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Growth fraction of human bladder tumors

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Summary. The growth fraction of bladder tumors was immunohistochemically assessed in situ using anti-DNA polymerase (Pol α) monoclonal antibody. This enzyme is known to be present in the nucleus of the cells in G1, S, and G2 phases. The percentage of labeled cells was expressed as the labeling index (LI). The average LI was 6.0% in normal epithelium and 17.8% in bladder tumors, this difference being significant. The labeled cells were distributed throughout both the basal and surface layers of bladder tumors. However, in some bladder tumors, the distribution of Pol α-labeled cells varied from area to area. The higher fraction of labeled cells was found in high grade or invasive tumors. Papillary and nodular bladder tumors showed a greater rate of cell proliferation than papillary tumors. These findings suggest that Pol α immunostaining could be a potent tool for easy and quick evaluation of proliferating cells in bladder tumors, thereby providing a supplement to conventional histological findings.

Key words: Bladder cancer – Growth fraction – Pol α immunostaining

It has been generally accepted that the histological grade based upon histological type is one of the most important factors for assessing the prognosis of patients with bladder tumor [5]. However, the biological behavior of a bladder tumor is not predictable from its histological features alone. If an integrated therapy for bladder tumors is to be designed, a more sensitive indicator of tissue biological potential is needed. We have already reported results of studies of S-phase cells in bladder tumors using a bromodeoxyuridine (BrdUrd) in vitro labeling method [12]; however not all tumor cells are present in the growth fraction. Therefore, further investigation is needed to obtain an estimate of cell proliferative potential in bladder tumors.

DNA polymerase α (Pol α) is one of the key mediating enzymes involved in DNA replication in eukaryotes [7, 9]. Monoclonal antibody Pol α recognizes one such protein present only in the nucleus of cycling cells, but not in that of resting cells [8, 10]. In the present study, we report the initial characterization of the growth fraction of bladder tumor using Pol α immunostaining. We also determined the relationship between the labeling index (LI) and biological behavior of bladder tumors.

Materials and methods

Material

The tumor samples were obtained from 45 patients with bladder tumor. There were 35 male and 10 female patients; the average age was 65.0 years with a range of 38–90 years. They were admitted to our hospital between 1985 and 1989. The examined specimens were all from transitional carcinomas. Their histological classification and staging were G1 ($n=8$), and G2 ($n=21$) and G3 ($n=16$), and Tis ($n=1$), T1 ($n=32$), T2 ($n=7$), T3 ($n=4$), and T4 ($n=1$), respectively. Patients were treated by transurethral resection (TUR; 20 patients) or total cystourethrectomy (25 patients). All specimens were obtained before any treatment was undertaken. Of these 45 tumors, 23 were used for the detection of Pol α and Ki-67 simultaneously; half of the tissue was used for Pol α frozen sections and the other half for Ki-67 frozen sections. Samples from six patients with benign urological disease served as controls.

Immunohistochemical staining for Pol α

Fresh specimens were embedded in OCT compound (Miles Scientific, Naperville, Ill.) immediately after surgical resection of tumors and frozen at −80°C. The cryostat sections were cut at 6 μm, mounted on slides, dried well, and then fixed in 4% paraformaldehyde (PFA) for 30 min at 4°C. The sections were exposed to 2% normal horse serum for 30 min to prevent nonspecific binding of the antibody to the sections after blocking of endogeneous peroxidase by 3% H_2O_2 for 20 min. The avidin-biotin peroxidase-complex (ABC) method [4] was used for Pol α staining. The sections were first incubated for 1 h with a 1:50 dilution of the monoclonal antibody Pol α (MBL, Nagoya, Japan) [8]. After a 3-min wash in phosphate buffered saline (PBS), a 1:100 dilution of biotinylated antimouse antibody (Vector Laboratories, Burlingame, Calif.) was applied to

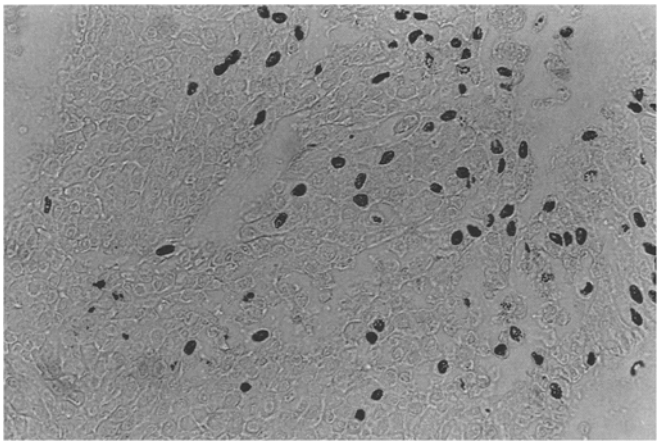

Fig. 1. Polymerase α positive cells are distributed throughout both the basal and surface layers of bladder tumor (Grade 2, T2), LI: 28.9%; ×200

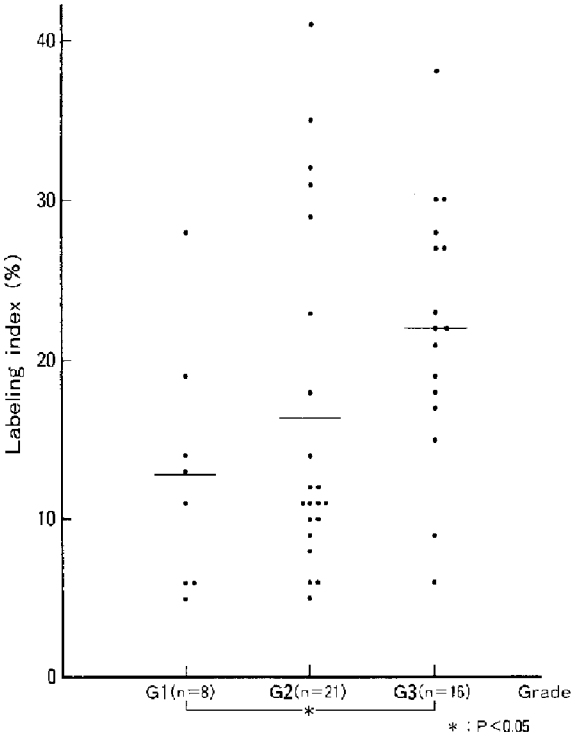

Fig. 2. Relationship between labeling index and tumor grade. Bars show mean level

the sections for 30 min. ABC (Vector Laboratories) was then applied, and sections were incubated with diaminobenzidine (DAB; Wako, Japan). Counterstaining was done using methyl green.

Ki-67 immunostaining

The staining method for Ki-67 was the same as that used for Pol α [2]. Briefly, the remaining half of tissue specimens was fixed in acetone for 20 min at 4°C. Sections were first exposed for 1 h to a 1 : 50 dilution of the monoclonal antibody Ki-67 (Dako, Denmark).

As for Pol α labeling above, sections were then treated with the biotin-labeled second antibody and with ABC. DAB was used for labeling.

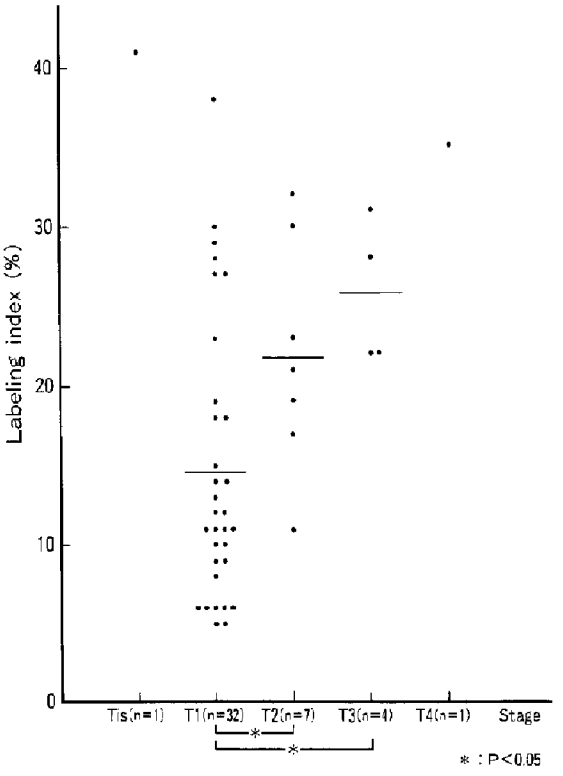

Fig. 3. Relationship between labeling index and tumor stage. Bars show mean level

Within each section four arbitrarily chosen, regions, each of which contained 100 cell nuclei, were examined and the LI (percentage of labeled nuclei divided by the number of cell nuclei scored) was determined.

Statistical methods

Student's *t*-test was used to analyze the difference between mean values. The level of statistical significance was 5% unless otherwise indicated. The correlation coefficient (r) and the regression equation (Pol α and regressed on Ki-67) were obtained using the least squares method.

Results

Labeled and unlabeled cells were easily distinguished due to the absence of background staining within specimens. In normal epithelium, a few labeled cells were found in the basal layers, but superficial cells were rarely labeled. By contrast, in the tumor samples most cells labeled for Pol α were equally distributed among the epithelial layers regardless of grade (Fig. 1). Within some bladder tumor samples, there was some variation in the distribution pattern of labeling depending upon the histological site. Staining for Pol α was confined to nuclei; it was not observed in the cytoplasm of cells. The LI for normal bladder and bladder tumors was determined. The average LI was 6.0% for normal epithelium and 17.8% for bladder tumors; this difference was statistically significant ($P < 0.01$). The LI for bladder tumors was determined separately for each histological grade (Fig. 2). the LIs for grades 1, 2 and 3 were 12.8 ± 7.8%, 16.4 ± 10.8% and 22.0

Table 1. Correlation between labeling index and tumor background factors

	n	Labeling index (%)[a]	Significant difference
1. Single	19	12.4 ± 7.5	$P < 0.01$
Multiple	26	21.7 + 9.6	
2. Size			
≧ 2 cm	30	21.1 ± 9.3	$P < 0.01$
< 2 cm	15	11.1 ± 7.4	
3. Shape			
Papillary	22	12.4 ± 6.6	$P < 0.01$
Nodular	8	24.9 ± 8.9	$P < 0.01$
Mixed	15	21.8 ± 10.4	
4. Cytology			
Positive	25	21.6 ± 9.9	$P < 0.01$
Negative	20	12.5 ± 7.6	
5. Treatment			
TUR	20	11.7 ± 6.4	$P < 0.01$
Cystourethrectomy	25	22.6 ± 9.5	
6. Lymph node involvement			
With	6	26.0 ± 9.3	n.s
Without	19	21.5 ± 9.5	

[a] Mean ± standard deviation.
n.s., not significant; TUR, transurethral resection

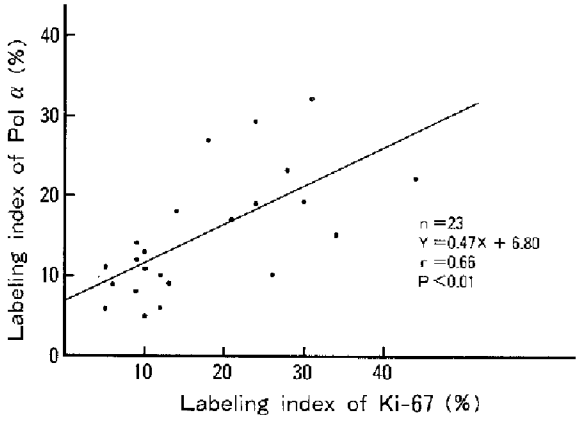

Fig. 4. Scattergram shows the relationship between Pol α and Ki-67 of labeling index for 23 of the tumor samples studied

± 8.1%, respectively. Thus, as tumor grade increased, so did the number of labeled cells within samples. The correlation between LI and histological stage was also determined (Fig. 3). The LIs for Tis, T1, T2, T3 and T4 were 41.0%, 14.6 ± 8.7%, 21.8 ± 7.3%, 25.8 ± 4.5% and 35.0% respectively. The LI for patients with T2 and T3 tumors was significantly higher than that for patients with T1 tumors.

The LIs determined for Pol α staining were compared according to the background factors for the bladder tumors (Table 1). There was a statistical relationship between the size of tumor and LI, and papillary and nodular tumors had greater rates of cell proliferation than did papillary tumors. The relationship between LI and subsequent patient prognosis was determined. The median follow-up period was 27.3 months. Two patients died of cancer during the follow-up period; their average tumor LI was 21.0 (%), higher, though not significantly, than that of survivors (17.6%; $n = 43$). Furthermore, the correlation between the LI determined for Pol α and Ki-67 staining was determined for 23 of the patients with bladder tumor (Fig. 4). Bladder tumors with a high Pol α LI generally also had a high Ki-67 LI.

Discussion

It is widely accepted that studies on cell kinetics in various tissue sections are important for achieving an understanding of the growth of normal and malignant cells. For the detection of proliferating cells, the technique of incorporating radioactively labeled DNA precursors into dividing cells has been employed [3]. However, the autographic procedure widely used for these studies has the disadvantages of handling difficulties and, occasionally, poor nuclear contrast. Its beneficial use in urological practice is therefore limited. Furthermore, not all cells are present in cycling phase [10]; a better understanding of cell proliferation in bladder tumors therefore requires detailed information about the growth fraction.

Recently, a monoclonal antibody, Pol α, which recognizes a nuclear antigen expressed in the G1, S and G2 phases of the human proliferating cell cycle, but is absent in the G0 (resting phase) [7, 9], was developed. Pol α immunostaining enables the detection of the growth fraction in situ in well-preserved tissue structures after PFA fixation without any pretreatment, also making it possible to compare cell proliferation with histological features.

In normal epithelium, Pol α-labeled cells were present in the basal layers. In normal epithelium, it may be the basal cells that divide, while the differentiated surface cells may be incapable of division. Most tumors, however, had many labeled cells, which were equally distributed among all epithelial layers. The LI for Pol α staining in normal epithelium and in the epithelium of cells of bladder tumors was higher than that determined using BrdUrd immunostaining [11, 12]. These findings might simply be explained by the fact that Pol α exists throughout all cell cycles of proliferative cells. In the present study, papillary and nodular bladder tumors were found to have significantly higher LIs than papillary tumors. These results remind us of the fact that poor prognosis is associated with certain shapes of bladder tumors [6].

Gerdes et al. [2] recently reported that proliferative cells in all cell cycles were successfully stained by the monoclonal antibody Ki-67. Although the reactivity of Ki-67 antibody seems to be similar to that of Pol α antibody, it remains to be determined whether the monoclonal Ki-67 and Pol α antibodies recognize the same antigen in cycling cells [1, 10]. In our study of specimens of bladder tumors, the Pol α LIs were found to be proportional to those of the Ki-67 LI.

In conclusion, we believe that Pol α immunostaining is a technique which is safe and reliable for use in the clinical labortories. It may be quite useful in the study of the proliferative activities of human bladder tumors.

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