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Scanning Electron Microscopy of Changes in the Urinary Bladder in Dogs Treated with N-Butyl-N-(4-Hydroxybutyl)Nitrosamine (BBN)

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Summary. The fine structures of the bladder mucosa during BBN carcinogenesis and of bladder tumors induced by BBN in dogs were examined by scanning electron microscopy. Normal dog bladder mucosa was covered with polygonal superficial cells with peaked microridges, but no microvilli. Microridges and uniform microvilli were observed in hyperplastic mucosa. In low grade papillary tumors induced by low doses of BBN, pleomorphic microvilli predominated. In dogs which received high doses of BBN, bizarre pleomorphic microvilli and blebs were observed in papillary lesions, whereas bumpy surfaces with thick short microvilli were observed in non-papillary lesions. These sequential changes which were observed in dog bladder mucosa during BBN carcinogenesis parallelled the changes that occurred in the process of chemical carcinogenesis in rodents, and dog bladder tumors were similar to those of rodents and of human bladder cancers.

Key words: Scanning electron microscopy, Dog, Bladder cancer, N-butyl-N-(4-hydroxybutyl)nitrosamine, Chemical carcinogenesis.

Introduction

The development of human urinary bladder cancer, recognized to have certain characteristics, can be classified into two major growth patterns – papillary-nonin vasive and non-papillary-invasive. Experimental models using chemical carcinogens in rodents, have been used widely to elucidate the natural history of bladder cancer [1, 2]. For a model of human bladder cancer, a larger animal model would be preferable, because it would allow casier examination. Okajima and collaborates [3] reported that dog bladder cancers induced by N-butyl-N-(4-hydroxybutyl)nitrosamine (BBN) are very similar to those of humans, both cystoscopically and histopathologically. The dog bladder cancers are papillary-noninvasive when the daily dosage of BBN is low and nonpapillary-invasive with high daily dosage of BBN.

Plenomorphism of microvilli on the luminal surface of bladder epithelium is a characteristic change during bladder carcinogenesis in rodents, and is thus considered a useful marker under scanning electron microscopy (SEM) for the development of bladder cancer.

The present SEM study was undertaken to observe the ultrastructural changes of dog bladder epithelium during BBN carcinogenesis.

Materials and Methods

Female beagle dogs weighing 7–10 kg (Fuji animals, Tokyo) were administered low dose (160 mg/day to 2 dogs), and high dose (500 mg/day to 2 dogs) of BBN (Izumi chemical, Yokohama) in gelatin capsules, given 6 times per week. The induction of urinary bladder cancers in dogs by BBN was described in detail by Okajima et al. [3]. Cytoscopic examination was performed periodically, and cold cup biopsies were undertaken transurethrally from the normal-appearing mucosa, from the hyperplastic mucosa during the period of BBN administration, and from bladder tumor and bladder mucosa adjacent to the tumor. Normal bladder mucosa specimens were obtained from 2 dogs before BBN treatment by cold cup biopsy, and from 2 mongrel dogs by cystectomy both in distended and shrunken conditions with in situ fixation.

The specimens were rinsed gently in phosphate buffer at pH 7.4 and fixed with 2.5% glutaraldehyde, post-fixed with 1% osmium tetroxide at 4 °C for 60 min, dehydrated with the ascending ethanol series, and replaced with isoamyl acetate. The specimens were dried by a critical point dryer (Hitachi, Tokyo) using liquid carbon dioxide, coated with 15 nm of gold by Ionsputter (Jeol, Tokyo), and then observed under scanning electron microscopy (Jeol, Tokyo).

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Results

Luminal surface epithelium of the normal dog urinary bladder was covered with flat orderly polygonal cells of relatively uniform size, 25–45 μm in diameter and clearly defined with intercellular ridges on the lateral border (Fig. 1). The luminal surface of superficial cells was covered with peaked microridges in a reticular arrangement, and with concave plaques between peaked mircoridges (Fig. 2). No microvilli, whether uniform or plenomorphic, were observed on the normal bladder surface epithelium. The luminal surface did not change with distension and shrinkage except for a flattening of concave plaques and a decrease in the number of peaked microridges in the distended case. SEM of normal bladder mucosa obtained by transurethral cold cup biopsy demonstrated intermediated appearance between those of the distended and the shrunken conditions.

Hyperplastic bladder mucosa was obtained from a beagle dog (NUDB-5), that received a low dose (160 mg) of BBN for 105 weeks. Petechia and slight dilatation of the subepithelial blood vessels were characteristic and mucosal proliferation was observed cystoscopically. The luminal surface of the hyperplastic foci were covered with pleomorphic cells, hemispherical or spindular in shape and variable in cell size. The luminal surfaces of these cells were covered with ropy microridges and uniform microvilli (Fig. 3). NUDB-5 developed a rice size papillary bladder tumor at 142 weeks, and was then observed without BBN treatment. Multiple papillary bladder tumors were observed at 420 weeks and diagnosed histopathologically as transitional carcinoma grade 2.

Normal appearing bladder mucosa was obtained from a beagle dog (NUDB-4), that received daily 160 mg of BBN for 132 weeks when small papillary bladder tumors was observed. The luminal surface was covered with rounded cells rather than polygonal cells, in a cobblestone arrangement. The intercellular ridge was thickened. The luminal surfaces of superficial cells were covered with ropy and leafy microridges and uniform microvilli. The fine structure of the surfaces displayed variability in the proportions of these fine structures, but uniform microvilli appeared to be predominant in most cells (Fig. 4). Multiple papillary bladder tumors were observed at 208 weeks in the same beagle dog (NUDB-4), and a multiple cold cup biopsy was performed from normal-appearing bladder mucosa, from hyperplastic mucosa adjacent to the bladder tumor, and from bladder tumor itself. The luminal surfaces of normal appearing mucosa were covered with rounded polygonal cells covered with both leafy microridges and uniform microvilli similar to those observed at 132 weeks (Fig. 5). The superficial cells of hyperplastic mucosa were pleomorphic with thick intercellular ridges. Uniform microvilli were predominant and peaked microridges were no longer observed (Fig. 6). The luminal surface of the dog bladder

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Fig. 1. Luminal surface cells of the dog urinary bladder of an untreated female beagle dog obtained by cold cup biopsy. Note the orderly polygonal shape with peaked mircoridges. x 750

Fig. 2. Higher magnification of Fig. 1. Peaked microridges and concave plaques are characteristic. x 10,000

Fig. 3. Luminal surface cells of slightly proliferated bladder mucosa obtained from beagle dog NUDB-5, which received BBN 160 mg/day for 105 weeks. The cells are irregular in size and shape. Ropy microridges and uniform microvilli are visible, and the intercellular ridges are marked. x 2,250

Fig. 4. Luminal surface cells of normal-appearing bladder mucosa obtained from beagle dog NUDB-4, which had a papillary bladder tumor. The cells are spindular or hemispherical in shape, with ropy and leafy microridges, and uniform microvilli. x 3,000

Fig. 5. Polygonal superficial cells in a cobblestone arrangement from a normal-appearing lesion in beagle dog NUDB-4 at 208 weeks. The cells are pentagonal, with leafy microridges and uniform microvilli. x 4,000

Fig. 6. Luminal surface cells obatined from an epithelial proliferating lesion adjacent to the papillary bladder tumor of beagle dog NUDB-4. Ropy microridges and pleomorphic microvilli are predominant. x 4,000

Fig. 7. Luminal surface of the papillary bladder tumor of beagle dog NUDB-4. The luminal surface is covered with small spindular and hemispherical cells with pleomorphic microvilli. x 3,000

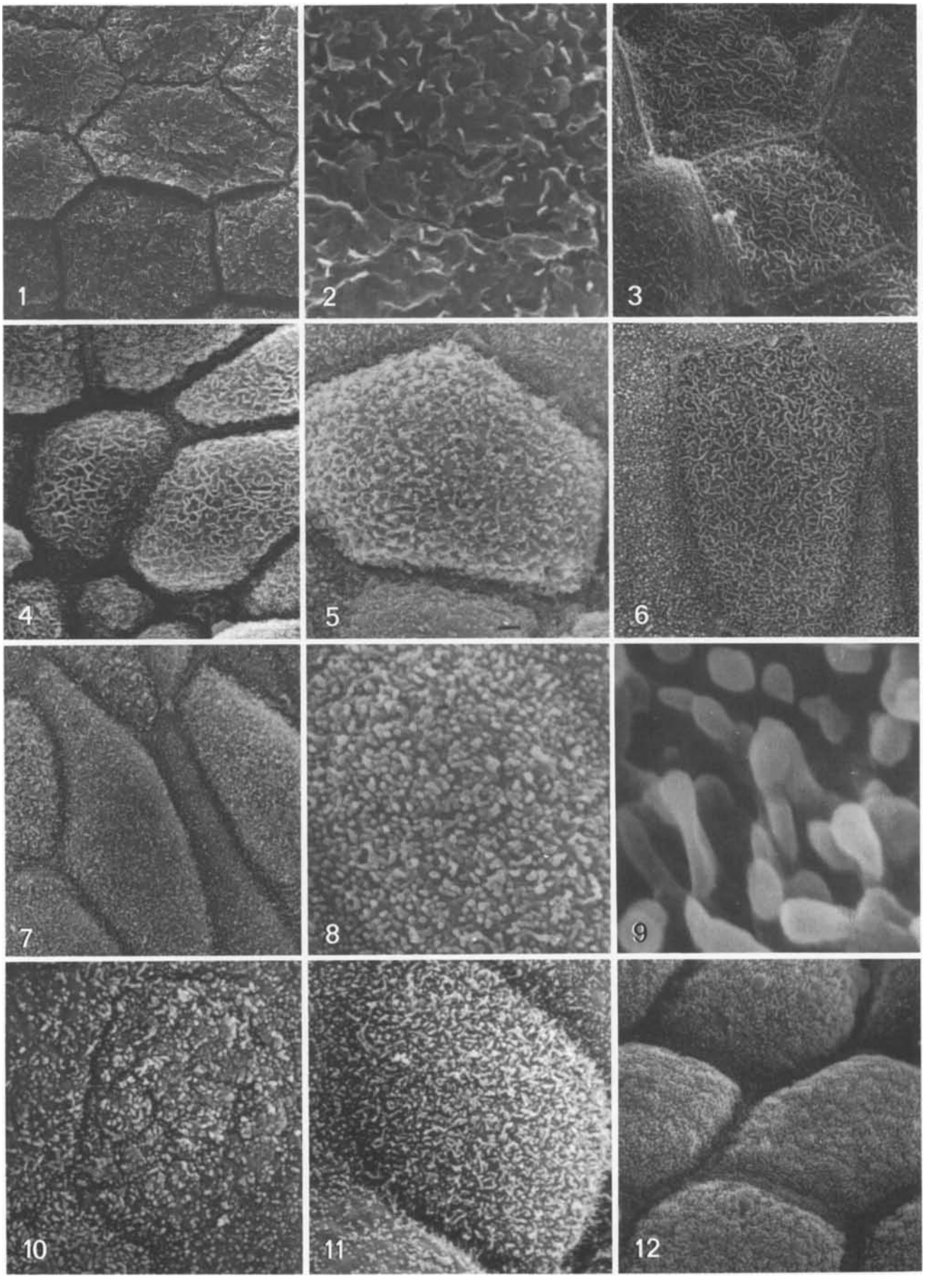
Fig. 8. Higher magnification of Fig. 7. Pleomorphisms of microvilli are marked, and microridges are seldom observed. x 7,500

Fig. 9. Higher magnification of pleomorphic microvilli of Fig. 7 and 8. x 57,000

Fig. 10. Superficial cells obtained from the papillary bladder tumor of beagle dog NUDB-12, which received BBN 500 mg/day for 210 weeks, have marked pleomorphic microvilli and blebs. x 7,500

Fig. 11. Superficial cells with bizarre pleomorphic microvilli from the same specimen as in Fig. 10. x 3,000

Fig. 12. Superficial cells obtained from non-papillary bladder tumor of beagle dog NUDB-12. Luminal surfaces of these cells are bumpy, corrugated, and covered with thick short microvilli, resembling the rind of a grapefruit. x 4,000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tumor was covered with relatively small pleomorphic cells, 10–25 μm in diameter and spindular or hemispherical in shape (Fig. 7). The luminal surfaces of these cells had pleomorphic microvilli rather than uniform microvilli without microridges (Fig. 8), and these microvilli were 0.1–0.45 μm in length and varied in shape (Fig. 9). The histopathological diagnosis of this bladder tumor was transitional cell carcinoma grade 2.

The beagle dog (NUDB-12) receiving a high dose (500 mg) of BBN developed bladder tumors showing the appearances of both papillary and non-papillary growth. Cold cup biopsy was performed form both lesions at 210 weeks. The luminal surface of the papillary lesion was covered with marked pleomorphic cells, while exfoliation of the surface cells was marked and intercellular ridges were obscure. The luminal surfaces of superficial cells displayed pleomorphic microvilli and blebs (Fig. 10). Some luminal surface cells had scattered short microvilli and/or bizarre pleomorphic microvilli (Fig. 11). The superficial cells of the non-papillary tumor were small, pleomorphic, and defined clearly with thickened intercellular ridges. The luminal surfaces of these cells were bumpy, corrugated, and covered with thick short microvilli rather than with pleomorphic microvilli, resembling the rind of a grapefruit (Fig. 12). NUDB-12 died of massive vesical bleeding, and histopathological examination revealed invasive transitional cell carcinoma grade 3. Similar morphologic changes were also observed in another beagle dog (NUDB-13) which received high daily dose of BBN.

Discussion

The characteristic fine structure of mature superficial cells of normal bladder epithelium includes asymmetric unit membranes and fusiform vesicles under transmission electron microscopy [4, 5], as well as peaked microridges and concave plaques under scanning electron microscopy [6–8]. These fine structures are common to all species of mammalia [9]. Ultrastructural changes in bladder epithelium during chemical carcinogenesis begin with the formation of microvilli in very early lesions before light microscopic changes occur and pleomorphic microvilli appear in irreversible lesions of various types caused by different carcinogens in rodents, [7, 10–12]. Pleomorphic microvilli are also observed in human bladder cancers [13, 14] and have been described as a morphological marker for irreversible lesions and bladder cancer itself [10, 15].

The present study demonstrated SEM changes on the luminal surface of dog bladder epithelium during BBN carcinogenesis. Early changes observed in normal-appearing mucosa were pleomorphism of superficial cells with peaked microridges, followed by covering with ropy and leafy microridges, and uniform microvilli on the luminal surface. The normal-appearing mucosa of the bladder tumor was covered with polymorphic cells in a cobblestone arrangement, with ropy microridges and microvilli. The mircovilli subsequently became predominant and more pleomorphic, as observed in hyperplastic foci and lesions adjacent to the bladder tumor. The superficial cells of papillary bladder tumors in dogs induced by low daily dosage of BBN had an abundance of uniform and/or pleomorphic microvilli. The superficial cells of papillary bladder tumors induced by high daily dosage of BBN had bizarre microvilli and blebs, but a poor surface structure with scattered pleomorphic and/or thick short microvilli on the surface cells of non-papillary bladder tumors. These fine structural changes in high grade human bladder cancers were reported by Jacobs et al. [14], who measured the number of the microvilli and the pleomorphism of human bladder cancers using a graphic data analysing system.

The sequential changes in dog bladder epithelium during BBN carcinogenesis seen by SEM are very similar to those observed in rodent bladder epithelia induced by several different carcinogens. The dog bladder cancers induced by BBN had similar fine structures on luminal surface clles as those observed both in humans and in rodents [10–12, 15]. These results demonstrate the use of the dog bladder cancer model in addition to the well established rodent models for elucidating the development and growth of human bladder cancers. However, plenomorphic microvilli were also observed in reversible hyperplasia caused by bladder damage such as surgical incision, freeze-ulceration, or formalin instillations in rat urinary bladder [16], or in chronic inflammatory mucosa in humans [17], so that the clinical significance of pleomorphic microvilli as a marker of bladder carcinogenesis needs further investigation.

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