Hiroshima J. Med. Sci.
Vol.44, No.4, 93～97, December, 1995
HIJM 44–15

A Mapping of Histology and Cell Proliferation in Human Bladder Cancer: An Immunohistochemical Study

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ABSTRACT

Transitional cell carcinoma of the urinary bladder is a multifocal disease and the whole bladder mucosa is more or less involved in the neoplastic process. A mapping study of 5 cystectomy specimens was performed, and cell proliferation was evaluated immunohistochemically using anti-proliferating cell nuclear antigen (PCNA) monoclonal antibody. A correlation between the pathological findings and the rates of PCNA-positive cells was observed in both the tumor and the surrounding mucosa in 4 of the 5 cases. The rates of PCNA-positive cells increased in areas with normal mucosa adjacent to those with dysplasia or transitional cell carcinoma, and in areas with normal mucosa or dysplasia adjacent to those with carcinoma in situ or transitional cell carcinoma in 2 cases. These features may indicate that a field change observed in the mapping study of a bladder with cancer is a manifestation of a stepwise progression of the mucosal disease.

Key words: *Mapping, Bladder cancer, Cell proliferation*

The high incidence of pathological findings in multiple mucosal biopsies of patients with carcinoma of the bladder makes it imperative to study the urothelium extensively when patients present with neoplastic disease[11)]. In bladder tumor disease, the multicentricity or multifocal appearance of neoplastic cells in normal-appearing mucosa is of the utmost importance[8)]. Mapping studies have been done to examine the occurrence and distribution of associated mucosal lesions, as well as to identify grossly invisible lesions which may influence prognosis[14)]. While the human urinary bladder epithelium has been shown to be very stable under normal conditions with regard to cell cycle distribution[7)], the cell proliferation, not only in the tumor but also in the surrounding mucosa, has been reported to increase with the cytologic or histologic grade of the tumor[8)]. Proliferating cell nuclear antigen (PCNA), which we evaluated immunohistochemically in the cystectomy specimens, has been known as cyclin[1,2)] or as an auxiliary protein for DNA polymerase δ[5,20,23)], and is directly involved in DNA synthesis[16)]. A linear correlation was found between PCNA and Ki67, which is a nuclear antigen expressed in all phases of the cell cycle, except G0 and early G1[6,19,21)]. The immunohistological method of assessing cell proliferation by using anti-PCNA monoclonal antibody has particular advantages over other techniques because of the maintenance of cellular and tissue architecture, and the relative simplicity of the methodology. The purpose of this study is to elucidate the characteristics of the field change associated with bladder cancer by evaluating both the extent of the pathological findings and the cell proliferation in total cystectomy specimens.

MATERIALS AND METHODS

We examined 5 cystectomy specimens by step-sectioning. None of the 5 patients had received chemotherapy or radiotherapy prior to total cystectomy. Each cystectomy specimen was opened along the midline of the anterior wall and then fixed in 10% buffered formalin after being pinned on a corkbord. After fixation, the entire bladder was cut into 2×0.5 cm strips with resected prostate. Each strip was embedded in paraffin and cut into 5 μm thick sections for hematoxylin and eosin staining and for PCNA immunostaining. The number of sections per cystectomy specimen was 72 in case 1, 98 in case 2, 84 in case 3, 63 in case 4 and 69 in case 5. The grading of the tumor was according to the criteria of Mostofi and associates[18)]. Tumor staging was defined according to the criteria of the International Union

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Against Cancer[10].

Sections from formalin fixed, paraffin embedded tissues were dewaxed, rehydrated and treated for 30 minutes with 0.3% H_2O_2 in methanol to quench endogenous peroxidase activity. Then, the sections were incubated with monoclonal anti-PCNA antibodies (DAKO, Denmark) at a dilution of 1 : 50 for 1 h at room temperature. Normal horse serum was used as a blocking agent and normal mouse serum as a negative control. Detection of bound antibody was performed with an avidin-biotin system (Vector Laboratories, Inc., Burlingame, CA), and visualized in 3,3'-diamminobenzidine tetrahydrochloride (0.5 mg/ml) with 0.03% H_2O_2. The sections were counterstained with methyl green, dehydrated and mounted. Two blinded observers scored the specimens according to the histological findings and the number of PCNA-positive cells. The number of proliferating cells was assessed by counting the cells with stained nuclei positive for PCNA among 500 to 1,000 cells, and the rate of PCNA-positive cells was determined by dividing the number of PCNA-positive cells by that of cells scored. The histological findings and the rate of PCNA-positive cells were mapped and illustrated diagrammatically in the entire internal surface of the bladder. The differences in the rate of PCNA-positive cells were analyzed using the Kruskal-Wallis test[15] against the histological findings for each cystectomy specimen.

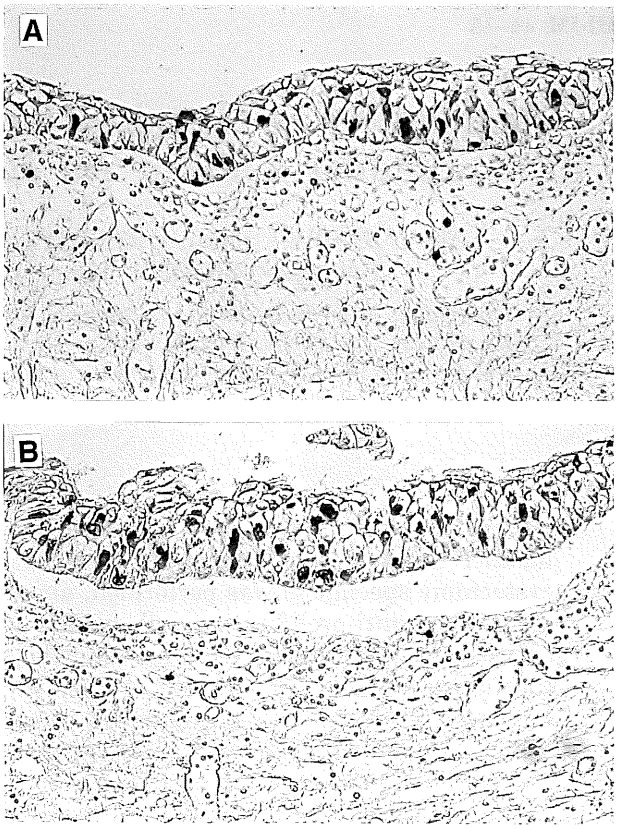

Fig. 1. PCNA immunostaining of dysplasia (A) and carcinoma in situ (B) showing positive staining in the nuclei. ×100.

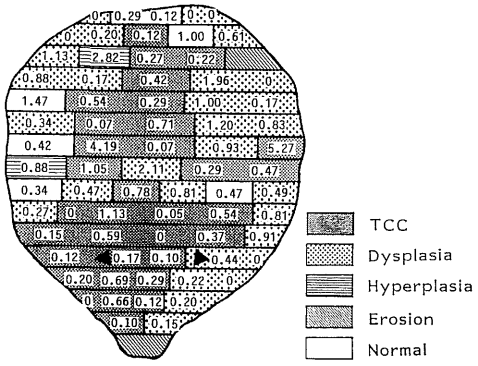

Fig. 2. Mapping results in a bladder removed from a 71-year-old man. Extensive distribution of dysplasia and transitional cell carcinoma. Numbers in the diagram indicate the rates of PCNA-positive cells in the corresponding areas. The rates of PCNA cells were represented similarly in the following figures.

RESULTS

PCNA staining was almost totally confined to the nucleus of the cells and showed a diffuse or granular pattern (Fig. 1). The histological findings and the rates of PCNA-positive cells were mapped simultaneously and the mean values of PCNA-positive cells were compared against the histological findings for each cystectomy specimen.

Case 1. A 71-year-old man had pT1, G2 transitional cell carcinoma (TCC). There was an extensive distribution of TCC and dysplasia (Fig. 2). The mean rates of PCNA-positive cells were 0.51% in the normal mucosa, 1.85% in the hyperplasia, 0.54% in the dysplasia, 0.15% in TCC, G1 and 0.66% in TCC, G2. There was no significant difference in the rate of PCNA-positive cells against these histological findings and no definite trend in the distribution of the rate of PCNA-positive cells.

Case 2. A 71-year-old woman had pT1, G3 TCC. Many areas with mucosal ablation were observed, and the distribution of dysplasia and carcinoma in situ (CIS) was extensive. The mean rates of PCNA-positive cells were 2.08% in the normal mucosa, 2.74% in the dysplasia, 25.52% in CIS and 29.70% in TCC, G3. The mean rates of PCNA-positive cells in CIS and TCC, G3 were significantly higher than those in the normal mucosa and dysplasia ($p<0.001$). No definite tendency was observed in the distribution of rates of PCNA-positive cells (Fig. 3).

Case 3. A 69-year-old man had pT1, G2 TCC. The normal mucosa was distributed widely in the

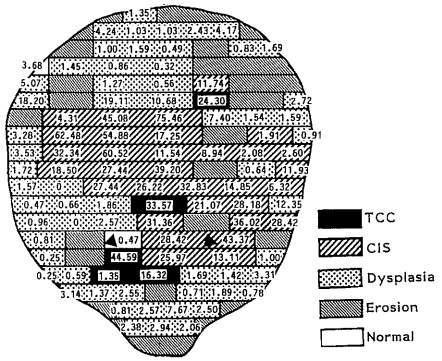

Fig. 3. Results of mapping of a bladder removed from a 71-year-old woman because of extensive carcinoma in situ.

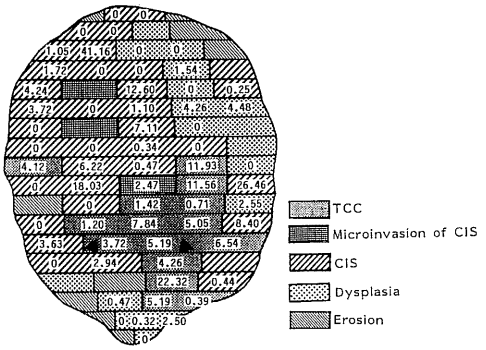

Fig. 5. Results of mapping of a bladder removed because of extensive carcinoma in situ and transitional cell carcinoma, grade 3. No area with normal mucosa was observed.

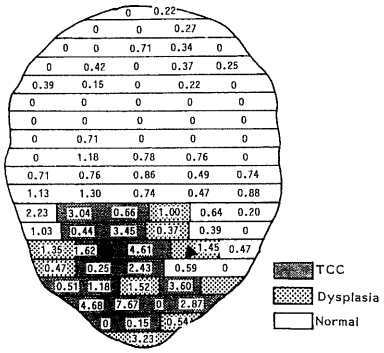

Fig. 4. Map of bladder showing the confined distribution of transitional cell carcinoma and dysplasia. The rate of PCNA-positive cells was high in the area with normal mucosa adjacent to the area with dysplasia and transitional cell carcinoma.

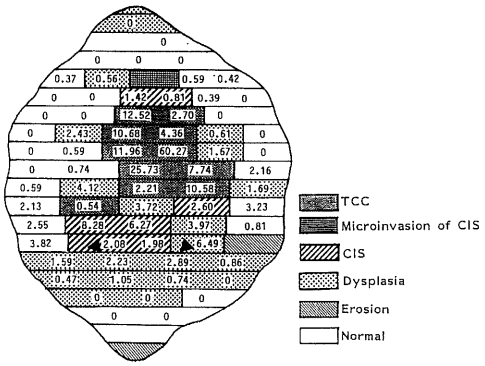

Fig. 6. Mapping results in a bladder removed from a 50-year-old man because of transitional cell carcinoma, grade 3 in the posterior wall. High rates of PCNA-positive cells were observed in the areas with normal mucosa or dysplasia adjacent to carcinoma in situ or transitional cell carcinoma, grade 3.

vesical cavity and the distribution of neoplastic changes was confined to the trigone and bladder neck. The mean rates of PCNA-positive cells were 0.35% in the normal mucosa, 1.24% in the dysplasia, 1.95% in TCC, G1 and 2.22% in TCC, G2. The rates of PCNA-positive cells showed a significant tendency to increase in proportion with the pathological findings ($p<0.01$). Furthermore, the rate of PCNA-positive cells in the area with normal mucosa was characterized by the distribution of relatively high values adjacent to the area with dysplasia and TCC (Fig. 4).

Case 4. A 67-year-old woman had pT3b, G3 TCC. No area with normal mucosa was observed. The CIS, dysplasia and TCC, G3 distributed widely in groups. The mean rates of PCNA-positive cells were 1.15% in the dysplasia, 4.24% in CIS and 6.10% in TCC, G3. The rate of one specimen with a microinvasion of CIS was 2.47%. The rates of PCNA-positive cells significantly increased with the progression of the lesions ($p<0.01$). No definite pattern was observed in the distribution of the rates of PCNA-positive cells in this cystectomy specimen (Fig. 5).

Case 5. A 50-year-old man had pT1, G3 TCC of the bladder. The main tumor was located in the posterior wall. The mean rates of PCNA-positive cells were 0.59% in the normal mucosa, 1.75% in the dysplasia, 3.30% in CIS, 1.36% in TCC, G2 and 16.28% in TCC, G3. The rates of PCNA-positive cells significantly increased in proportion with the pathological findings ($p<0.01$). Furthermore, high rates of PCNA-positive cells in the normal mucosa and dysplasia were observed in the areas adjacent to CIS and TCC, G3 (Fig. 6).

DISCUSSION

The whole bladder mucosa is more or less involved in the neoplastic process in many bladder cancer patients[8]. Mapping studies of the bladder

have acquired additional significance because abnormalities of the bladder epithelium, not identifiable by the urologist, may play a major role in the prognosis of bladder tumors[14]. The wide spectrum of human bladder cancer from papillary superficial tumors to nonpapillary invasive tumors is well recognized. Field changes, including CIS and atypical hyperplasia or dysplasia in apparently normal mucosa, are also widely recognized[13,17,22]. Moreover, the extensive distribution of mucosal abnormalities observed in this study seems to indicate that bladder cancer is the manifestation of a field change in the bladder mucosa. No relationship was observed between PCNA-positive cells and pathological findings in one cystectomy specimen (Case 1), and the low rate of PCNA-positive cells in the transitional cell carcinoma may indicate the indolent characteristics of the tumors in the bladder of this case. A significant relationship between the rates of PCNA-positive cells and the pathological findings was observed in both the tumor and the surrounding mucosa in 4 (Cases 2, 3, 4 and 5) of the 5 cystectomy specimens. Furthermore, the rates of PCNA-positive cells demonstrated a tendency to increase in the area with normal mucosa adjacent to TCC or dysplasia and the areas with normal mucosa or dysplasia adjacent to CIS or TCC, G3, respectively. A dramatic increase in the immunohistologically detectable PCNA-containing cells was reported in histopathologically normal tissues adjacent to tumors[9]. This was observed in breast lobules adjacent to breast tumors, as well as in pancreatic parenchyma adjacent to tumors of the pancreas[9]. Since growth factors were demonstrated to induce increased PCNA mRNA stability and consequently PCNA expression[3,4,12], a hypothesis has been postulated as follows: some of the tumors are actively secreting platelet derived growth factor, or similar growth factors, that are stabilizing the PCNA mRNA and thus inducing PCNA protein accumulation in the surrounding normal cells[9]. Such a hypothesis is consistent with our observations made in the mapping study of bladder cancer. Autocrine or paracrine growth factor mediated regulation of cell proliferation may explain the extent of mucosal neoplastic changes and tumors which are recognized as a field change in the bladder.

Normal urothelium becomes hyperplastic, then dysplastic, then CIS develops and then frank bladder carcinomas develop. The number of cells with PCNA protein accumulation increased as these changes in the urothelium took place. Furthermore, PCNA protein accumulation was found in the non-malignant tissue adjacent to the bladder cancers. These results seem to indicate that the extent of neoplastic changes in the bladder is a manifestation of a stepwise progression of mucosal disease, and that, clinically, analysis of the proliferation rates provides useful information for the choice of appropriate therapy.

(Received July 18, 1995)
(Accepted September 28, 1995)

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