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# Morphological changes of microvilli on different surfaces of epithelial cells in the rat bladder treated with *N*-butyl-*N*-(4-hydroxybutyl) nitrosamine

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**Abstract** This study investigates morphological changes of microvilli occurring on different surfaces of epithelial cells, including luminal surfaces, intercellular surfaces, and basal cell surfaces adjacent to the basement membrane (BM), in the rat bladder treated with *N*-butyl-*N*(4-hydroxybutyl) nitrosamine (BBN) by transmission electron microscopy (TEM). The present results showed that the microvilli were observed not only on the luminal surfaces of epithelial cells but also on the intercellular surfaces and basal cell surfaces adjacent to the BM following administration of BBN. There were a variety of morphological changes of microvilli on the luminal surfaces, intercellular surfaces and basal cell surfaces adjacent to the BM, respectively. It is suggested that the microvilli occurring on the luminal surfaces may not represent characteristics of the microvilli occurring on the intercellular surfaces and on the basal cell surfaces adjacent to the BM, and that occurrence of the microvilli on the epithelial cell surfaces may reflect a higher metabolic activity of proliferative epithelial cells and may play an important role in the processes of tumor growth during bladder carcinogenesis.

**Key words** Microvilli · Morphology · Carcinogenesis · Epithelial cell surfaces · Rat bladder · Transmission electron microscopy

## Introduction

Microvilli are usually found on the surface structures of normal small intestine, whose principal function is absorption as well as of malignant cells [9–11]. The microvilli occurring on the luminal surfaces of bladder tumor cells usually displayed a nonuniform appearance and were referred to as the pleomorphic microvilli in experimental situations and human bladder tumors [10, 12, 19]. The presence of pleomorphic microvilli has been demonstrated to be a reliable marker for the development of irreversible proliferative changes in urothelial cells [5, 15]. With the aid of transmission electron microscopy (TEM), many investigations concerning the microvilli alterations on the luminal surfaces of epithelial cells in bladder tumor have been finished [2]; whereas no research concerning morphological changes of microvilli both on the intercellular surfaces and on the basal cell surfaces adjacent to the basement membrane (BM) in bladder tumor has been reported.

According to previous studies, it is believed that occurrence of microvilli on the epithelial cell surfaces of the bladder may reflect existence of bladder pathological changes. Therefore occurrence of microvilli on different surfaces of the bladder epithelial cells, including luminal surfaces, intercellular surfaces, and basal cell surfaces adjacent to the BM, may be imagined. So the following problems are focused upon in this study: (1) whether the microvilli may occur or not on the different cell surfaces simultanuously following administration of *N*-butyl-*N*-(4-hydroxybutyl) nitrosamine (BBN), which has been widely used in the study of bladder tumor in the animal model [13, 14]; (2) whether the different characteristics of the microvilli may be observed or not on the different cell surfaces with the aid of TEM; and (3) what role the microvilli may play during bladder carcinogenesis.

## Materials and methods

Forty male Wistar rats (Kurea, Osaka, Japan), 7 weeks of age at the start of the experiment, were divided into a control group (12 rats) and a BBN group (28 rats). Rats were housed three to a polycarbonate cage, which were placed in an environment-controlled room illuminated for 12 h/day. BBN (Kasei, Tokyo, Japan) was

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administered to the rats at a dose of 0.05% in drinking water in the BBN group. Seven rats of the BBN group and three rats of the control group were killed at 4, 8, 12, and 20 weeks from the beginning of the experiment, respectively. The obtained specimen of each rat bladder was longitudinally cut into two parts, and each of them was prepared for the procedures of light microscopy (LM) and TEM.

For LM, the specimens were placed in 10% formalin and processed for paraffin embedding. Each paraffin block was step-sectioned and stained with hematoxylin and eosin. Multiple sections of each specimen were examined. For TEM, the specimens were cut into $3 \times 3 \times 3$ mm cubes and fixed with 2.5% glutaraldehyde. Following a phosphate buffer wash, the specimens were postfixed in 1% osmic acid for 2 h, stained with 2% tannic acid for 1 h, and dehydrated in graded ethanols. Then, the specimens were treated with propylene oxide and embedded in Epon 812. Ultrathin sections were cut on an LKB ultramicrotome by a diamond knife and double-stained with uranyl acetate and lead citrate. They were examined with a Hitachi JEM 1200EX TEM.

In this study, epithelial cell surfaces of the rat bladder were subdivided into the following three types. The luminal surfaces were designated as the surfaces of superficial cells facing the bladder lumen. The intercellular surfaces were identified as the surfaces of epithelial cells facing each other. The basal cell surfaces adjacent to the BM were described as the surfaces of basal cells only facing the BM. Additionally, the bladder lesions were histologically classified as hyperplasia, papilloma, and carcinoma, as prescribed previously [6, 8]. The hyperplasia was divided into three types: mild, moderate, and severe hyperplasia. The bladder epithelium was three to five layers thick in mild hyperplasia, six to eight layers thick in moderate hyperplasia, and over nine layers thick in severe hyperplasia.

## Results

### LM findings

In the control group, the rat bladder epithelium showed normal appearance in all 12 rats during 20 weeks of observation. In the BBN group, all seven

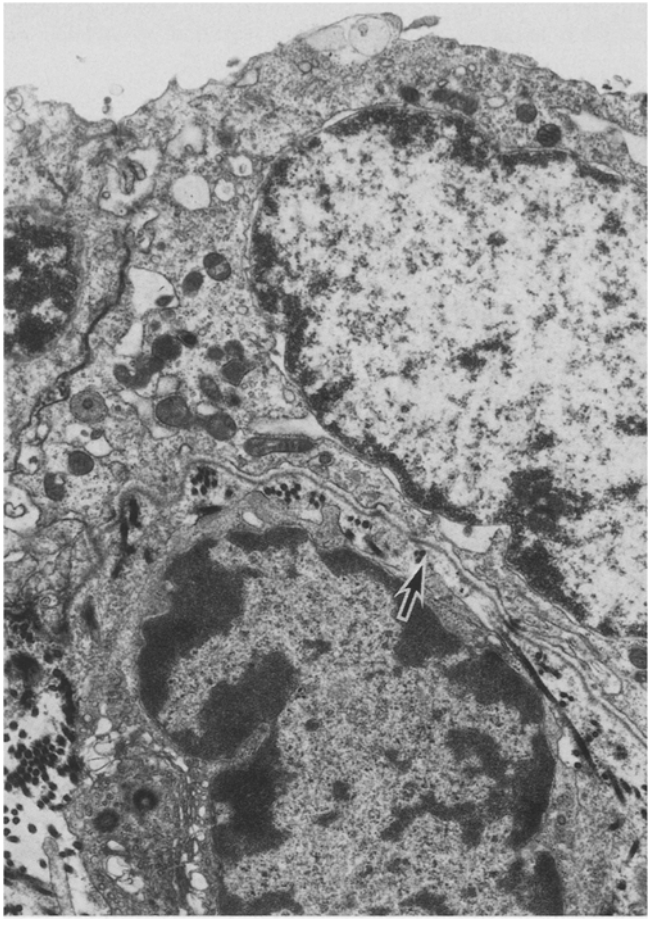

**Fig. 1** No microvilli are found on the different cell surfaces, including luminal, intercellular, and basal cell surfaces adjacent to the basement membrane ( → ), (in the control group at 20 weeks, using TEM ×10000

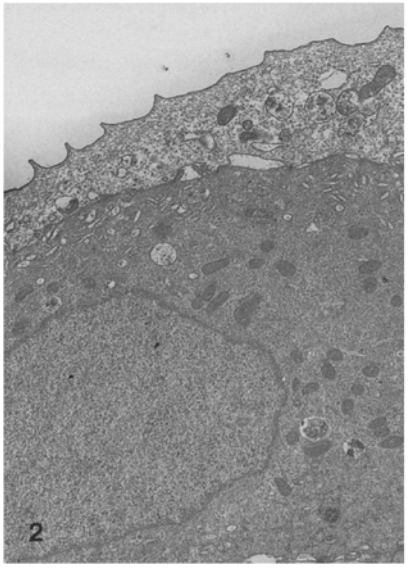


**Fig. 2** Luminal surfaces of the rat bladder epithelium in mild hyperplasia. Short, uniform microvilli are characteristic in the BBN group at 4 weeks, using TEM ×5000

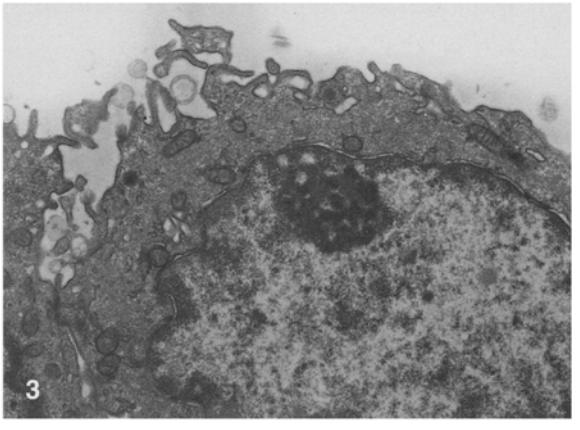


**Fig. 3** Luminal surfaces of the rat bladder in noninvasive transitional cell carcinoma. Short and long irregular microvilli are marked. BBN group at 20 weeks, using TEM ×8000

killed rats displayed mild hyperplasia at 4 weeks and moderate hyperplasia at 8 weeks. At 12 weeks, severe hyperplasia was found in five rats and papilloma in two rats. Noninvasive transitional cell carcinoma (TCC) of the rat bladder epithelium was observed in all seven rats at 20 weeks.

### TEM findings

In the control group, no microvilli, whether uniform or pleomorphic, were observed on the luminal surfaces under TEM. Although some few cell processes were observed on the intercellular surfaces and basal cell surfaces adjacent to the BM, no microvilli were found on them (Fig. 1).

In the BBN group, the luminal surfaces were covered with short, uniform microvilli at 4 and 8 weeks (Fig. 2), then both short and long irregular microvilli were found at 12 and 20 weeks (Fig. 3). The intercellular surfaces were covered with both long and short microvilli during 20 weeks (Figs. 4, 5). On the basal cell surfaces adjacent to the BM, both round, strong and thin, long microvilli were found at between 4 and 20 weeks (Figs. 6–9), and the round, strong microvilli were observed more than thin, long ones. Following administration of BBN, the number of the microvilli occurring on each cell surface of the different cell layers was increased by degrees. On the other hand, the microvilli occurring on the luminal and intercellular surfaces contained microfilaments, while the microvilli occurring on the basal cell surfaces adjacent to the BM consisted of microfilaments and some secretory granules.

Table 1 displays characteristics of the microvilli occurring on the different surfaces of epithelial cells in the rat bladder treated with BBN as examined by TEM.

## Discussion

In normal conditions, extensions of the cell cytoplasm covered by the cell membrane are collectively referred to as cell processes, which were thought to be involved in the transport of materials into the cells and in the transport of secretory products of cellular metabolism out of the cells [9]. In pathological and experimental situations, the cell processes are frequently referred to as microvilli or microvillus-like tumor cell protrusions [1, 4, 9].

Some reports interpreted the presence of pleomorphic microvilli on luminal surfaces as an indicator of abnormal or neoplastic proliferative changes in the bladder epithelium of experimental animals [15] and humans [16]. It was suggested that long microvilli were associated with the malignancy of the bladder tumor in rat bladder carcinogenesis [3]. However, short microvilli were also reported as the characteristic of bladder tumors in dog [12] and of gallbladder tumors in humans [18]. In this study, both short and long irregular microvilli were observed on the luminal superficial cell surfaces in severe hyperplasia and noninvasive TCC of the rat bladder induced by administration of BBN. These findings also demonstrate that the pleomorphisms of microvilli on the luminal surfaces may be regarded as surface microfeatures of irreversible and malignant proliferative lesions in the bladder epithelium; although it is difficult to find out which may be much more closely related to the malignancy of the bladder tumor between the short microvilli and the long ones on the luminal surfaces.

Our present results showed that there were a variety of morphological changes in microvilli occurring on the different surfaces, including the luminal, intercellular, and basal cell surfaces adjacent to the BM, of the rat bladder epithelium. Some authors reported that microvillous cellular processes could be found on the deeper cells of bladder tumor exposed by ultrasonic wave forces using scanning electron microscopy, and the microvillous cellular processes of deeper cells in bladder tumor were more pleomorphic than those of deeper cells in the nonneoplastic bladder mucosa [23]. Our results showed that the microvilli covering either the intercellular surfaces or the basal cell surfaces adjacent to the BM possessed different morphological characteristics to the microvilli occurring on the luminal surfaces at between 4 and 20 weeks. It was found that, although short and uniform microvilli were observed on the luminal surfaces at 4 and 8 weeks, the microvilli were long and short on the intercellular surfaces as well as round and strong and thin and long on the basal cell surfaces adjacent to the BM at between 4 and 20 weeks. Based on these findings, it is suggested that the microvilli occurring on the luminal surfaces may not share characteristics of the microvilli occurring both on the intercellular surfaces and on the basal cell surfaces adjacent to the BM in the rat bladder tumors induced by BBN.

It is well known that the BM components are synthesized by the cells resting upon them [17, 21]. In our other study the thickened BM has been found in the rat bladder epithelium during bladder carcinogenesis induced by BBN [25]. The present results showed that the microvilli occurring on the basal cell surfaces adjacent to the BM were greatly different, morphologically, from those occurring on the luminal and intercellular surfaces of epithelial cells in the rat bladder. Most of them exhibited the round, strong characteristic, and consisted of microfilaments and some secretory granules. It is thought that these round, strong microvilli closely correlate with neosynthesis of the BM components during carcinogenesis.

Some authors suggested that an increase in microvilli closely correlates with the growth potential and metastatic ability of tumor cells [22]. Other reports also revealed that microvilli may be characteristic of active

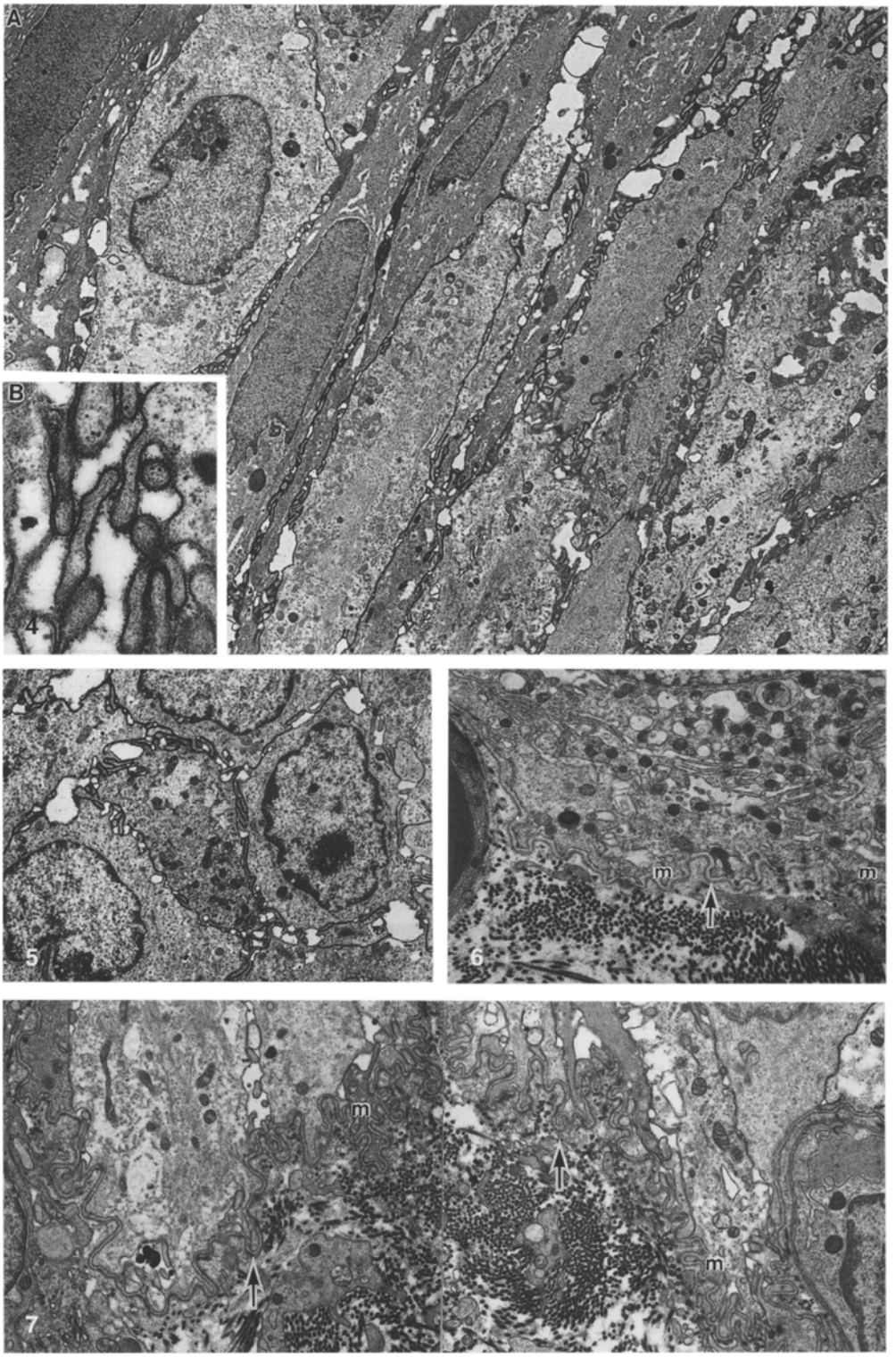


**Fig. 4** **A** Intercellular surfaces of the rat bladder epithelium in mild hyperplasia. A lot of long and short microvilli are observed. BBN group at 4 weeks, using TEM. ×2000, **B** Higher magnification of **A**. ×25 000

**Fig. 5** Intercellular surfaces of the rat bladder epithelium in severe hyperplasia. Both long and short microvilli occur on them. BBN group at 12 weeks, using TEM ×5000

**Fig. 6** Basal cell surfaces adjacent to the basement membrane (BM) of the rat bladder epithelium in mild hyperplasia. Round, strong microvilli (*m*) are found as well as the thickened BM (→). BBN group at 4 weeks, using TEM ×6000

**Fig. 7** Basal cell surfaces adjacent to the BM of the rat bladder epithelium in moderate hyperplasia. Both round, strong and thin, long microvilli (*m*) are found on the basal cell surfaces adjacent to the thickened BM (→). BBN group at 8 weeks, using TEM ×6000

cells in general [24], or occurrence of the microvilli represented increased secretory and metabolic activity in tumor cells [18]. The present findings indicate that occurrence of the microvilli on epithelial cell surfaces of the rat bladder may result from a transformation of normal epithelial cells to a malignant situation during carcinogenesis. Following the administration of BBN, the normal epithelial cells of the rat bladder were replaced by the benign and malignant proliferative epithelial cells with much higher metabolic activity. In

**Table 1** Characteristics of microvilli on different surfaces of epithelial cells in the rat bladder

| | | Control group | BBN group | | | |
|---|---|---|---|---|---|---|
| | | 4 w–20 w | 4 w | 8 w | 12 w | 20 w |
| LM | | Normal appearance (12) | Mild HP (7) | Moderate HP (7) | Severe HP (5) Papilloma (2) | Noninvasive TCC (7) |
| TEM | Luminal | No microvilli (12) | Short UM (7) | Short UM (7) | Irregular M (7) | Irregular M (7) |
| | Intercellular | No microvilli (12) | Long & short M (7) | Long & short M (7) | Long & short M (7) | Long & short M (7) |
| | Basal cell | No microvilli (12) | Round strong & thin long M (7) | Round strong & thin long M (7) | Round strong & thin long M (7) | Round strong & thin long M (7) |

Notice: 4 w = 4 weeks, and it is the same as other ones; Figure in ( ) represents the number of rats; HP = hyperplasia; UM = uniform microvilli; M = microvilli

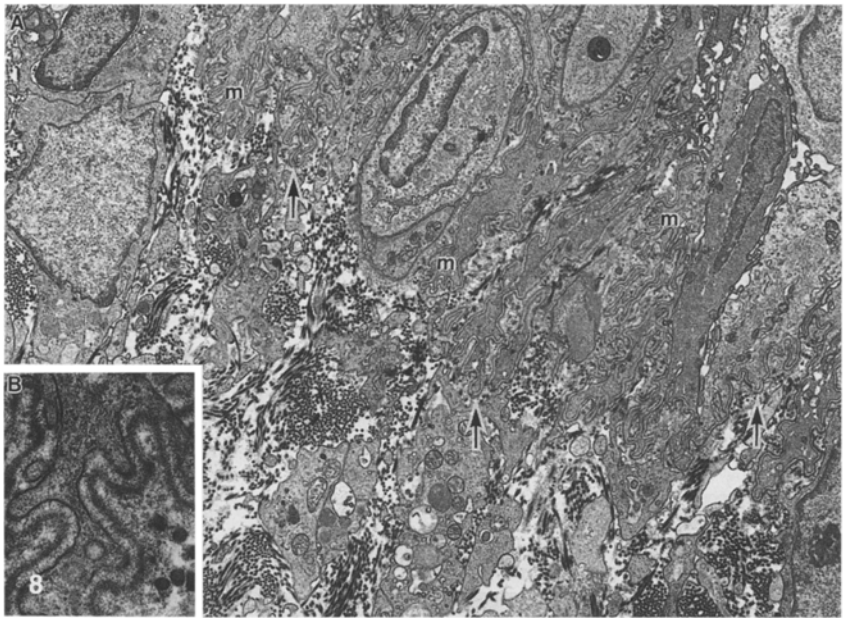


**Fig. 8** **A** Basal cell surfaces adjacent to the BM of the rat bladder epithelium in severe hyperplasia. Numerous microvilli (*m*), including round, strong and thin, long ones, are observed on the basal cell surfaces adjacent to the BM (→). BBN group at 12 weeks, using TEM. ×2000. **B** Higher magnification of **A**. ×25 000

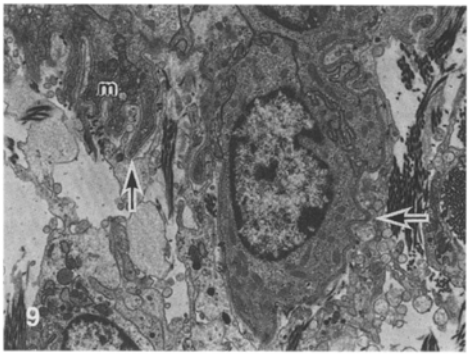


**Fig. 9** Basal cell surfaces adjacent to the BM of the rat bladder in noninvasive transitional cell carcinoma. The thickened BM (→) snakes along the microvilli (*m*) occurring on the basal cell surfaces. BBN group at 20 weeks, using TEM ×4000

order to respond to these new needs, numerous microvilli occurred on the different surfaces of proliferative epithelial cells. However, in other reports, the microvilli, including pleomorphic microvilli, were also observed in reversible hyperplasia caused by bladder damage such as surgical incision, freeze-ulceration, or formalin instillations in rat urinary bladder [7], or in chronic inflammatory mucosa in humans [20]. According to these findings, it is also suggested that occurrence of the microvilli may reflect a higher metabolic activity of proliferative epithelial cells and may play an important role in the processes of tumor growth during carcinogenesis.

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## Editorial comment

The bladder was not inflated in situ with a fixative before cutting to two halves in this experiment. Therefore, the bladder was not examined in the dilated condition. This reviewer thinks that the bladder wall is observed differently between in the dilated condition and in the contracted condition by both of light microscopy and transmission electron microscopy. For example, tumor grade and tumor stage may be clearly observed light microscopically and microvilli may be more clearly distinguishable by TEM in the dilated condition. Koss [1] reported that study that deep crevices or canals extending from the surface of the cell into the depth of the cytoplasm were frequently observed in contracted bladders. On the other hand, such intracytoplasmic canals were few and shallow in dilated bladders.

In addition, the authors noted that hyperplasia was divided into three types: mild, moderate and severe hyperplasia according to epithelial cell layer thickness. However, nodulopapillary hyperplasia defined as exophytic (intraluminal) and/or endophytic (downward) growth with minimal atypia should be also classified as neoplastic lesions in such investigation [2].

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