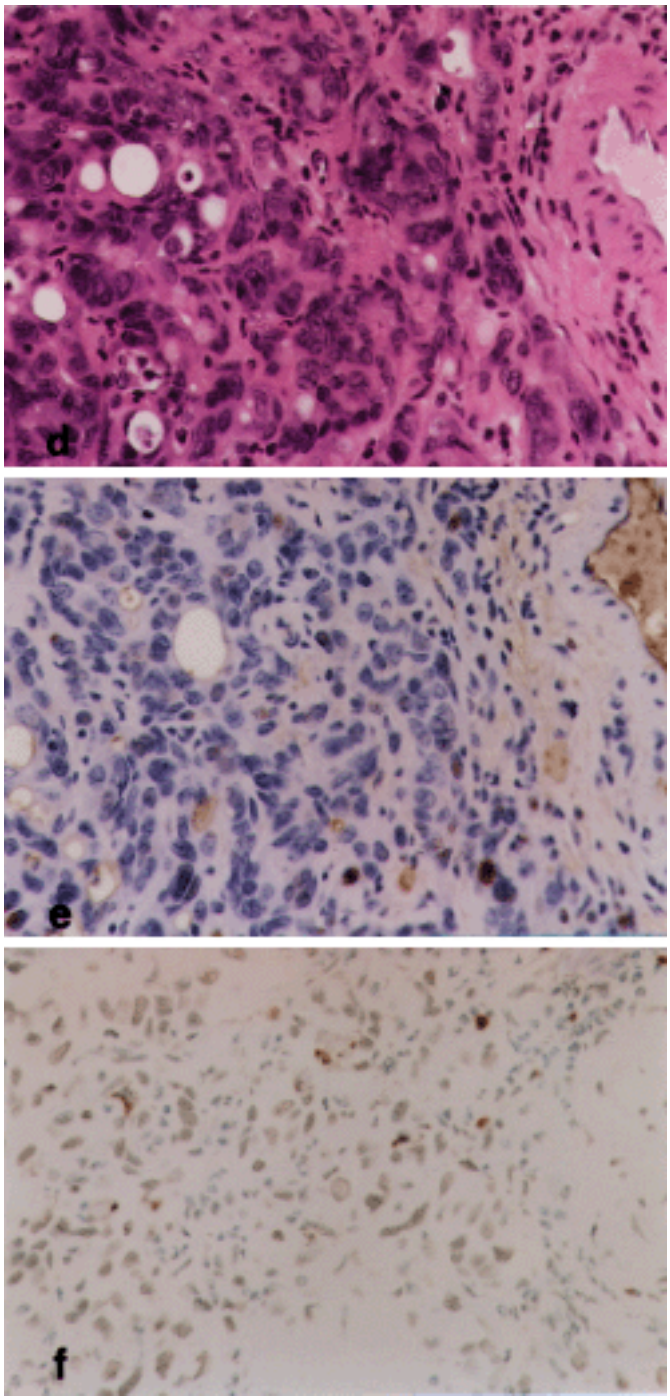
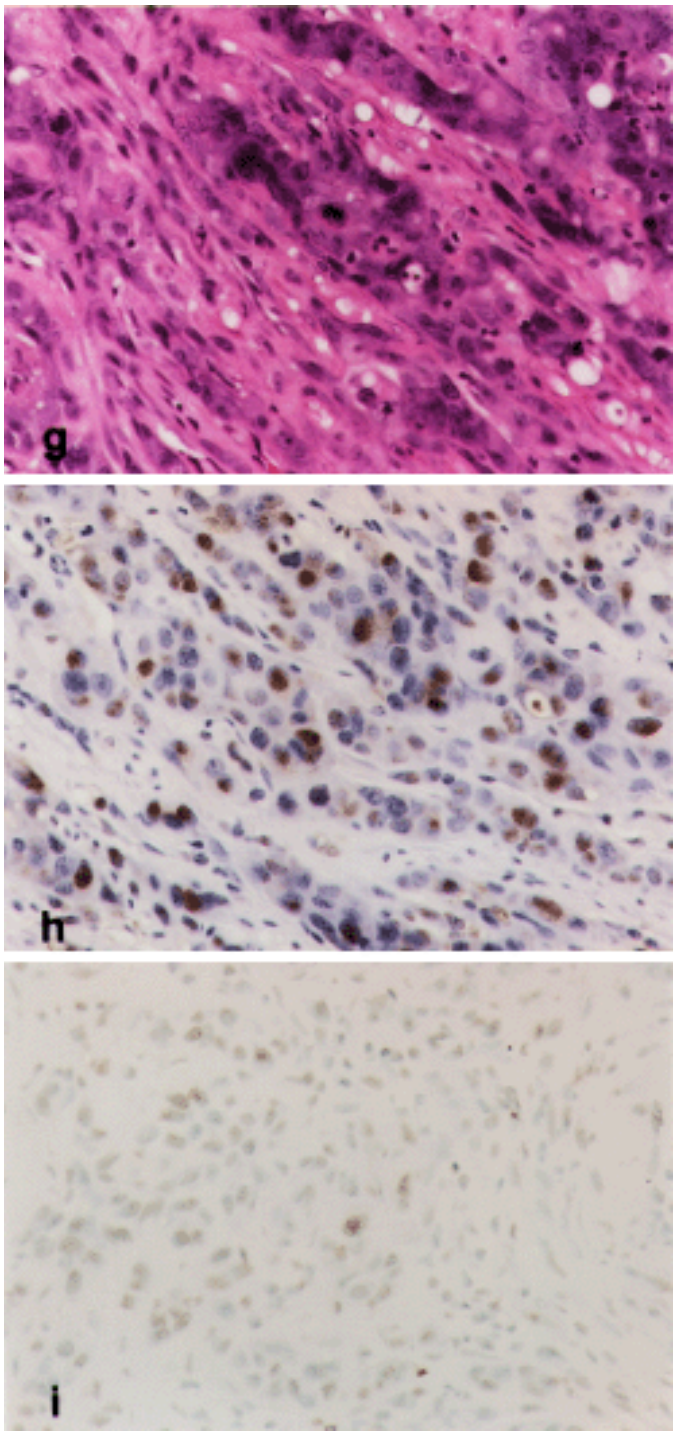


Fig. 3. A Grade-3 invasive bladder carcinoma in Group I. **d:** Hematoxylin and eosin staining. **e:** Proliferating cell nuclear antigen-positive cells shown by immunohistochemical staining. **f:** Some apoptotic cells shown by terminal-deoxynucleotidyl-transferase (TdT)-mediated dUTP-biotin nick end labeling, × 200.

coagulation, but its prognosis is good. The latter frequently shows metastasis and has a very poor prognosis and many problems. Most of the previous studies reported the sex hormone influence on urinary bladder carcinogenesis in the rat model. In all BBN-treated rat strains, bladder carcinomas are usually papillary, multiple and superficial. Thus bladder carcinomas in rats are a satisfactory model for studying papillary superficial bladder carcinomas in humans (Ito et al., 1975). On the other hand, mice whose bladder epithelium has changed to dysplasia, carcinoma in situ and invasive tumor by BBN are good models for human invasive bladder carcinomas (Hirose et al., 1976). Thus, mice were selected in the present investigation as models for invasive bladder carcinomas in humans.

In Experiment I, the occurrence of carcinomas was significantly higher in Group III and significantly lower in Group I than in the other groups. In Experiment II, the tumor induction time was significantly shorter in Group III and significantly longer in Group I than in the other groups. A previous report showed that both surgical castration and luteinizing hormone-releasing hormone agonist treatment significantly reduced the occurrence of carcinomas as compared with controls; thus, surgery and ether anesthesia had little influence on carcinoma occurrences (Imada et al., 1997). Similarly, intramuscular injection was not found to affect induction of carcinogenesis (Kono et al., 1975). Our findings also showed that an overdose of testosterone promotes the occurrence of blad-



der carcinomas in BBN-induced mice and that suppression of testosterone decreases it.

Previously, the effects of testosterone on bladder carcinogenesis in animal models were considered to suppress the detoxication of BBN, induce a stimulation pathway for BBN-proximate carcinogen in the liver (Bertram and Craig, 1972) or increase cell growth in premalignant lesions of the bladder epithelium (Okajima et al., 1975). Recently, Imada et al. (1997) demonstrated the existence of androgen receptors on the bladder epithelium of mice and rats using an immunohistochemical staining. Noronha and Rao (1986) also reported that sex hormone receptors were identified in human advanced transitional cell carcinomas, and dihydrotestosterone and testosterone receptors were found more frequently than estrogen receptors. Although there is no clear evidence, testosterone may act on bladder mucosa and promote bladder carcinogenesis as it does in prostate cancer.

Previous studies have shown significant correlations between PCNA and histological stages or grades in human bladder cancer (Waldman et al., 1993; Skopeliou et al., 1997). PCNA expression in rats was also found to gradually increase as histological stages and grades advance (Yamashi, 1996). The present study yielded similar results, and our findings agreed significantly with those of the previous reports. In Group III,

Fig. 4. A Grade-3 invasive bladder carcinoma in Group III. **g:** Hematoxylin and eosin staining. **h:** Proliferating cell nuclear antigen-positive cells shown by immunohistochemical staining are significantly more stained than in Group I (Fig. 3e). **i:** Apoptotic cells are almost equal in quantity to in Group I, shown by terminal-deoxynucleotidyl-transferase (TdT)-mediated dUTP-biotin nick end labeling (Fig. 3f), × 200.

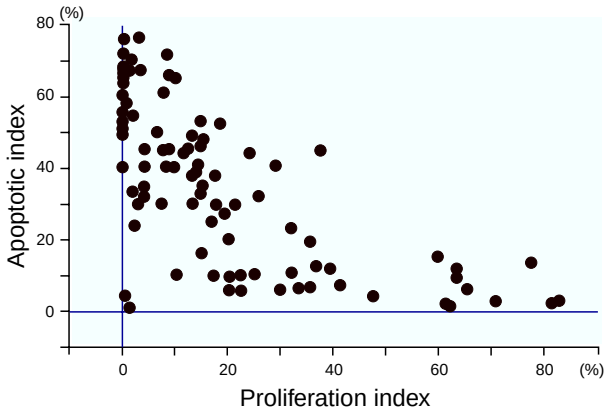


Fig. 5. Correlation between the proliferation index and the apoptotic index. A significant negative correlation is found ($r = -0.7$, $P < 0.001$, Pearson's test).

PCNA showed the highest expression, and in Group I, the lowest expression among the 3 groups. These findings support that testosterone promotes increased cell proliferation in BBN-induced mouse bladder carcinogenesis.

Apoptosis is one of the most important molecular mechanisms in the process of carcinogenesis. Originally, apoptosis is characterized by a marked reduction in cell volume and increase in density. Apoptotic bodies are histologically characterized by their small size, nuclear chromatin condensation, DNA fragmentation and compactness of cytoplasmic organelles (Kerr et al., 1972, 1994). TUNEL was introduced by Gavriel et al. (1992), and shown to be able to detect apoptosis cells in routine formalin-fixed, paraffin-embedded sections. The extent of apoptosis has been examined in several experimental tumor types (Aihara et al., 1994; Tatebe et al., 1996). The previous studies reported that the apoptotic index in bladder tumors, such as cell proliferation, indicates a positive correlation between histological stages and grades in humans (Koyuncuoglu et al., 1998) or a negative correlation in rats (Shirai et al., 1995). In the present study, the occurrence of apoptosis in a BBN-treated mouse showed a negative correlation with histological stage, grade or proliferation index. These different results may be influenced by differences in numerous oncogenes and tumor suppressor genes, including *bcl-2*, c-*myc*, *p53*, etc. among the various models.

This is the first study that indicates a correlation between the effects of testosterone in BBN-induced mouse bladder carcinogenesis and cell proliferation and apoptosis. Among the 3 groups, the proliferation index of invasive carcinomas was significantly higher in Group III and lower in Group I than in other groups; on the other hand, no significant difference was observed in apoptotic index. These results suggest that one of the effects of testosterone in BBN-induced mouse bladder carcinogenesis increases cell proliferation rather than decreasing apoptosis, and that this effect promotes mouse bladder carcinogenesis.

However, the exact mechanisms by which testosterone promotes bladder carcinogenesis are not known. Thus, it is necessary to investigate the relationship between testosterone and growth factor production in the process of bladder carcinogenesis. In the future, it might be possible to perform a chemoprevention trial on bladder cancer with hormonal therapy, provided that further investigations clarify the mechanisms related to the effects of testosterone on human bladder carcinogenesis.

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