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**Basement membrane and tumor invasion: ultrastructural observations in the basement membrane of rat bladder with invasive transitional cell carcinoma induced by N-butyl-N-(4-hydroxybutyl)nitrosamine**

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**Abstract** This study describes ultrastructural alterations in the basement membrane (BM) of rat bladder with invasive transitional cell carcinoma (TCC) induced by N-butyl-N-(4-hydroxybutyl)nitrosamine. Various alterations including thickening, degradation and neosynthesis were found in the bladder BM of one rat with invasive TCC. Focal destruction of both the BM lamina zones was found in addition to partially degraded BMs showing focal degradation and loss of only the BM lamina rara. Neosynthesis of complete BM including the lamina rara and lamina densa was observed surrounding the nests of carcinoma cells deep in the stroma, while neosynthesis of incomplete BM including only a lamina densa-like structure was also found around carcinoma cells which had just crossed the BM into the adjacent stroma from the original tumor masses. There was an increased hemidesmosomal frequency in some areas of thickened BM, and focal loss of hemidesmosome in the areas of degraded BM. It is suggested that BM degradation may take place in two steps, and that BM neosynthesis may also be a two-step process in invasive TCC of rat bladder.

**Key words** Basement membrane · Tumor invasion · Transitional cell carcinoma · Degradation · Neosynthesis · Hemidesmosome

Basement membrane (BM) comprises a ubiquitous extracellular matrix found at the boundary of the epithelial cells and connective tissue stroma. At the level of pathological anatomy, the BM can be understood as the structural barrier to the invasion and metastatic behavior of malignant cells. Tumor invasion can be defined as the active migration of neoplastic cells out of their tissue of origin and into different types of adjacent tissue. During the transition from in situ to invasive carcinoma, tumor cells penetrate the epithelial BM and enter the underlying interstitial stroma [17]. As a general feature of all types of carcinoma [1], defects in the BM are associated with the tumor invasion [15]. Although the loss of BM in invasive carcinoma has received much attention, several ultrastructural studies have demonstrated tumor invasion in the presence of an essentially complete BM [5, 8] and a thickened BM [6]. N-Butyl-N-(4-hydroxybutyl)nitrosamine (BBN) has been proved to be an effective organ-specific carcinogen on urinary bladder of rat, and is widely used in the study of bladder tumor in animal models [13, 14]. This study reports various changes in the BM of rat bladder with invasive TCC induced by administration of BBN for 28 weeks using transmission electron microscopy (TEM).

**Materials and methods**

Twenty male Wistar rats (Kurea, Osaka, Japan), 7 weeks of age at the start of the experiment were divided into a BBN group (15 rats) and a control group (5 rats). Rats were housed three to a polycarbonate cage, placed in an environmentally controlled room illuminated for 12 h/day. BBN (Kasei, Tokyo, Japan) was administered at 0.05% in drinking water for 28 weeks in the BBN group. All 15 rats in the BBN group and the 5 rats in the control group were put to death 28 weeks from the start of the experiment. The specimens of rat bladder obtained were examined by light microscopy and TEM, respectively. For light microscopy one part of the bladder was 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 bladder were examined. For TEM observation the specimens were cut into 4×4×4-mm blocks and fixed with 2.5% glutaraldehyde for 4 h at room temperature. Following a phosphate buffer wash, the specimens were post-fixed in 1% osmium acid for 2 h, then dehydrated in graded ethanol, treated with propylene oxide and embedded in Epon 812. Ultrathin sections were cut on a LKB ultramicrotome with a diamond knife and examined with a Hitachi JEM-1200 EX TEM.
Fig. 1 The BM is smoothly arranged at the junction of the basal epithelia and interstitial stroma. Two distinct BM zones, the lamina rara and the lamina densa, are of the same thickness (→). The hemidesmosomes are also observed on the plasma membrane of basal cells. Control group, 28 weeks, TEM, ×20000

Fig. 2 Focal thickening in the BM (→) of rat bladder with invasive TCC. The lamina rara is thickened to the same extent as the lamina densa. BBN group, 28 weeks, TEM, ×15000

Fig. 3 Focal destruction is observed in the BM of rat bladder with invasive TCC. Both BM lamina zones are degraded (→). BBN group, 28 weeks, TEM, ×8000

Fig. 4 Partially degraded BM and focal degradation of the lamina rara in the BM (→) of rat bladder with invasive TCC. An increased hemidesmosomal frequency is observed in the thickened BM area. BBN group, 28 weeks, TEM, ×5000

Fig. 5 Partially degraded BM, showing focal loss of only the BM lamina rara, is found in the BM of rat bladder with invasive TCC. The carcinoma cells are covered only by the BM lamina densa (→). BBN group, 28 weeks, TEM, ×6000

Fig. 6 Neosynthesis of incomplete BM with only a lamina densa-like structure (→) is found surrounding the tumor cells which have just crossed the BM into the stroma from the original tumor masses. BBN group, 28 weeks, TEM, ×3000

Results

Light microscopic findings

In the control group the rat bladder epithelium appeared normal in all five rats. In the BBN group invasive TCC of the bladder was observed in all 15 rats. The bladder tumor had focally invaded into the muscle layers, but had not gone beyond the rat bladder wall.