PATTERNS OF EPITHELIAL PROLIFERATION REVEALED BY CONTINUOUS ADMINISTRATION OF BROMODEOXYURIDINE DURING URINARY BLADDER CARCINOGENESIS IN RATS

Masae Tatematsu, Shoji Fukushima, Toyohiko Aoki, Yukinori Mera, Tadashi Insue and Nobuyuki Ito
First Department of Pathology, Nagoya City University Medical School, Mizuho-cho, Mizuho-ku, Nagoya 467

The patterns of epithelial proliferation in the urinary bladder during carcinogenesis were examined sequentially in rats given drinking water supplemented with 0.05% N-butyl-N-(4-hydroxybutyl)nitrosamine (BBN) for 12 weeks and then water without BBN for 18 weeks. Animals received 120 μg of bromodeoxyuridine (BrdU) per hour continuously via an osmotic minipump for 4 days before sacrifice and labeled cells were detected immunohistochemically using monoclonal antibody against BrdU. Two types of BBN-induced epithelial lesions were distinguishable: reversible changes characterized by proliferation of basal cells only, and irreversible changes with high and irregularly distributed incorporation of label throughout the epithelium. Simple hyperplasia, and papillary or nodular hyperplasia consisted of areas of reversible and/or irreversible changes, whereas papilloma and cancer consisted of areas of irreversible changes.

Key words: Cell proliferation — Urinary bladder — N-Butyl-N-(4-hydroxybutyl)nitrosamine — Bromodeoxyuridine
planted subcutaneously into the back of the rats and 120 µg of BrdU per hour (Sigma Chemical Co., St. Louis, Mo.) was injected for 4 days before sacrifice.

The urinary bladder was inflated and fixed with 10% phosphate-buffered formalin (pH 7.2) for 24 hr and then routinely processed for histological and immunohistochemical examination. The ABC method7 was used to detect BrdU incorporated into DNA. Affinity-purified biotin-labeled horse anti-mouse immunoglobulin, IgG and avidin-biotin-peroxidase complex (Vectastain ABC Kit, PK 4002) were obtained from Vector Laboratories Inc. (Burlingame, Calif.). Before immunostaining, the sections were treated with 4 N hydrochloric acid for 20 min at 37°C and neutralized with boric acid-borate buffer at pH 7.6. Then sections were treated with 0.04% actinase (Kaken Kagaku, Tokyo) for 3 min at 37°C. For immunohistochemical staining, sections were treated sequentially with normal horse serum, monoclonal mouse anti-BrdU (Becton Dickinson, Mountain View, Calif.) (1:100), biotin-labeled horse anti-mouse IgG (1:400) and avidin-biotin-peroxidase complex (ABC). Sites of peroxidase binding were detected by the diaminobenzidine method of Graham and Karnofsky.8 Sections were then counter-stained with hematoxylin for microscopic examination. Pre-immune mouse serum was used as a negative control for the specificity of anti-BrdU antibody.

Light microscopic studies showed that the sequential changes of the bladder epithelium were simple hyperplasia, PN hyperplasia, papilloma and cancer.1,3 Simple hyperplasias, from week 5 to week 8, showed a regular arrangement of labeled cells in the basal layers (Fig. 1). After week 8, dysplastic areas with slight irregularity of nuclear arrangement appeared within areas of simple hyperplasias. In some of these dysplastic areas, labeled cells were distributed irregularly from the base to the surface (Fig. 2). Although, the labeling index of simple hyperplasias including dysplastic areas significantly decreased after week 18, some dysplastic areas maintained a high rate of BrdU incorporation. PN hyperplasias appeared from week 8. The two types of PN hyperplasias were distinguishable on the basis of differences in their patterns of epithelial proliferation. One (type A) showed a regular distribution of labeled cells limited

Fig. 1. Immunohistochemical demonstration of BrdU-positive cells regularly distributed in basal layers of simple hyperplasia (week 8). ×200.

Fig. 2. BrdU-positive cells distributed irregularly throughout the basal and surface layers of a dysplastic focus within an area of simple hyperplasia (week 12). BrdU immunohistochemistry, ×200.

Fig. 3. PN hyperplasia showing a regular distribution of labeled cells in basal layers (week 12). BrdU immunohistochemistry, ×200.
to the lower epithelial layers (Fig. 3). The mean numbers of type A hyperplasias per 10 cm of basement membrane (BM) were 12.5 (week 8), 22.6 (week 12), 21.5 (week 18) and 17.2 (week 30). The other (type B) contained focal proliferating areas consisting of labeled cells distributed irregularly throughout both the basal and surface layers (Fig. 4). The mean numbers of type B hyperplasias per 10 cm of BM were 2.2 (week 8), 4.2 (week 12), 2.6 (week 18) and 1.2 (week 30). From week 18, the number of labeled cells in areas of type A PN hyperplasia began to decrease, but those in areas of type B did not decrease. Although the numbers of type A and type B PN hyperplasias decreased from week 18, type B seemed to develop into papilloma, which appeared from week 18, because type B showed a similar BrdU incorporation pattern to that of papilloma. Papillomas and cancers were found to consist of mixtures of areas with a high rate of BrdU incorporation and areas with only slight labeling (Fig. 5). Areas of cancer showed more irregular patterns of

Fig. 4. PN hyperplasia demonstrating irregular distribution of BrdU-incorporating cells throughout the whole epithelium (week 12). BrdU immunohistochemistry, ×200.

Fig. 5. Well-differentiated transitional cell carcinoma consisting of areas of high and low labeling. BrdU immunohistochemistry, ×200.

Table I. Labeling Indices of Bladder Epithelial Cells after Continuous Exposure to BrdU for 4 Days in Rats Given BBN

<table>
<thead>
<tr>
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<th>Weeks after beginning of experiment</th>
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<tr>
<td></td>
<td>4</td>
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<tr>
<td>Normal epithelium (Control)</td>
<td></td>
</tr>
<tr>
<td>Simple hyperplasia</td>
<td>37.5 ± 5.0&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>PN hyperplasia</td>
<td></td>
</tr>
<tr>
<td>Type A&lt;sup&gt;a&lt;/sup&gt;</td>
<td>42.4 ± 5.6</td>
</tr>
<tr>
<td>Type B&lt;sup&gt;b&lt;/sup&gt;</td>
<td>69.5 ± 6.8&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>Papilloma</td>
<td>45.6 ± 6.4</td>
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<tr>
<td>Cancer</td>
<td></td>
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<sup>a</sup> Mean ± SD.
<sup>b</sup> Significantly different from the value in week 12 at $P<0.01$.
<sup>c</sup> Significantly different from the value in week 12 at $P<0.05$.
<sup>d</sup> Significantly different from the value for type A at $P<0.01$.
<sup>e</sup> Lesion showing regular distribution of labeled cells limited to the lower epithelial layers.
<sup>f</sup> Lesion containing focal proliferating areas consisting of labeled cells distributed irregularly throughout both the basal and surface layers.
BrdU incorporation than areas of papilloma, but there was no significant difference between their patterns. The labeling indices of papillomas and cancers did not decrease with time. Table I summarizes the labeling indices of the different lesions.

Degeneration and regeneration of target cells, which are changes induced by both carcinogenic and noncarcinogenic agents, are usually observed soon after the administration of carcinogens. Although these non-specific lesions may be confused with true preneoplastic changes, they stop proliferating when carcinogen administration is stopped. Indeed, a marked decrease of the labeling indices of simple hyperplasia and type A PN hyperplasia after cessation of BBN administration was observed. Sequential observation of urinary bladder carcinogenesis has suggested that two types of simple hyperplasias and PN hyperplasias may exist, one reversible and the other irreversible. The present investigation demonstrated that these two types of lesions could be differentiated on the basis of different patterns of distribution of labeled cells. That is, irreversible papillomas and cancers and PN hyperplasias not showing regression contained irregularly distributed areas of BrdU incorporation independent of basal or surface location, whereas regular labeling limited to the basal layers was associated with simple hyperplasia and reversible PN hyperplastic lesions. In addition to indicating a direct link between dysplastic foci arising within areas of simple hyperplasia and more advanced lesions, the present method should be useful for assessing the urinary bladder carcinogenic potential, as opposed to non-specific effects, of exogenous chemicals.

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