Proliferating cell nuclear antigen expression in papillary thyroid carcinoma

H Ando, H Funahashi, M Ito, T Imai, H Takagi

Abstract

Aims—To correlate the expression of pro-liferating cell nuclear antigen (PCNA) with various clinicopathological features of papillary thyroid carcinoma.

Methods—Sections from 131 formalin fixed, paraffin wax embedded papillary thyroid carcinomas were stained with a monoclonal antibody (PC10) directed against PCNA using the avidin-biotin immunoperoxidase (ABC) method. PCNA immunoreactivity was based on the PCNA labelling index (LI) following evaluation of at least 1000 tumour cells, and expressed as follows: grade A (LI < 10%), grade B (10% ≤ LI < 25%), and grade C (LI ≥ 25%). The relation between PCNA expression in these three groups and other clinicopathological factors, such as sex, age, tumour size, nodal metastases, and histological differentiation, were examined.

Results—Based on the labelling index, 57 (43.5%) cases were graded as A, 46 (35.1%) as B, and 28 (21.4%) as C. The female-:male ratios were 6.13:1 for group A, 2.83:1 for group B, and 2.11:1 for group C. The mean (SD) ages of the patients were 39.0 (16.1) in group A, 53.5 (14.4) in group B, and 55.8 (13.3) years in group C. The correlation between age and PCNA grade was strongest in women.

Conclusions—PCNA immunoreactivity is correlated with sex and age in patients with papillary thyroid tumours.

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Department of
Surgery II, Nagoya
University School of
Medicine, Nagoya,
Japan
H Ando
H Funahashi
T Imai
H Takagi

Department of
Pathology
M Ito

Correspondence to:
Dr Masafumi Ito,
Department of Pathology,
Nagoya University Hospital,
65 Tsurumai-cho, Showa-ku,
Nagoya 466, Japan.

The prognosis of patients with papillary thyroid carcinoma is generally good, even if surgical resection is not performed early in the clinical course. However, there is a subset of patients with a poorer prognosis, in whom the tumour infiltrates the surrounding cervical tissues which cannot be cured even with radical treatment. These patients do not always present with histologically poorly differentiated or anaplastic carcinomas.

Recently, there have been several studies examining the proliferative activity of various malignant tumours using immunohistochemical methods. Few such studies have examined the proliferative activity of papillary thyroid carcinomas. These studies suggest that there is a relation between general clinical course and proliferative activity in papillary thyroid can-cer.[1 2] However, there are patients who follow a clinically malignant course although they may have favourable histology. Therefore, we studied proliferating cell nuclear antigen (PCNA) expression in papillary thyroid carcinoma during various clinical stages and related this expression to the other clinicopathological features.

Methods

Patients (n = 131) surgically treated for papillary thyroid carcinoma at the Department of Surgery II, Nagoya University Hospital between 1981 and 1993 were studied. Papillary carcinoma was confirmed histologically in all 131 patients. Tumours that underwent ana-plastic transformation and were associated with follicular carcinomas were excluded. The mean (SD) age of the patients overall was 47.7 (16.7) years (range 7–83 years) and of the 102 female and 29 male patients were 45.2 (15.8) and 56.7 (16.8) years, respectively. Surgical treatment comprised total thyroidectomy (123 patients), subtotal thyroidectomy (one patient), lobectomy (four patients), and tumour excision (four patients).

IMMUNOSTAINING
Specimens were fixed routinely in 10% formalin and embedded in paraffin wax. Sections, 3 μm thick, were mounted on poly-L-lysine coated glass slides and deparaffinised using xylene and graded alcohol. The sections were then washed with water and incubated with normal goat serum for 10 minutes to reduce non-specific antibody binding. The primary monoclonal mouse anti-PCNA antibody (NCL-PCNA, PC10; Novocastra Laboratories Ltd, UK) was diluted 1 in 200 and applied to the sections overnight at 4°C. Sections were rinsed in phosphate buffered saline (PBS) and then immersed in 0.03% H_2O_2 in methanol for 20 minutes to block endogenous peroxidase activity. The secondary antibody, a biotinylated horse anti-mouse immunoglobulin, was applied for 40 minutes, sections were rinsed in PBS, and an avidin-biotin peroxidase complex reagent (Vectastain ABC kit, Vector Laboratories, Burlingame, California, USA) applied for 30 minutes at room temperature. Diaminobenzidine (Sigma, St Louis, Missouri, USA) was used as the chromogen. Sections were lightly counterstained with Mayer's haematoxylin.

Quantitative analysis of PCNA expression was conducted by determining the ratio of positively to negatively stained cells (labelling index, LI). At least five representative high power fields (×400) were examined by light

Table 1 Clinicopathological features according to PCNA grade. Results are expressed as mean (SD) unless stated othewise

PCNA grade	Female:male ratio (female/male)	Mean age (years)	Mean age by sex		Tumour size (cm)	Cases of poorly differentiated carcinoma (%)
			Male	Female		
A	6.13: 1 (49/8)	39.0 (16.1)	50.3 (24.1)	37.2 (4.0)	3.3 (4.0)	10/57 (17.5)
B	2.83: 1 (34/12)	53.5 (14.4)	61.8 (11.8)	50.5 (14.2)	3.4 (2.5)	13/46 (28.3)
C	2.11: 1 (19/9)	55.8 (13.3)	55.6 (14.2)	56.2 (12.8)	3.7 (2.8)	6/28 (21.4)

*$p < 0.05$.

A = Labelling index (LI) < 10%; B = 10% ≤ LI < 25%; C = LI ≥ 25%.

microscopy. More than 1000 tumour cell nuclei were counted in each specimen, and the percentage of PCNA positive cells was calculated. Tumours were assigned to one of three groups based on the LI (group A, LI < 10%; group B, 10% ≤ LI < 25%; group C, LI ≥ 25%). Some of the tumours in each group were also immunostained with the MIB-1 monoclonal antibody (MBL, Nagoya, Japan) for comparison. MIB-1 immunostaining was performed as for PCNA immunostaining except that the specimens were pretreated in a microwave oven and the antibody was diluted 1 in 50. Statistical analysis was performed using Fisher's exact probability test and Student's *t* test; $p < 0.05$ was considered significant.

Results

Based on the labelling index, 57 (43.5%) tumours were assigned to group A, 46 (35.1%) to group B, and 28 (21.4%) to group C (fig 1). Figure 2 shows the distribution of all 131 patients according to both PCNA grade and sex. The female patients were divided into two groups based on age (0–49 years, 50 years and older). The ratios of female:male patients in each group were: 6.13:1 in group A, 2.83:1 in group B and 2.11:1 in group C. The percentage of female patients aged 0–49 years was 70.2% in group A, 30.4% in group B and 17.9% in group C. The mean (SD) age of patients in each group was 39.0 (16.1) in group A, 53.5 (14.4) in group B, and 55.8 (13.3) years in group C. When female patients were considered alone, there was a good correlation between the mean age at diagnosis and the PCNA grade. This was not the case for men (table 1).

There was no significant difference in the average maximal diameters of the tumours assigned to the three groups (table 1). There was no significant correlation between PCNA grade and the presence or absence of lymph node metastases (table 2). Twenty nine (22.1%) tumours were histologically poorly differentiated papillary carcinomas with either a solid or trabecular, or both, pattern. PCNA reactivity by group for these poorly differentiated carcinomas was 17.5% (10/57) in group A, 28.3% (13/46) in group B and 21.4% (six of 28) in group C. PCNA immunoreactivity was uniform in tumours with papillary and solid/trabecular patterns (table 1).

Disease recurred in 23 (17.6%) patients (12 men and 11 women) following surgery; more
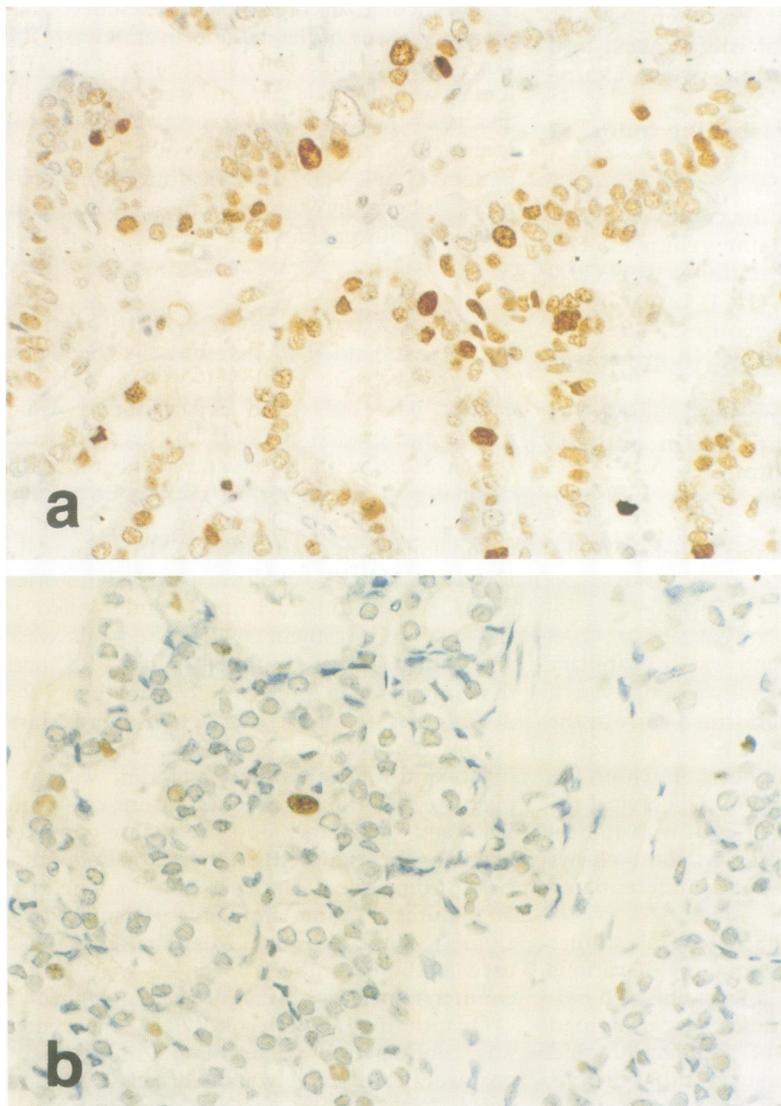

Figure 1 (a) Strong (grade C) and (b) weak (grade A) PCNA immunostaining.
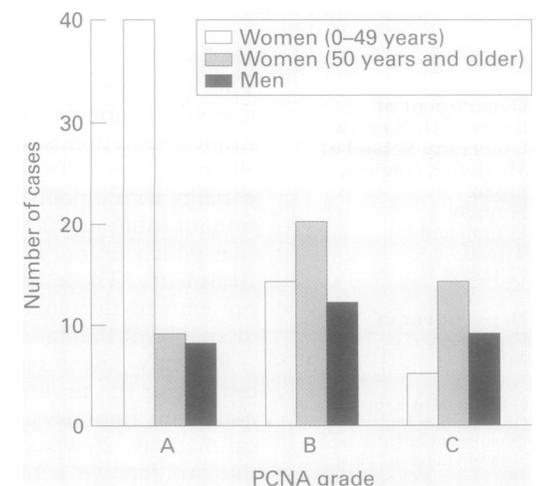

Figure 2 Distribution of patients according to sex and PCNA grade. Women are divided into two groups based on age (0–49 years, 50 years and older).

Table 2 *Relation between lymph node status and PCNA grade. Results expressed as n (%)*

Lymph node status	PCNA grade		
	A	B	C
pN0	6 (11.5)	8 (26.7)	2 (9.5)
pN1	46 (88.5)	30 (73.3)	21 (90.5)
Total	52	38	23

pN0 = regional lymph node metastases absent.
pN1 = regional lymph node metastases present.

men (41.4%) than women (10.8%) relapsed. The recurrence ratios according to PCNA grade were as follows: 10.5% (six of 57) in group A, 28.3% (13/46) in group B and 14.3% (four of 28) in group C. The prognosis for patients in group A was the most favourable of the three groups, although there was no correlation between PCNA expression and overall prognosis.

Ten cases were selected at random from each group and immunostained with MIB-1; however, MIB-1 positivity was rarely observed regardless of the PCNA grade.

Discussion

PCNA is a factor for DNA polymerase delta, is synthesised during the late G1 to S phase of the cell cycle and has been confirmed to be cyclin.[3][4] Recently, many investigators have applied PCNA immunostaining to clinical specimens in order to estimate the malignant nature or prognosis of various neoplasms. In the current study, we found that some cases of papillary thyroid carcinoma stained strongly for PCNA. Previous studies have suggested that the proliferative activity of papillary carcinomas in terms of the PCNA positive ratio is generally less than 10%.[2] However, we found several cases with PCNA immunostaining labelling indexes of more than 50%.

One of our major findings was that PCNA expression in papillary thyroid carcinoma correlates with the age and sex of the patients. The female:male ratio was lower, and the mean age of patients was higher in tumours strongly expressing PCNA than those with weak expression. The correlation between age and PCNA expression was especially strong for women. This result is interesting because the most important prognostic factors in papillary carcinoma are the age and sex of the patient, and therefore PCNA grade cannot be used as

an independent prognostic factor. Other clinicopathological features such as a tumour size, lymph node metastases and the degree of differentiation[5] did not correlate with PCNA expression.

It has been suggested that PCNA activity is not necessarily associated with DNA synthesis, but may be involved in unscheduled DNA repair throughout the cell cycle.[6][7] The proportion of PCNA positive cells found in many tumours exceeds that expected for the proportion of proliferating cells as assessed by other methods.[8] We also found that some papillary carcinomas overexpressed PCNA and that MIB-1 immunostaining indicated the real proliferative activity of the tumour.[9] Some authors have suggested that PCNA overexpression is a result of cytokine action.[10][11] In the current study, there was no correlation between PCNA expression and lymphocytic infiltration. In conclusion, as yet unknown factors regulated by the age and sex of patients with papillary thyroid carcinoma may be related to PCNA expression.

1 Yoshida A, Kamma H, Asaga T, Masuzawa C, Kawahara S, Miura T, *et al.* Proliferative activity in thyroid tumors. *Cancer* 1992;**69**:2548–52.

2 Shimizu T, Usuda N, Yamada T, Sugenoya A, Iida F. Proliferative activity of human thyroid tumors evaluated by proliferating cell nuclear antigen/cyclin immunohistochemical studies. *Cancer* 1993;**71**:2807–12.

3 Bravo R, Frank R, Blundell PA, MacDonald-Bravo H. Cyclin/PCNA is the auxiliary protein of DNA polymerase-delta. *Nature* 1987;**326**:515–17.

4 Prelich G, Tan CK, Kostura M, Mathews MB, So AG, Downey KM, *et al.* Functional identity of proliferating cell nuclear antigen and a DNA polymerase-delta auxiliary protein. *Nature* 1987;**326**:517–20.

5 Sakamoto A, Kasai N, Sugano H. Poorly differentiated carcinoma of the thyroid: A clinicopathologic entity for a high-risk group of papillary and follicular carcinomas. *Cancer* 1983;**52**:1849–55.

6 Toschi L, Bravo R. Changes in cyclin: proliferating cell nuclear antigen distribution during DNA repair synthesis. *J Cell Biol* 1988;**107**:1623–28.

7 Shivji KK, Kenny MK, Wood RD. Proliferating cell nuclear antigen (PCNA) is required for DNA excision repair. *Cell* 1992;**69**:1–20.

8 Hall PA, Levison DA, Woods AL, Yu CCW, Kellock DB, Watkins JA, *et al.* Proliferating cell nuclear antigen (PCNA) immunolocalization in paraffin sections: an index of cell proliferation with evidence of deregulated expression in some neoplasms. *J Pathol* 1990;**162**:285–94.

9 Rose DSC, Maddox PH, Brown DC. Which proliferation markers for routine immunohistology? A comparison of five antibodies. *J Clin Pathol* 1994;**47**:1010–14.

10 Hall PA, Hart I, Goodlad R, Cortes PJ, Lane DP. Expression of proliferating cell nuclear antigen in non-cycling cells. *J Pathol* 1992;**168**:97A.

11 Monaghan P, Perusinghe NP, Nicholson RI, McClelland RA, O'Hare MJ, Lane DP. Growth factor stimulation of proliferating cell nuclear antigen (PCNA) in human breast epithelium in organ culture. *Cell Biol Int Rep* 1991;**15**:561–70.