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Proliferative Cell Index in Endometrial Adenocarcinoma of Different Nuclear Grades

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Proliferative cell indices (PCI) in 20 cases of endometrial adenocarcinoma were obtained by staining DNA polymerase α. The PCI ranged from 11.1 to 42.1, averaging 24.7±8.7. Four cases of stage III all exhibited fairly large PCI (30.4–32.9). In contrast, in 16 cases of stage I the values were spread over a wide range. In 13 cases with histological grade (HG) 1 of stage I, larger PCI were obtained in the nuclear grade (NG) 2 group; the mean PCI values of HG 1-NG 1 and HG 1-NG 2 were 16.5±4.6 and 25.6±5.2, respectively. Because of the good correlation between PCI and HG or NG, PCI may be useful as an additional prognostic factor in endometrial adenocarcinoma, especially in stage I cases.

Key words: Proliferative cell index — Endometrial adenocarcinoma — Nuclear grade

Prognosis of endometrial carcinoma is usually evaluated on the basis of three independent factors: clinical stage, histological grade (HG)[4] and depth of myometrial invasion.[1,2] Nuclear grade (NG) was also recently reported to influence the prognosis of endometrial carcinoma[3,4] as well as other types of adenocarcinoma.[5–7]

We stained DNA polymerase α (Pol α) in endometrial adenocarcinoma cells with the monoclonal anti Pol α antibody. The first aim of this study was to obtain the percentage of proliferative cells in endometrial adenocarcinoma with different histological grades. Secondly, we wanted to find out whether nuclear grade correlates with proliferative activity of endometrial adenocarcinoma.

MATERIALS AND METHODS

Twenty cases of endometrial adenocarcinoma were studied (Table I). They were all operated in the Department of Obstetrics and Gynecology of Nagoya National Hospital. Sampling was done from a part of the tumor macroscopically judged to be characteristic. Histological grading was performed in compliance with the system of the International Federation of Gynecologists and Obstetricians (FIGO).[8] Nuclear grading was performed according to Christopherson's criteria.[3] Tumors with well differentiated cells having elongated or oval nuclei, a fine chromatin pattern and inconspicuous or very small nuclei were considered to be NG 1. Usually, only a few mitoses were found in these tumors. Cells with round, enlarged nuclei containing large, at times irregular and multiple, and numerous mitoses were considered NG 3. Those with nuclear changes greater than NG 1, but less than NG 3 were coded NG 2 (Fig. 1).

Procedures for the fixation of specimens and immunohistochemical detection were described in our pre-

Table I. Cases of Endometrial Adenocarcinoma

Case	Age	Stage	HG	NG	PCI
1	42	I	1	1	11.1
2	52	I	1	1	13.3
3	66	I	1	1	16.4
4	42	I	1	1	17.5
5	57	I	1	1	11.5
6	59	I	1	1	22.5
7	61	I	1	1	23.5
8	46	I	1	1	16.0
9	45	I	1	2	30.7
10	56	I	1	2	30.5
11	47	I	1	2	24.6
12	66	I	1	2	18.1
13	75	I	1	2	24.2
14	56	I	2	2	29.6
15	65	I	2	2	42.1
16	78	I	3	2	33.3
			Mean±SD: 22.8±8.8[a]		
17	62	III	1	2	30.4
18	51	III	2	2	32.5
19	59	III	2	2	32.9
20	61	III	2	3	32.9
			Mean±SD: 32.2±1.2		
	Total		Mean±SD: 24.7±8.7		

a) $P<0.01$ compared with the stage III group.

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[4] Abbreviations used are: HG, histological grade; NG, nuclear grade; Pol α, DNA polymerase α; PCI, proliferative cell index.

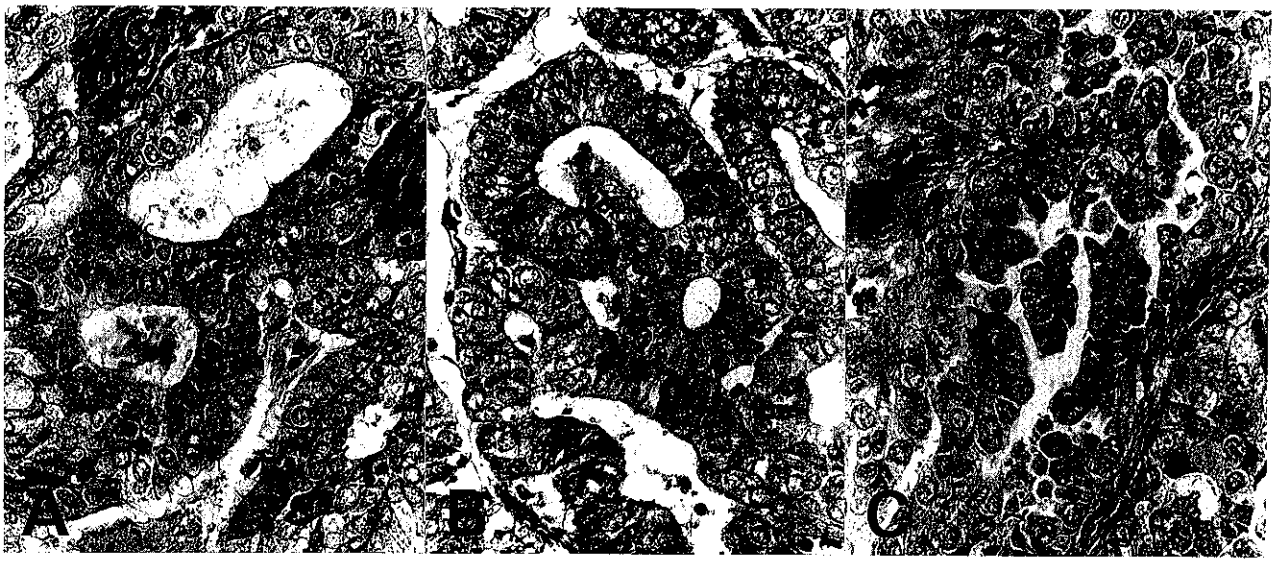

Fig. 1. Hematoxylin and eosin staining. A, nuclear grade 1 (case 4); B, nuclear grade 2 (case 18); C, nuclear grade 3 (case 20). ×320.

vious report.[9] Briefly, fresh specimens were fixed in 2% PLP (2% paraformaldehyde, 10 mmol/liter $NaIO_4$ and 75 mmol/liter lysine in a 50 mmol/liter sodium phosphate buffer, pH 7.4) for 12 h at 4°C. They were washed serially with 50 mmol/liter sodium phosphate buffer, pH 7.4, containing 10%, 15% and 20% sucrose for 24 h each at 4°C, then embedded in OCT compound. Sections 5 μm thick were cut from the frozen specimens. Pol α was stained using the indirect immunoperoxidase technique with monoclonal anti Pol α antibody (CL22-2-42) obtained from MBL (Nagoya). The characterization of the antibody was reported previously.[10]

The percentage of Pol α-positive cells was determined by counting about 2000 cells. Proliferative cell index (PCI) was defined as the percentage of Pol α-positive cells in tissues.

RESULTS

Figures 2 and 3 show, respectively, the staining patterns of Pol α in well-differentiated adenocarcinoma (HG 1-NG 1, case 4 in Table I) and moderately differentiated adenocarcinoma (HG 2-NG 2, case 15). Pol α was localized in nuclei of cancer cells, except for cytoplasmic localization in the M-phase (Fig. 4), in agreement with our previous results.[9]

Table I lists the estimated PCI of 20 cases of endometrial adenocarcinoma. PCI ranged from 11.1 to 42.1 with an average of 24.7 ± 8.7. Firstly, we looked at the distribution of PCI in different stages. Large PCI (30.4–32.9) were observed in all 4 cases of stage III. However, the values were distributed over a wide range in 16 cases of stage I. Secondly, we checked the correlation beween PCI and HG or NG. Table II lists the PCI in HG 1 to 3 and NG 1 to 3. The lowest average PCI (20.7 ± 6.8) was for HG 1. Larger values, 34.0 ± 4.7 and 33.3, were obtained for HG 2 and 3, respectively. The lowest average PCI (16.5 ± 4.6) was obtained for NG 1, with larger values, 29.9 ± 6.1 and 32.9, for NG 2 and 3, respectively. The differences in mean PCI between HG 1 and HG 2, and between NG 1 and NG 2 are statistically significant ($P < 0.01$). Thirdly, the differences in HG and NG in stage I were classified into 4 groups (Table III). An average PCI of 16.5 ± 4.6 was obtained for HG 1-NG 1. Larger values, 25.6 ± 5.2, 35.9 ± 8.8 and 33.3, were obtained for HG 1-NG 2, HG 2-NG 2 and HG 3-NG 2, respectively.

DISCUSSION

We previously reported a technique for detection of proliferative cells in cancer of the uterine cervix by immunological staining of Pol α.[9] In our present study, we determined the proliferative cell index, defined as the percentage of Pol α-positive cells, of 20 cases of endometrial adenocarcinoma. The average PCI, 24.7, was significantly smaller than that (53.7) in cervical squamous cell carcinoma.[9] The meaning of this difference is unknown; nevertheless, our data seem reasonable because the same tendency was reported by Fettig and Sievers[11] using ^{3}H-thymidine autoradiography.

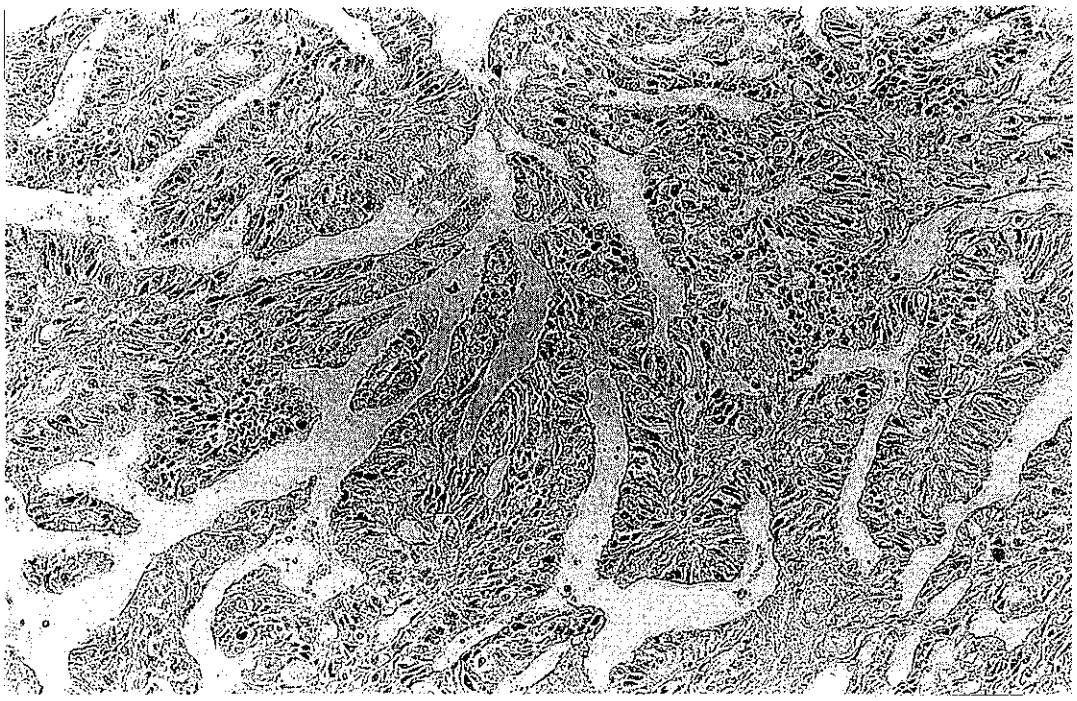

Fig. 2. Pol α staining of well differentiated adenocarcinoma (HG 1-NG 1, case 4). Immunoperoxidase with DAB, counterstained with methyl green. $\times 200$.

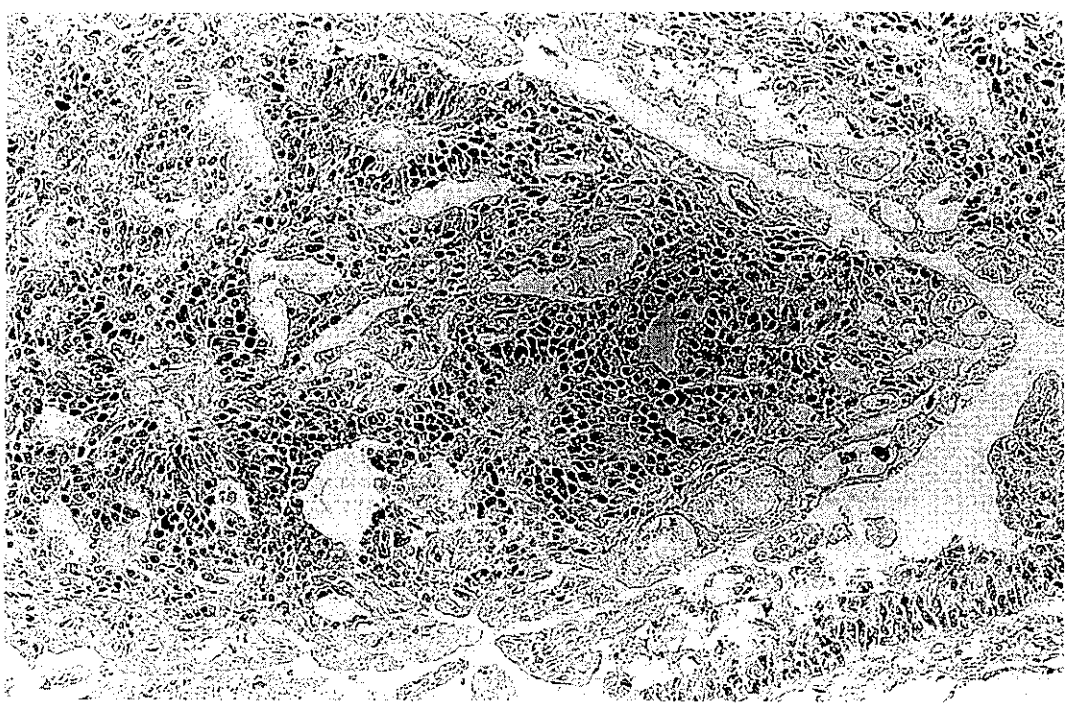

Fig. 3. Pol α staining of moderately differentiated adenocarcinoma (HG 2-NG 2, case 15). Immunoperoxidase with DAB, counterstained with methly green. $\times 200$.

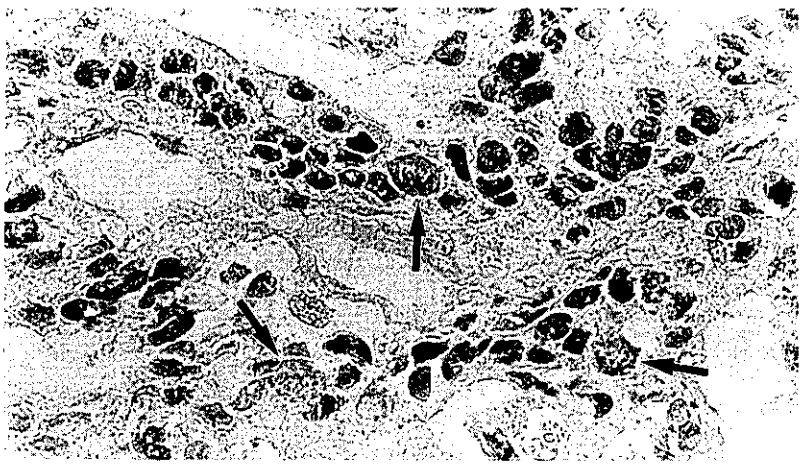

Fig. 4. Pol α staining (HG 2-NG 2, case 15). Nuclear localization of Pol α in cancer cells, except for cytoplasmic localization in the M-phase (arrows). $\times 600$.

Table II. PCI in Endometrial Adenocarcinoma by Histological Grade and Nuclear Grade

Group	n	PCI	
		Mean±SD	(range)
HG 1	14	20.7±6.8	(11.1–30.7)[a]
HG 2	5	34.0±4.7	(29.6–42.1)
HG 3	1	33.3	
NG 1	8	16.5±4.6	(11.1–23.5)[b]
NG 2	11	29.9±6.1	(18.1–42.1)
NG 3	1	32.9	

a) $P<0.01$ compared with the HG 2 group.
b) $P<0.01$ compared with the NG 2 group.

Table III. PCI in Endometrial Adenocarcinoma of Stage I by Histological Grade and Nuclear Grade

Group	n	PCI	
		Mean±SD	(range)
HG 1-NG 1	8	16.5±4.6	(11.1–23.5)[a]
HG 1-NG 2	5	25.6±5.2	(18.1–30.7)
HG 2-NG 2	2	35.9±8.8	(29.6–42.1)
HG 3-NG 2	1	33.3	

a) $P<0.01$ compared with the HG 1-NG 2 group.

The PCI of 14 cases with HG 1 varied widely from 11.1 to 30.7. In contrast, those of 5 cases with HG 2 were distributed from 29.6 to 42.1. An important finding is the higher PCI in HG 2 (34.0±4.7) than in HG 1 (20.7±6.8). A previous report found no difference in labeling indices between cases with HG 1 and 2.[12] Our results seem more reasonable because, according to the differential definition, cancer cells in HG 1 are more highly differentiated than in HG 2, in agreement with the results of Iversen[13] showing a higher flow cytometric DNA index in HG 2.

We also found that NG 1 group had the lowest average PCI (NG 1, 16.5±4.6; NG 2, 29.9±6.1; and NG 3, 32.9). Thus, higher HG or NG correlates with larger PCI. We also evaluated the PCI of HG 1 group (n=13) by nuclear grade of stage I. Average PCI of HG 1-NG 2 (25.6±5.2) was significantly larger than that of HG 1-NG 1 (16.5±4.6). We can conclude that endometrial adenocarcinoma of HG 1-NG 2 possesses higher proliferative activity than that of HG 1-NG 1. Thus, supplementing NG, PCI could also become a convenient parameter for determining proliferative activity of endometrial adenocarcinomas.

Since prognosis is clearly better for cases of stage I than for those of stage III,[1,2] a PCI in stage I does not possess the same meaning in terms of prognosis as the same PCI in stage III. We have now started to evaluate whether PCI can be used as a prognostic factor in endometrial adenocarcinoma of stage I.

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