Expression of Proliferating Cell Nuclear Antigen(PCNA) in biopsy and autopsy specimens of gastric carcinoma

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Abstract: Although proliferating cell nuclear antigen (PCNA) is known to be an indicator of malignant potential in tumors, the biological and clinicopathological significance of PCNA in tumor tissue is controversial. Methods: Immunohistochemical expression of PCNA was examined in 58 gastric carcinoma tissues obtained at autopsy to test the clinicopathological significance. In addition, in 24 of the 58 tumor tissues we compared immunohistochemical expression of PCNA in biopsy and autopsy specimens from the same patient in order to know whether the proliferating activity of tumor cells is stationary from the early stage to the end of tumor growth. Results: 1. PCNA was undetectable in some tumor tissues (12.5% in biopsy and 10.3% in autopsy specimens). 2. the frequency of PCNA positive cases and labeling index (LI) (%) of PCNA in tumor tissues were not significantly different between biopsy and autopsy specimens. 3. the intensity of PCNA reaction was not related to prognosis. 4. PCNA positive cases and LI did not correlate with survival condition. Conclusion: It is hard to say whether PCNA is a reliable indicator in predicting malignancy and prognosis of gastric cancer. J. Med. Invest. 44: 149-153, 1998

Key Words: *PCNA, gastric cancer, immunohistochemistry*

INTRODUCTION

Study of the cell proliferative state has yielded information concerning the proliferative activities of malignant tumors which contribute to the understanding the biological behavior of cancer. Examination of tritiated thymidine in vivo or in vitro and bromodeoxyuridine [BUdR] labeling index of the proliferative state of tumor cells in gastric carcinomas and breast carcinomas was believed to be meaningful in judging prognosis of cancer (1, 2, 3). Yonemura et al. (4) reported that tumors having a high Ki-67 labeling rate had a high frequency of vessel invasion, with consequent poor prognosis and survival. Proliferative cell nuclear antigen (PCNA/cyclin) is a 36KD acidic nuclear protein associated with cell proliferation and expressed mainly, but not absolutely, in the cell nuclus (5).

Compared with BUdR, although the mechanism is almost identical, examination of PCNA is more convenient and can be used on archival specimens. Many studies of PCNA in different tumors have been carried out, but the conclusions are inconsistent (6, 7, 8, 9). Some authors believe that PCNA is a valid marker of proliferation in gastric and other tumors and may be a significant prognostic indicator (10, 11). Tumors with high proliferative activity show a more malignant clinical course and poorer prognosis than those with low proliferative activity (2). Other authors believed that there remain some doubts as to the relationship between PCNA expression and cell proliferation in the context of at least some forms of neoplasia (12). Thus the significance of PCNA immunoreactivity in tumors has yet to be fully established (13). Moreover, few papers have studied the relationship of PCNA expression between biopsy and autopsy in the same patient. We examined and compared PCNA expression in biopsy and autopsy to determine whether the proliferative activity of both specimens determined by PC10, a mouse monoclonal antibody to PCNA, would provide useful and reliable information for the clinical outcomes of patients with gastric cancer. Also we wanted to clarify the differences in biological activity between longer and shorter survival cases in addition to the relation to sex and age.

MATERIALS AND METHODS

Fifty-eight autopsy specimens and 24 biopsy specimens of gastric cancers were chosen from the files of Tokushima University Hospital, Japan, from 1968 to 1989 without any bias. The diagnosis was confirmed by histological examination and pathological criteria. The ages of patients ranged from 27 to 88 years old (mean, 63 years old), male 38, female 20. Of the biopsy specimens 11 out of 24 died of cancer including 2 who died of cancer bleeding. Of the autopsy specimens, 33 out of 58 underwent surgery and had recurrence. Both biopsy and

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autopsy specimens were available for study in 24 of the 58 patients. All sections were cut from formalin-fixed, paraffin-embedded blocks. Anti-PCNA monoclonal antibody PC 10 from Dakopatts (Copenhagen, Denmark) was used.

Immunostaining

Immunostaining was performed on the sections using the Avidin-Biotin-Peroxidase complex (ABC) technique. After deparaffinization by incubation at 60°C for 30 minutes and dehydration in a graded ethanol series, the sections were incubated sequentially with the following solutions : 1. 0.3% hydrogen peroxide in absolute methanol for 30 minutes to remove endogenous peroxidase activity ; 2. normal horse serum for 30 minutes to reduce background staining ; 3. PC10 dilution of 1 : 100 with overnight incubation at 4°C was found to be optimal ; 4. ABC complexes (Dakopatts) diluted 1 : 200 at room temperature for 40 minutes. 5. 0.03% 3, 3-diaminobenzidine tetrahydrochloride solution to which hydrogen peroxide (0.02%) was added just before use. Between each step of the immunostaining procedure the sections were washed in phosphate buffered saline (PBS). The intensity of positive nuclear reaction for PCNA varied from weak to strong.

In order to know what relationship exists between PCNA intensity and patients' prognosis, age and sex, we counted the labeling index (LI) twice : firstly, the cells with strong nuclear reaction were counted ; secondly, not only the cells with strong but also weak nuclear reaction were counted. Figure 1 shows PCNA staining in a gastric tumor. Immunoreaction was observed as strong reaction (long arrow) or weak reaction (short arrow). The LI of the cells with a strong nuclear reaction was 26.0% and the LI of both the cells with strong and weak reaction was 66.0% in this case. We used this standard to evaluate all sections.

Tumors with positive cells for PCNA were regarded as positive cases regardless of the number of positive cells. In the labeling index the data were presented as mean ±SD which was statistically analyzed by Student's t test. The frequency of positive cases was statistically analyzed by the Mann-Whitney test.

RESULTS

Immunoreactivity was observed in nuclei with only occasional cytoplasmic staining in both biopsy and autopsy specimens of gastric cancer. Figure 2 shows hematoxylin & eosin (A) and PCNA staining (B) in the same part of a gastric tumor (biopsy specimen). Only nuclear staining was counted as positive. The results of immunostaining in biopsy and autopsy specimens of all gastric tumors are shown in Table I. Of the biopsy specimens, 21 out of 24 cases (87.5%) were PCNA positive. The autopsy specimens of these 24 patients showed the same PCNA-positive ratio (87.5%). Of the all 58 autopsy specimens, 52 (89.7%) had PCNA-positive tumor cells. No significant difference of frequency of PCNA positive cases between biopsy and autopsy specimens was found. The LI ranged from 0% to 50% (mean 14.6%) in biopsy specimens at first counting and from 0% to 75% (mean 30.6%) at second counting ; The LI ranged from 0% to 59.5% (mean 10.1%) in autopsy specimens at first counting and from 0% to 87% (mean 33.4%) at second counting. In neither the first counting nor the second counting was any significant difference in

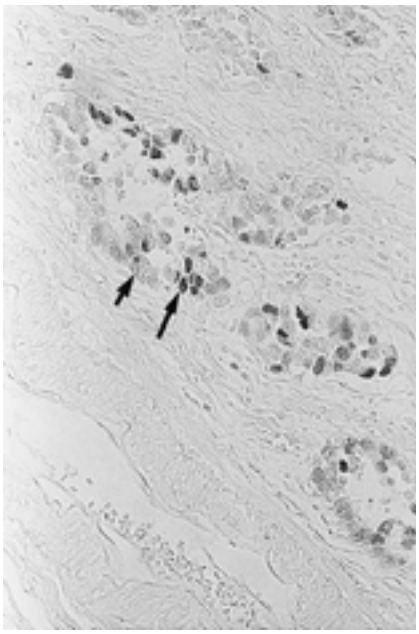

Fig.1. Photomicrograph of gastric carcinoma (autopsy specimen) stained with proliferating cell nuclear antigen (PCNA) monoclonal antibody PC10. Immunoreaction is observed with strong (long arrow) and weak reaction (short arrow). (original magnification x 231).

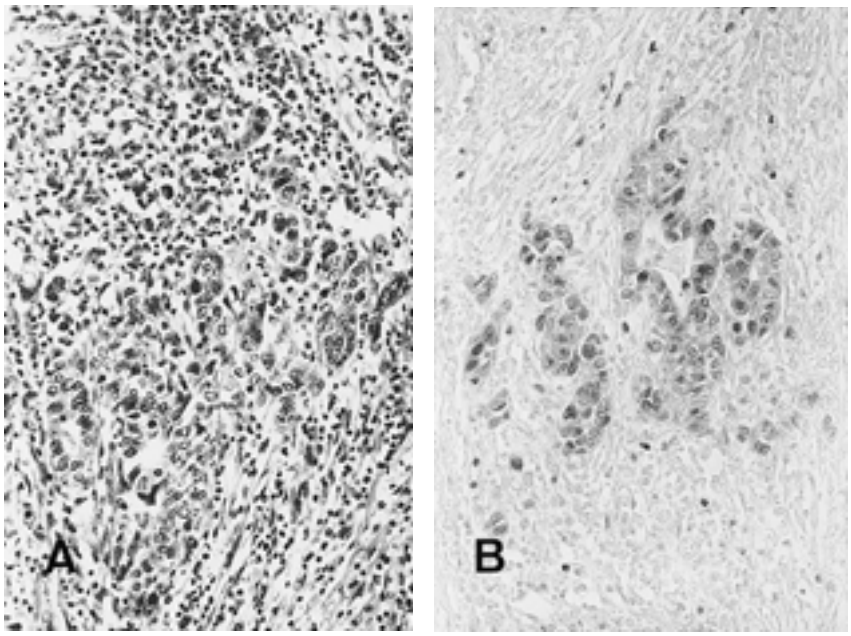

Fig.2. Photomicrograph of gastric carcinoma (biopsy specimen) stained with H & E (A) and with proliferating cell nuclear antigen (PCNA) monoclonal antibody PC10 (B). Only nuclear staining is regarded as a PCNA-positive reaction. (original magnification x 231).

Table I. PCNA Expression in Biopsy and Autopsy specimens of Gastric cancer

	No. of cases	PCNA Positive Cases (%)	Labeling Index (%) Mean±SD	
			1 st	2 nd
Biopsy	24	21(87.5)	14.6±25.2	30.6±24.8
		NS	NS	NS
Autopsy	58	52(89.7)	10.1±13.5	33.4±24.5

NS : No significant difference.
1 st : First counting ; 2 nd : Second counting.

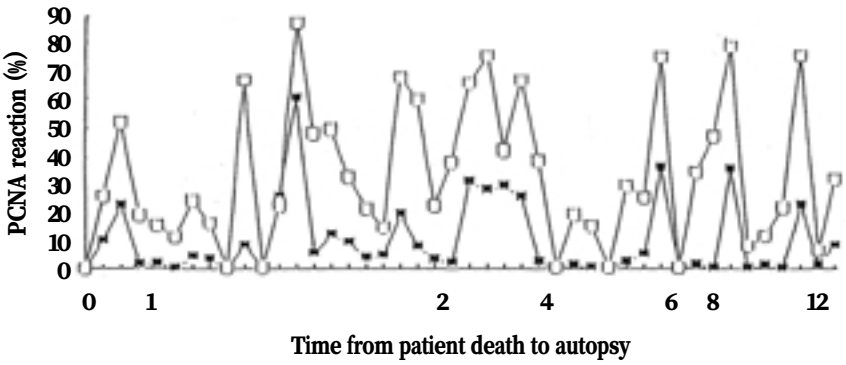

Fig.3. The relationship between duration to tissue fixation and PCNA reaction. ■ : first counting and □ : second counting.

LI between biopsy and autopsy found. We checked the duration from patients death to autopsy, which ranged from 30minutes to 12 hours, against PCNA reaction. Figure 3 shows the relationship between post-mortem delay and PCNA reaction, from which we found PCNA reaction of samples was not influenced by shorter or longer duration.

Table II shows PCNA reaction in groups of different survival condition, sex and age. Examining the survival period, the shortest period from biopsy to death was one half month ; the longest 9 years. The cases with shorter survival period from biopsy to death did not show higher incidences of PCNA expression. In the biopsy specimens, the frequencies of PCNA positive cases in the shorter and longer groups were 83.3% (15/18) and 100% (6/6) and in the autopsy specimens, the frequencies of PCNA positive cases were 94.4% (17/18) and 66.7%, respectively (4/6). The LI of these two groups showed no significant difference among autopsy

Table II. PCNA Expression in Different Survival Period, Sex and Age Groups

Case	No.	PCNA Positive Cases (%)		Labeling Index (%) (Mean±SD)			
		Biopsy	Autopsy	Biopsy		Autopsy	
				1 st	2 nd	1 st	2 nd
Shorter Group (<12Mon.)	18	15/18(83.3)	17/18(94.4)	11.0±15.3	23.9±24.1	11.0±15.8	34.6±23.7
		NS	NS	*	*	NS	NS
Longer Group (>12 Mon.)	6	6/6(100)	4/6(66.7)	25.5±8.9	50.5±15.0	1.4±1.30	17.0±14.7
Male	38	17/18(94.4)	36/38(94.7)	15.7±15.9	31.0±24.5	11.5±14.2	36.2±24.7
		NS	NS	NS	NS	NS	NS
Female	20	4/6(66.7)	16/20(80.0)	11.6±13.8	29.2±28.0	7.5±11.9	28.9±26.4
Younger Group (<35)	4	3/3(100)	4/4(100)	24.2±24.5	32.7±29.1	28.1±23.3	61.5±30.9
		NS	NS	NS	NS	*	*
Older Group (>35)	54	18/21(85.7)	49/54(90.7)	13.3±13.7	30.3±25.0	8.8±11.8	31.0±23.3

* : $p<0.05$.
NS : No significant difference.
1 st : First counting ; 2 nd : Second counting.

specimens at either the first or second counting, but the difference in biopsy specimens was statistically significant. Namely, LI was significantly higher in the longer survival group than in the shorter group. In the different sex groups we found no significant difference between the frequency of PCNA positive cases or LI and sex. Between older and younger groups, in autopsy specimens the LI was significantly different both in first and second counting. Namely, LI was significantly higher in the younger group than that in the older group.

DISCUSSION

It is very important to look for a reliable method to judge biological activity of tumors. The prognosis in patients with gastric cancer has been thought to be dependent on the stage of disease, which include gross finding, nodal and organ metastasis, histologic type and vessel invasion, etc. (14). But these parameters appear less valuable in determining of prognosis, especially in the early stages. Therefore we need to obtain information about the level of tumor aggression.

Malignant tumors are in a highly proliferative state, and examination of the proliferative activity of the tumor cell has thus been believed to be a promising method for predicting malignancy and prognosis (15, 16). Amadori et al. (2) reported that in the study of proliferative activity of primary gastric tumors using tritiated thymidine labeling technique the survival rate (3 years) of patients with tumors having a low labeling index was higher (50%) than those with a high labeling index (13%). Several similar results associated with gastric cancer as well as prostatic cancer, breast cancer and astrocytic neoplasm have been reported (11, 17, 18, 19). But Hall et al. thought that the significance of PCNA immunoreactivity in tumors is at present uncertain (13). PCNA immunostaining with PC10 from formalin-fixed, paraffin embedded prostatic cancers has little prognostic value (20), and PCNA staining of stored paraffin sections is of little prognostic value in patients with gastric cancer (21). In our study, we examined and compared both biopsy and autopsy specimens of gastric tumors in 24 cases, and found that the frequency of PCNA positive cases and PCNA-LI in biopsy specimens did not significantly differ from that in autopsy specimens. We therefore are of the opinion that both frequency of PCNA positive cases and LI should be considered in evaluating the meaning of PCNA. Our present study showed 87.5% (21/24) of biopsy specimens and 89.7% (52/58) of autopsy specimens to have a PCNA-positive reaction. The reason why all malignant tumor cells were negative for PCNA in some cases is not clear. We supposed that the negative PCNA staining may be the result of inactivity due to a post-mortem delay before fixation, but our investigation showed this to be unfounded. Hattori et al. (22) reported that the patients with an index >12% (the mean of all of PCNA values) had a worse prognosis than those with an index <12%, when weakly PCNA-stained nuclei were excluded. In our study we counted twice : first time only strong PCNA-stained nuclei was counted and second time both strong and weak PCNA-stained nuclei was counted. However the results of this double counting did not show a similar relationship. Moreover, in biopsy, the LI of PCNA was significantly higher in the longer survival group than in the shorter group at both the first and second countings. On the other hand, the LI of autopsy specimens was significantly higher in the younger group than in the older group at both countings. These findings suggest that the intensity of PCNA reaction is not so important in the judgment of prognosis of tumor, and that the survival period of the patients can not be predicted by the intensity of PCNA reaction, LI or PCNA-positive cases. There was little relationship between PCNA expression and survival period, either in biopsy or autopsy. The duration from biopsy to death ranged from one half month to 9 years. According to some other reports, a strong PCNA reaction means poor prognosis and short survival period in some cancers ; and those carcinomas with good prognosis usually demonstrate a weak reaction (14,15). The results of our study differed from these conclusions. We could not confirm any the rule that the prognosis of tumors can be predicted by PCNA reaction, or that PCNA is a reliable prognostic factor. Our results suggested that the frequency of PCNA positive cases, LI and the intensity of PCNA reaction are not directly related to the activity of tumor growth. We can not say that examination of PCNA is meaningless in the study of gastric tumors, but we think that care is necessary when imposing clinical and clinicopathological values on PCNA.

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