Virchows Archiv (1994) 424:533–537

ORIGINAL ARTICLE

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Proliferative cell nuclear antigen expression in follicular tumours of the thyroid with special reference to oxyphilic cell lesions

Received: 23 December 1993 / Accepted: 10 March 1994

Abstract The expression of proliferative cell nuclear antigen (PCNA) in follicular tumours of the thyroid was examined by immunohistochemistry. Both usual nonoxyphilic cell follicular tumours (non-OCT) and oxyphilic cell tumours (OCT) were subdivided into benign, indeterminate, encapsulated carcinoma, and widely invasive carcinoma types. Among non-OCT the percentages of PCNA-positive cells in benign tumours, encapsulated carcinomas, and widely invasive carcinomas was 2.5%–8.6%, 11.8%–39.1%, and 18.6%–20.0%, respectively. There was a statistically significant difference between benign tumours and encapsulated or widely invasive carcinomas, as in previous studies. A value of 10% was appropriate to distinguish benign from malignant lesions. PCNA-positive cells in indeterminate-type non-OCT were not significantly different from those in benign tumours, ranging from 4.3%–19.6%, and occurring at more than 10% in three of six tuours. Among OCT the positivity was less than 10% in benign tumours (4.5%–7.8%) and more than 10% in malignant tumours (14.1%–35.9%) and all the eight indeterminate tumours (12.5%–27.3%), with a statistically significant differences between the benign tumour and each of the latter types. These results indicate that the examination of PCNA is valuable in diagnosis of thyroid follicular tumours and that the use of similar diagnostic criteria may be warranted in both non-OCT and OCT.

Key words Thyroid · Follicular tumour · Oxyphilic cell tumour · PCNA · Immunohistochemistry

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Introduction

Histological diagnosis of follicular tumours of the thyroid still poses a challenging problem. The criteria for malignancy including the presence of capsular and/or vascular invasion, have been well established, but may require painstaking examination of the capsule and special stains to identify blood vessels [8, 27]. Furthermore, the criteria in oxyphilic (Hürthle) cell tumours (OCT), whose cell population is exclusively or predominantly made up of oxyphilic cells [18], remains controversial [1, 5, 10, 11, 12, 25]. Recently, Carcangiu et al. [6] divided OCT into three categories; malignant, indeterminate, and benign types based on the degree of capsular and/or blood vessel invasion, growth pattern, nuclear atypia, and necrosis.

Proliferative cell nuclear antigen (PCNA) appears in the nucleus during the synthetic phase of the cell cycle [2, 22]. Monoclonal antibodies to PCNA applicable for routinely processed tissue have been produced and have been shown to be useful in the evaluation of cellular proliferation [17, 24]. Recently, a few reports have suggested that the percentage of PCNA-positive cells of follicular carcinoma is significantly higher than that of follicular adenoma [21, 23]. Although not exactly stated, these studies seem to have included only ordinary (nonoxyphilic cell) follicular tumours (non-OCT). In the present study, using the similar immunohistochemical method, we have confirmed this difference in non-OCT by more detailed analysis and also analysed OCT.

Materials and methods

Thyroid tumour specimens with adequate fixation time were examined. They included 28 non-OCT and 24 OCT. Blocks of each tumour had been fixed in 10% buffered formalin for an average of 24 h; a few non-OCT cases in which the fixation time had exceeded 48 h were excluded. Sections of paraffin-embedded tissues were stained with haematoxylin and eosin and elastic-van Gieson. The non-OCT were selected from the file of our pathology department, which included cases from the affiliated hospitals as well as Nagoya City University Hospital. The OCT were consecutive cases at Nagoya City University Hospital between 1976 and 1990. The non-

OCT and OCT were divided according to the criteria of World Health Organization (WHO) [14] and also Carcangiu et al. [6] with some modification; indeterminate categories were made in non-OCT as well as in OCT. There were 19 benign tumours (10 non-OCT and 9 OCT), 14 indeterminate tumours (6 non-OCT and 8 OCT), 16 encapsulated carcinomas (9 non-OCT and 7 OCT), and 3 widely invasive carcinomas (non-OCT only). Widely invasive carcinoma showed unequivocal invasion of the surrounding thyroid or extrathyroid tissues. The encapsulated carcinoma showed obvious (full-thickness) capsular invasion and/or obvious blood vessel invasion although they were well-encapsulated macroscopically. The indeterminate tumours exhibited partial (but not transcapsular) invasion or tumour cell nests in the fibrous capsule. The benign tumours lacked the histological features described above. Growth patterns (solid/trabecular or follicular) were variable and did not differ in the four groups. No significant necrosis was found in tumours in any group in the present series.

Immunohistochemical staining for PCNA was performed on paraffin sections by the streptavidin-biotin imunoperoxidase method [9] using the IMMU-MARK immunostaining kits (ICN ImmunoBiologicals, Lisle, Ill., USA) and monoclonal anti-PCNA antibody PC10 (dilution, 1: 100) (Novocastra Laboratories, Burlingham, La., USA). The peroxidase activity was visualized with diaminobenzidine, followed by counterstaining with methyl green. As negative controls, normal mouse IgG was substituted for the primary antibodies. The percentage of PCNA-positive cells was determined by counting at least 1000 tumour cells in randomly selected fields using a high-resolution colour video camera (Ikegami, model MKC-385, Tokyo, Japan) and a tablet measure unit (Olympus, model VM-30, Tokyo, Japan). Two of us (H.T. and Y-P.Y.) assessed the judgment of the positivity by viewing the high-resolution display and counting the positive cells independently of the histological diagnosis of the tumours.

Student's *t*-test was used for statistical analysis. The difference was considered to be significant when the *P* value was less than 0.05.

Results

The clinical data of the patients are summarized in Tables 1 and 2. The follow-up data of all patients, except for a few patients with non-OCT, were available extending from 1 year to 12 years, with a median of 5 years. One patient (case 27) with widely invasive non-OCT carcinoma was alive with a lung metastasis. All the other patients were alive with no evidence of tumour, although one patient with indeterminate OCT (case 39) had a past history of thyroidectomy 24 years before (the pathological diagnosis was not available).

Tumour cells positive for PCNA showed nuclear staining with a granular or diffuse pattern with variable degrees of intensity (Figs. 1–3); all identifiable staining was regarded as positive. Positive staining for PCNA was also seen in the nuclei of proliferating cells in germinal

Table 1 Nonoxyphilic cell follicular tumours (*non-OCT, PCNA* proliferative cell nuclear antigen, *M* male, *F* female, *NED*, no evidence of disease)

Case number	Age	Sex	Operation	PCNA (%)	Follow-up
Benign non-OCT (follicular adenoma)					
1	27	F	Lobectomy	2.7	Lost to follow-up
2	25	F	Enucleation	5.2	Lost to follow-up
3	50	F	Lobectomy	8.6	Alive NED after 11 years
4	38	F	Lobectomy	4.0	Alive NED after 10 years
5	49	M	Lobectomy	4.5	Alive NED after 10 years
6	31	F	Lobectomy	2.5	Alive NED after 3 years
7	61	M	Lobectomy	6.8	Alive NED after 2 years
8	45	M	Lobectomy	5.2	Alive NED after 1 year
9	47	M	Lobectomy	6.8	Alive NED after 1 year
10	45	F	Lobectomy	3.9	Alive NED after 1 year
Indeterminate non-OCT					
11	22	F	Lobectomy	6.9	Alive NED after 4 years
12	26	F	Lobectomy	5.2	Alive NED after 4 years
13	58	F	Lobectomy	4.3	Alive NED after 4 years
14	30	F	Lobectomy	19.6	Alive NED after 10 years
15	34	F	Lobectomy	12.9	Alive NED after 4 years
16	38	M	Lobectomy	16.2	Alive NED after 4 years
Malignant non-OCT (encapsulated follicular carcinoma)					
17	67	F	Lobectomy	24.6	Lost to follow-up
18	66	F	Lobectomy	14.6	Alive NED after 6 years
19	44	F	Lobectomy	12.1	Alive NED after 5 years
20	36	F	Lobectomy	19.7	Alive NED after 1 year
21	63	F	Lobectomy	16.4	Alive NED after 1 year
22	34	F	Lobectomy	36.9	Alive NED after 1 year
23	40	F	Lobectomy	11.8	Alive NED after 1 year
24	64	F	Lobectomy	39.1	Alive NED after 1 year
25	29	F	Lobectomy	29.3	Alive NED after 1 year
Malignant non-OCT (widely invasive follicular carcinoma)					
26	41	F	Lobectomy	19.3	Lost to follow-up
27	57	F	Total thyroidectomy	20.0	Alive with distant metastasis after 5 years
28	72	F	Lobectomy	18.6	Alive NED after 5 years

Table 2 Oxyphilic cell tumours (*OCT*)

Case number	Age	Sex	Operation	OCNA (%)	Follow-up
Benign OCT (oxyphilic adenoma)					
29	42	F	Lobectomy	6.7	Alive NED after 10 years
30	28	F	Lobectomy	7.7	Alive NED after 9 years
31	20	M	Lobectomy	5.8	Alive NED after 9 years
32	24	F	Lobectomy	4.7	Alive NED after 8 years
33	51	M	Lobectomy	7.3	Alive NED after 8 years
34	58	M	Lobectomy	7.3	Alive NED after 7 years
35	75	M	Lobectomy	5.8	Alive NED after 4 years
36	49	F	Lobectomy	4.5	Alive NED after 4 years
37	45	F	Lobectomy	7.2	Alive NED after 3 years
Indeterminate OCT					
38	75	F	Lobectomy	14.8	Alive NED after 10 years
39	58	F	Lobectomy	23.4	Alive NED after 9 years
40	48	F	Lobectomy	12.5	Alive NED after 5 years
41	43	F	Lobectomy	27.3	Alive NED after 4 years
42	69	F	Lobectomy	19.0	Alive NED after 3 years
43	55	F	Lobectomy	15.1	Alive NED after 3 years
44	15	F	Lobectomy	13.5	Alive NED after 3 years
45	21	F	Lobectomy	13.3	Alive NED after 2 years
Malignant OCT (encapsulated oxyphilic cell carcinoma)					
46	52	F	Lobectomy	14.1	Alive NED after 12 years
47	54	F	Lobectomy	31.3	Alive NED after 9 years
48	43	F	Lobectomy	28.4	Alive NED after 5 years
49	49	F	Lobectomy	22.3	Alive NED after 4 years
50	21	F	Lobectomy	19.8	Alive NED after 4 years
51	43	F	Lobectomy	20.5	Alive NED after 3 years
52	74	F	Lobectomy	35.9	Alive NED after 3 years

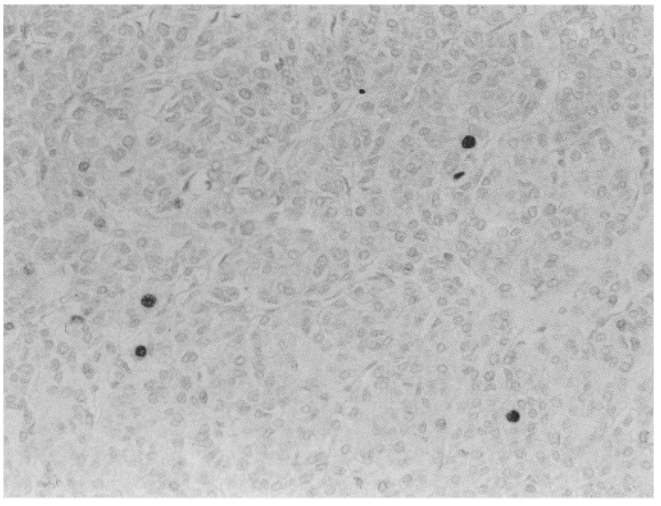

Fig. 1 Benign oxyphilic cell tumour (case 35). A few nuclei of the tumour cells stain positive for proliferative cell nuclear antigen (PCNA). Streptavidin-biotin (SAB) method with methyl green counterstain, ×250

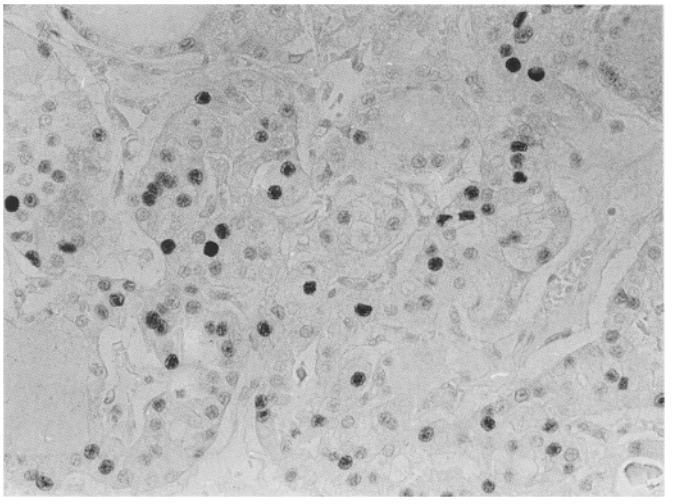

Fig. 2 Encapsulated oxyphilic cell carcinoma (case 46). Many nuclei of tumor cells stain positively for PCNA. SAB method with methyl green counterstain, ×250

centres of reactive lymphoid follicles which were sometimes present in and around the tumours.

The percentages of PCNA-positive tumour cells in non-OCT types are shown in Table 1. The values in benign non-OCT (follicular adenomas) ranged from 2.5% to 8.6% (mean, 5.0±1.9%), all lower than 10%. Those in encapsulated follicular carcinoma and widely invasive follicular carcinoma ranged from 11.8% to 39.1% (mean, 22.7±10.3%) and 18.6% to 20.0% (mean, 19.3±0.7%), respectively; all were higher than 10%. The differences in the values between follicular adenoma and encapsulated follicular carcinoma and between follicular adenoma and widely invasive follicular carcinoma were statistically significant ($P<0.001$, $P<0.001$, respectively), while that between encapsulated follicular carcinoma and widely invasive follicular carcinoma was not. The values in indeterminate non-OCT ranged from 4.3% to 19.6% (mean, 10.8±6.3%), three tumours (cases 11, 12, 13) were

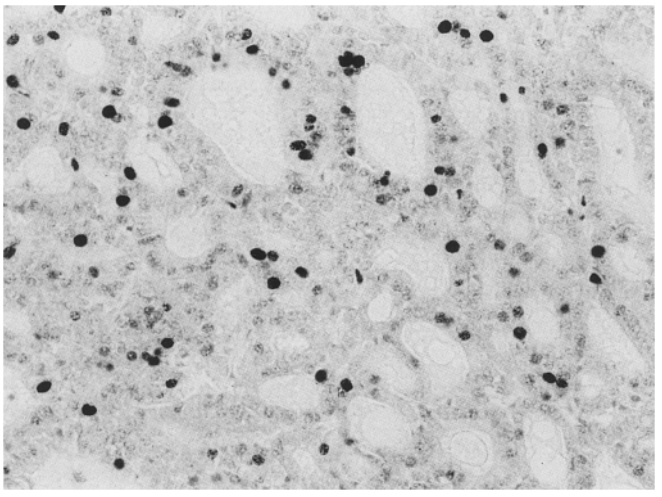

Fig. 3 Indeterminate oxyphilic cell tumour (case 40). Many nuclei of tumour cells stain positive for PCNA. SAB method with methyl green counterstain, ×250

lower than 10% and another three (cases 14, 15, 16) higher. Histologically, the capsule of the three tumours in low-value group had a tendency to hyalinization around the tumour cell nests, but exact distinctions between the two groups was difficult in the haematoxylin and eosin stained sections. There was no significant differences in the PCNA values between follicular adenoma and indeterminate tumours as a whole.

The percentages of PCNA-positive tumour cells in OCT are shown in Table 2. Like non-OCT, the values were less than 10% in benign lesions (4.5%–7.8%, mean 6.3±1.2%; Fig. 1) and more than 10% in malignant OCT (14.1%–35.9%, mean 23.7±7.4%; Fig. 2). There was statistically significant difference in the values between these two types of tumours ($P<0.001$). All indeterminate-type OCT showed percentages higher than 10% (12.5%–27.3%, mean 24.5±7.5%; Fig. 3) and there was also statistically significant difference in the values between benign-type and indeterminate-type tumours ($P<0.001$). There was no significant difference in the values between malignant-type and indeterminate-type tumours.

Discussion

The recent WHO classification [14] defines follicular adenoma as a tumour with well-defined fibrous capsule and encapsulated follicular carcinoma as a grossly encapsulated tumour with histologically unequivocal vascular invasion and/or capsular invasion that penetrates the full thickness of the capsule. When the invasion is not distinct or does not penetrate the full thickness of the capsule, the tumour is diagnosed as follicular adenoma. Widely invasive follicular carcinoma shows widespread infiltration of blood vessels and/or adjacent thyroid tissue and often lacks complete encapsulation. WHO specifies OCT as a variant of non-OCT, using the same histological criteria [14]. However, uncertainty exists as to the true nature of OCT. Some authors regard all these tumours as potentially malignant, since several patients with histologically benign OCT died from their disease [25]. Carcangiu et al. [6] described Hürthle (oxyphilic) cell carcinomas as a highly aggressive neoplasm when compared with non-oxyphilic cell follicular carcinoma.

Recently, more objective methods have been tried in the evaluation of thyroid follicular tumours. The usefulness of DNA histograms (flow cytometry) was limited because DNA aneuploidy was observed in follicular adenoma, follicular carcinoma, and OCT [4, 15]. The immunohistochemical demonstration of PCNA, a 36 kDa acidic nuclear protein essential for DNA synthesis, has been very useful in estimating cell proliferation and malignant potential in certain tumours [2, 17, 22]. The PC10 monoclonal anti-PCNA antibody is applicable for formalin-fixed paraffin-embedded tissues, provided that the fixation time is adequate [13, 24, 26]. The materials used in this study were within appropriate conditions [13]; reactive lymph follicles when present showed many positive cells.

Only two studies using PCNA on thyroid tumours have been reported [21, 23]. A statistically significant difference of PCNA labelling index was shown between follicular adenoma and follicular carcinoma, although one report is in abstract form only [23] and neither described indeterminate type tumours separately. Moreover, OCT are apparently not included in either series. In this study the percentages of PCNA-positive cells in encapsulated or widely invasive follicular carcinomas of non-OCT type were significantly higher than those in follicular adenomas, in agreement with previous results [21, 23]. Based on the ranges of PCNA counts in the carcinoma group and adenoma, a value of 10% was estimated to be appropriate for the separating line between benign and malignant lesions. In our study, non-OCT with partial capsular invasion or with tumour cell nests in the capsule were designated as indeterminate tumours following Carcangiu et al.'s criteria [6] for OCT. Among six indeterminate non-OCT, three showed PCNA indices lower than 10% and another three higher than 10%.

Malignant tumours of OCT type likewise showed significantly higher percentages of PCNA-positive cells than benign tumours, with the cut-off level of 10%. In addition, all indeterminate OCT also showed values more than 10%, significantly higher than those of benign OCT. In view of the proliferative activity these indeterminate tumours (half of non-OCT and all of OCT in this study) may be carcinomas which cannot be distinguished from adenomas in conventional histology. It is possible to conclude that the capsular invasion, even if not transcapsular, may be an important histological feature suggesting active proliferation, more so in OCT than in non-OCT. In Carcangiu et al.'s series [6], all the OCT of benign and indeterminate types behaved in a benign fashion clinically; all our cases also showed a good clinical course. However, a longer follow-up period may be needed, since one of our cases had a past history of thy-

roidectomy 24 years before. In Evans' series [7], three patients with encapsulated follicular tumour showing nests, cords, or nodules of tumour cells located within the capsule died of the tumour.

This study, like previous reports, indicates that the incidence of malignancy in OCT is higher than that in ordinary follicular tumours. A review of the files of Nagoya City University Hospital from 1976 to 1990 showed that 7 (4.2%) of 165 non-OCT were carcinoma (unpublished data), similar to the figures reported by others; 2.3% by Schroder et al. [20] and 2.4% by Lang et al. [16]. The incidence of malignancy in OCT ranges from 18.3% to 62.3% in the literature [3, 5, 18], and in the present series is 29% (7 of 24 OCT) during the same period (1976–1990) at Nagoya City University Hospital. The rate becomes 62.5% (15 of 24 OCT) when the indeterminate tumours with high PCNA index is considered malignant. While this high incidence of malignancy may explain previous concerns about OCT in general, the present PCNA study supports the use of similar criteria in the diagnosis of non-OCT and OCT.

Acknowledgements The authors thank Ms. Keiko Sano, Ms. Takako Ohshima and Ms. Yoshiko Fukushima for technical assistance and Ms. Michiyo Yokoi for manuscript preparation.

Addendum

Since submission of ths manuscript Ruschoff et al. [19] have reported silver nucleolar organiser region (AgNOR) staining for thyroid follicular tumours; the AgNOR distribution score showed significant difference between benign adenomas and malignant follicular tumours. Their results, accord with ours although oxyphilic cell tumours were not included.

References

1. Bondeson L, Bondeson A-G, Ljungberg O, Tibblin S (1981) Oxyphil tumors of the thyroid. Follow-up of 42 surgical cases. Ann Surg 194: 677–680
2. Bravo R, Frank R, Blundell PA, MacDonald-Bravo H (1987) Cyclin/PCNA is the auxiliary protein of DNA polymerase-delta. Nature 326: 515–517
3. Bronner MP, LiVolsi VA (1988) Oxyphilic (Askanazy/Hurthle cell) tumors of the thyroid: microscopic features predict biologic behavior. Surg Pathol 1: 137–150
4. Bronner MP, Clevenger CV, Edmonds PR, Lowell DM, McFarland MM, LiVolsi VA (1988) Flow cytometric analysis of DNA content in Hurthle cell adenomas and carcinomas of the thyroid. Am J Clin Pathol 89: 764–769
5. Caplan RH, Abellera M, Kisken WA (1984) Hurthle cell tumors of the thyroid gland. A clinicopathologic review and long-term follow up. JAMA 251: 3114–3117
6. Carcangiu ML, Bianchi S, Savino D, Voynick IM, Rosai J (1991) Follicular Hurthle cell tumors of the thyroid gland. Cancer 68: 1944–1953
7. Evans HL (1984) Follicular neoplasms of the thyroid. A study of 44 cases followed for a minimum of 10 years, with emphasis on differential diagnosis. Cancer 54: 535–540
8. Franssila KO, Ackerman LV, Brown CL, Hedinger CE (1985) Follicular carcinoma. Semin Diagn Pathol 2: 101–122
9. Garcia RL, Coltrera MD, Gown AM (1989) Analysis of proliferative grade using anti-PCNA/cyclin monoclonal antibodies in fixed, embedded tissue. Comparison with flow cytometric analysis. Am J Pathol 134: 733–739
10. Gonzalez-Campora R, Herrero-Zapatero A, Lerma E, Sanchez F, Galera H (1986) Hurthle cell and mitochondrion-rich cell tumors. A clinicopathologic study. Cancer 57: 1154–1163
11. Gosain AK, Clark OH (1984) Hurthle cell neoplasms. Malignant potential. Arch Surg 119: 515–519
12. Gundry SR, Burney RE, Thompson NW, Lloyd R (1983) Total thyroidectomy for Hurthle cell neoplasm of the thyroid. Arch Surg 118: 529–532
13. Hall PA, Levison DA, Woods AL, Yu CC-W, Kellock DB, Watkins JA, Barnes DM, Gillett CE, Camplejohn R, Dover R, Waseem NH, Lane DP (1990) Proliferating cell nuclear antigen (PCNA) immunolocalization in paraffin sections: an index of cell proliferation with evidence of deregulated expression in some neoplasms. J Pathol 162: 285–294
14. Hedinger CE (1988) Histological typing of thyroid tumours, 2nd edn. Springer, Berlin Heidelberg New York
15. Joensuu H, Klemi P, Eerola E (1986) DNA aneuploidy in follicular adenomas of the thyroid gland. Am J Pathol 124: 373–376
16. Lang W, Georgii A, Stauch G, Kienzle E (1980) The differentiation of atypical adenomas and encapsulated follicular carcinomas in the thyroid gland. Virchows Arch [A] 385: 125–141
17. Robbins BA, Vega D de la, Ogata K, Tan EM, Nakamura RM (1987) Immunohistochemical detection of proliferating cell nuclear antigen in solid human malignancies. Arch Pathol Lab Med 111: 841–845
18. Rosai J, Carcangiu ML, DeLellis RA (1992) Tumors of the thyroid gland. In: Atlas of tumor pathology, fascicle 5, series 3. Armed Forces Institute of Pathology, Washington, DC, pp 161–182
19. Ruschoff J, Prasser C, Cortez T, Hohne HM, Hohenberger W, Hofstadter F (1993) Diagnostic value of AgNOR staining in follicular cell neoplasms of the thyroid: Comparison of evaluation methods and nucleolar features. Am J Surg Pathol 17: 1281–1288
20. Schroder S, Pfannschmidt N, Dralle H, Arps H, Bocker W (1984) The encapsulated follicular carcinoma of the thyroid. A clinicopathologic study of 35 cases. Virchows Arch [A] 402: 259–273
21. Shimizu T, Usuda N, Yamanda T, Sugenoya A, Iida F (1993) Proliferative activity of human thyroid tumors evaluated by proliferating cell nuclear antigen/cyclin immunohistochemical studies. Cancer 71: 2807–2812
22. Takasaki Y, Deng J-S, Tan EM (1981) A nuclear antigen associated with cell proliferation and blast transformation. Its distribution in synchronized cells. J Exp Med 154: 1899–1909
23. Takeda K, Asano G, Yamada N (1992) Thyroid follicular lesions: immunohistochemical study using anti-proliferating cell nuclear antigen antibody (abstract; in Japanese). Trans Soc Pathol Jpn 81: 91
24. Tateyama H, Mizuno T, Tada T, Eimoto T, Hashimoto T, Masaoka A (1993) Thymic epithelial tumours: evaluation of malignant grade by quantification of proliferating cell nuclear antigen and nucleolar organizer regions. Virchows Arch [A] 422: 265–269
25. Thompson NW, Dunn EL, Batsakis JG, Nishiyama RH (1974) Hurthle cell lesions of the thyroid gland. Surg Gynecol Obstet 139: 555–560
26. Waseem NH, Lane DP (1990) Monoclonal antibody analysis of the proliferating cell nuclear antigen (PCNA). Structural conservation and the detection of a nucleolar form. J Cell Sci 96: 121–129
27. Yamashina M (1992) Follicular neoplasms of the thyroid. Total circumferential evaluation of the fibrous capsule. Am J Surg Pathol 16: 392–400