in neoplasms. Dawson *et al.* (1990) compared the positive rate of PCNA in breast carcinomas to those in benign breast lesions, and revealed the increased expressions in carcinomas (3). Louis *et al.* (1991) reported the higher rate of PCNA expression in higher grade brain tumors (6–15%). They also recognized the tendency for the increased expressions of PCNA as the grade of tumor progressed (10). Visakorpi *et al.* pointed out that the labeling index is higher in the poorly differentiated prostatic carcinomas (16). Furthermore, Jain *et al.* (1991) examined 93 cases of gastric carcinomas and reported the worse prognosis of tumors with higher expressions (7). Thus, PCNA was shown to be a good indicator for evaluating malignant neoplasms, however, reports for functioning endocrine tumors are rare.

Tumors arising from endocrine organs, such as parathyroid glands and adrenal glands, have problems

for their properties. One problem is the difficulty of determining the characteristics of tumors. Whether a tumor is carcinoma, adenoma, or hyperplasia, could not easily be determined clinically and even histologically. Another problem is to classify the varieties of clinical or histological appearances of parathyroid adenomas. Microscopic appearances show many variations. In some adenomas cells scattered diffusely and in other adenomas duct formations are seen. The degree of pleomorphism differs in each case. Our previous study demonstrated the possibility of further classifying parathyroid adenomas using lectins and oncogene analysis (4, 12). However, there is still no definitive histological indicator explaining variation of clinical symptoms and data.

Our results showed that PCNA might also give a new criterion for further classifying parathyroid adenomas, since a correlation exists between the rate of positive cells on paraffin sections and preoperative serum calcium levels, which is one of the most significant factors. It has been known that the weight of the parathyroid adenoma is correlated with the serum calcium level (9), which is not contradictory to our result. However, the weight does not reflect the proliferation in our result. Rather, the serum calcium level reflects the proliferation. It is interesting that the serum calcium level, reflecting the excessive secretion of PTH, is also an indicator of the cell growth, but its meaning is still unknown. Furthermore, we could significantly classify the cases of parathyroid adenoma into two groups according to the level of the rates of PCNA.

The other factors, plasma PTH levels, blood ionized calcium levels, nephrogenic cyclic adenosine monophosphate (c-AMP) could not be measured in many cases because of methodological problems in the

past.

In our study, 5 cases were available for the comparison between the positive rate on paraffin-embedded sections and that on frozen sections. It was revealed that frozen sections seem better for detecting expressions of PCNA, which might reflect the loss of activity during the fixation and embedding procedures. However, it is difficult for small parathyroid adenoma to collect samples as a routine work. Wolf reported that this antigen is preserved even in overfixed paraffin-embedded specimens and obtained stable results (18). In our experiment, reproductivity was confirmed using a paraffin-embedded blocks obtained for a routine pathological diagnosis, which were fixed for 24–48 hr in 10% formalin. We consider that this method using routine paraffin sections will be useful for evaluating the proliferation of parathyroid adenoma.

The property of antibodies might be taken into account. Antibodies for PCNA were initially found in the serum of SLE patients (11). Monoclonal antibodies were obtained by Ogata *et al.* (14), and there are now two clones of monoclonal antibodies commercially available (17). There were no reports referring to the difference between each kind of antibodies, but these antibodies might recognize different epitopes.

In our report, the rate for PCNA appeared to be lower than those in other reports. This fact might be due to the difference of antibody previously described or the proliferation in benign tumors might be slower than that in malignant tumors. The problem is whether this lower positive rate could be useful. The differential diagnosis between adrenal carcinoma and adenoma is mainly made by the rate of mitotic cells. The cut-off line is usually 5 mitoses per 50 high power fields (15). Moreover, anaplastic seminoma is also defined by the mitotic rate, which is more than thirty mitoses in a total of 10 high power fields (13). These rates seem to be lower than 1.0%. Taking these facts into account, our data could be useful as a practical criterion.

Our study showed the possibility of classifying parathyroid adenomas and further studies will deal with the histological criteria reflecting the clinical conditions.

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