Chemically Induced Smooth Muscle Tumors of the Mouse Urinary Bladder


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SUMMARY
Occasional submucosal tumors of indeterminate origin and without definite connection to the overlying mucosa have been reported in mice given various carcinogenic agents. Two Swiss mice fed niridazole developed such tumors. Electron microscopy of 1 of the tumors revealed thin myofilaments with oval dense bodies, marginal dense plaques, and gap junctions, identifying the tumor as originating from smooth muscle cells. No desmosomes, tonofilaments, secretory granules, striated muscle, or collagen were present in the tumor cells. Since the biology of these lesions is unknown, classification as benign or malignant cannot be made at this time. Their recognition is important in evaluating possible bladder carcinogens in mice.

INTRODUCTION
Numerous chemicals have demonstrated carcinogenic activity toward the mouse urinary bladder inducing predominantly tumors of epithelial origin (2). Occasionally, sarcomas have also been induced, usually of the spindle cell variety. Uncommonly, a histologically undifferentiated tumor is observed in the submucosa of the mouse urinary bladder without apparent connection to the overlying epithelium. Such lesions were termed “vegetative lesions” by Bonser and Jull (1). Tumors of this type developed in 2 Swiss mice given niridazole in the diet for 52 and 72 weeks. This agent, used widely in the treatment of schistosomiasis (11), has been demonstrated to promote bladder tumors in mice given various carcinogenic agents. Two Swiss mice fed niridazole developed such tumors. Electron microscopy of 1 of the tumors revealed thin myofilaments with oval dense bodies, marginal dense plaques, and gap junctions, identifying the tumor as originating from smooth muscle cells. No desmosomes, tonofilaments, secretory granules, striated muscle, or collagen were present in the tumor cells. Since the biology of these lesions is unknown, classification as benign or malignant cannot be made at this time. Their recognition is important in evaluating possible bladder carcinogens in mice.

MATERIALS AND METHODS
The 2 mice in which the submucosal tumors were induced were part of an experiment described in detail elsewhere (14), in which a diet containing niridazole was administered to 10-week-old Swiss mice for 30 weeks followed by the control diet for 6 weeks, after which the diet containing one-half of the amount of the initial dose of niridazole was fed until death of the animals. Three groups, each containing 30 female and 30 male mice, were fed niridazole at doses of 0.1, 0.05, and 0.025%, respectively. Transitional cell carcinomas with squamous metaplasia were found in 4 of 30 and 1 of 30 female mice at the 2 higher doses, respectively, but not at the lower dose or in male mice. The 2 submucosal tumors described here occurred in male mice, 1 receiving 0.05% and 1 receiving 0.025% of niridazole, which died after 72 and 52 weeks of the experiment, respectively.

At autopsy the bladders of all animals were fixed in 10% formalin and then bisected, totally embedded in paraffin, and sectioned. Histological sections were stained with hematoxylin and eosin and examined. Serial sections through 1 of the bladders containing submucosal tumor were prepared and examined. The paraffin block of the 2nd bladder was processed at St. Vincent Hospital for examination of the submucosal tumor by electron microscopy. The block was melted and the tissues were placed in 100% xylene for 2 hr. It was then placed briefly in 50% xylene/ethanol, then in 100% ethanol, and then taken through a descending ethanol series to water. It was fixed in 1% OsO$_4$ in 0.1 M sodium phosphate buffer, pH 7.4; rapidly dehydrated in an ascending ethanol series; and embedded in Epon 812. One-$\mu$m-thick sections were cut and stained with toluidine blue, and selected areas were then sectioned, stained with uranyl acetate and lead, and examined in a Zeiss EM 9A electron microscope.

RESULTS
In the hematoxylin- and eosin-stained sections, both tumors were located near the trigone of the urinary bladder and were confined entirely within the subepithelial connective tissue without evidence of mucosal or muscular invasion. Serial sections through 1 of the tumors demonstrated no connection of the tumor with the epithelium or muscular layers. The luminal surface of the bladder was slightly elevated over the tumor areas when compared with the adjoining mucosa, but the mucosa appeared normal microscopically. By light microscopy (Fig. 1) the tumors were sharply circumscribed and consisted of round to oval, bizarre, markedly pleomorphic cells with large, hyperchromatic nuclei (Fig. 2). Many nuclei had irregular shapes with numerous angulations, and large nucleoli were usually present.
Considerable eosinophilic cytoplasm was present in most cells. Numerous inflammatory cells, lymphocytes and polymorphonuclear leukocytes, were present at the deep margin of the tumor, and occasionally they were present within the stromal stroma of the tumor.

Although the tissue of the tumor examined by electron microscopy was originally fixed in formalin and embedded in paraffin, the detail seen by electron microscopy was sufficient to identify the cells. By electron microscopy, the cellular features seen by light microscopy were confirmed. The cells and nuclei were markedly pleomorphic, with the nuclei having numerous angulations and bizarre shapes, hyperchromatism, and large nucleoli. Within the cytoplasm were large amounts of rough, dilated endoplasmic reticulum, numerous free ribosomes, moderate numbers of poorly preserved mitochondria, similar in size to those in normal bladder smooth muscle, and occasional thin myofilaments approximately 50 Å wide (Fig. 3). Within some of the clusters of myofilaments were occasional oval dense bodies. Marginal dense plaques adjoining the plasmalemma were also occasionally seen, and gap junctions were present between cells (Fig. 4). In addition, within the cytoplasm of several cells were bundles of filaments with a regular periodicity of approximately 200 Å (Figs. 4 and 5). Evaluation of the tumor cells for pinocytotic vesicles and basement membranes was not possible because of the artifacts of the fixation process. Both components appeared to be focally present, but artifactual causation could not be ruled out. Tonofilaments, desmosomes, collagen, cilia, and striated myofilaments were not observed within the tumor.

DISCUSSION

Tumors of the urinary bladder in animals induced by chemicals usually originate from the transitional cell mucosal epithelium. Rarely, tumors of nonepithelial tissue also occur (2). The submucosal tumors reported in the present study are uncommon and have been described only in the mouse (G. T. Bryan, O. Yoshida, and A. M. Pammukcu, personal communications) following the administration of carcinogenic substances either p.o. or by the implantation of pellets within the bladder. The tumors that we have characterized in this report resemble those described in mice by Bonser and Jull (1) and Roe (10) as “vegetative epithelial changes”. The lesions described by these authors, except for 1 instance, occurred at suture lines in the bladder dome after implantation of pellets into the bladders. In their cases the lesions contained pleomorphic cells with rare mitoses, and they were circumscribed within the submucosa except in 1 instance. Our lesions also were composed of highly pleomorphic cells but no mitoses were observed and there was no evidence of invasion of mucosa or muscle layer. The cells of these tumors are large and range in appearance from epithelial-like to undifferentiated.

In our electron micrographs the presence of myofilaments in parallel array, occasional oval dense bodies within the clusters of myofilaments, marginal dense plaques, and gap junctions are all consistent with an origin of these tumor cells from smooth muscle (4-6, 8, 9, 12, 13). Dilated endoplasmic reticulum is not found in normal smooth muscle cells but is occasionally found in smooth muscle tumors (8). We were unable to demonstrate clearly the presence of basement membranes and pinocytotic vesicles because of the original fixation and embedding process. There was no evidence of desmosomes, tonofilaments, secretory granules, collagen, or striated skeletal muscle which would be characteristic of epithelial or other nonepithelial differentiation. The banded inclusions present in some of the cells have not been described in leiomyomas or leiomyosarcomas, but similar structures have been described in a mesocolonic leiomyoblastoma (presumably also of smooth muscle origin) in a human patient (3).

Submucosal tumors such as we described above have not been reported in mice used as controls in bladder carcinogenesis studies. Their incidence in experimental animals has been very small, and the biology of these tumors is not established. At this time classification as either benign or malignant would not be appropriate. It is important to recognize their nonepithelial nature, however, and to distinguish them from invasive carcinomas or malignant connective tissue tumors when the results of carcinogenesis studies are being tabulated. This point would be particularly significant in the testing of weak carcinogens with a low tumor yield.

REFERENCES

Fig. 1. Urinary bladder containing submucosal tumors without connection to overlying epithelium or underlying muscle layer. Arrow, moderate inflammatory cell infiltrate. H&E, × 100.

Fig. 2. Higher magnification of submucosal tumor demonstrating normal overlying epithelium and pleomorphism of tumor cells. H&E, × 400.

Fig. 3. Tumor cells containing myofilaments (arrows) with dense bodies. × 5400.

Fig. 4. Two tumor cells joined by gap junction (Arrow 1) with marginal plaques (Arrows 2) and crystalline inclusions (Arrow 3). × 19,000.

Fig. 5. Higher magnification of crystalline inclusions with periodicity of approximately 200 Å. × 56,000.
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