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Transplantation Characteristics, Morphologic Features, and Interpretation of Preputial Gland Neoplasia in the Fischer 344 Rat

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Preputial gland neoplasms in the Fischer 344 rat are relatively uncommon tumors with a prevalence of approximately 3% in the National Toxicology Program data base. They occur late in life, are well differentiated, and rarely metastasize. Based on studies through 4 serial passages, 10 well-differentiated preputial gland neoplasms transplanted into the mammary fat pads of syngeneic recipients grew to 30 mm within 10 weeks. Recipients died or were sacrificed with large transplanted tumors within 6 months. The morphologic features of the transplanted neoplasms were similar to those of the primary neoplasms through the four passages. Proliferative lesions of the preputial glands comprise a morphological continuum and separation of these growths into categories of hyperplasia, adenoma, and carcinomas is based largely on cytological features and the degree of altered growth patterns. Morphologic features to assist in diagnosis of preputial gland neoplasms and recommendations for interpreting treatment-associated increases of these neoplasms are presented.

Introduction

Certain mammals, particularly rodents, have specialized glands that secrete pheromonelike substances that are associated with sexual attraction and arousal, dominant and submissive social behavior, and territorial marking (*1–4*). Such specialized sebaceous glands include the preputial or clitoral glands in rats and mice, the flank organ of the hamster, and the ventral gland of the gerbil (*5*).

The dorsoventrally flattened, paired preputial glands of the male rat are located in the subcutis of the preputial skin on either side of the penis. They are yellowish tan to brown and may reach 15 mm in length and 5 to 8 mm in width in a sexually mature male rat. Their growth and function is under hormonal control, especially that of adrenocorticotropic hormone (ACTH) and testosterone (*6–7*).

Although a specialized type of sebaceous gland, the preputial gland is unique in that it is highly developed and has a branched duct system and glandular sebaceous glands (*8*). The ducts are lined by stratified squamous epithelium and are frequently distended by accumulated secretion. The glandular cells are arranged in acini. Each acinus is delimited by a thin basement membrane and contains many granule-filled secretory cells and a small number of reserve or basal cells with dense nuclei and condensed cytoplasm. The latter are usually flattened against the basement membrane. The acini vary from 25 to 100 μm in diameter and are separated by a small amount of collagen and occasional reticulum fibers.

The perinuclear secretory granules found in preputial gland acinar cells are single, membrane-bound primary lysosomes containing β-glucuronidase and acid phosphatase (*9*). The same hydrolases are present in the sebum of the preputial gland and the perinuclear granules are believed to be secretory lysosomes that, after discharge from the disaggregating cell, release their acid hydrolases into the sebum. The β-glucuronidase is possibly involved in the breakdown of amino sugars of mucopolysaccharides and can act on numerous mucosubstances present in the genitourinary tract such as those found in sperm acrosomes or the cervical mucus (*10*).

Inflammatory and degenerative changes are frequently observed when preputial glands are examined histologically (*11*), and adenomas of the rat preputial gland have been reported in up to 8.7% of 24- to 30-month-old Fischer 344 rats (*12*). Since most preputial gland neoplasms are well differentiated and metastases are rare, the basis for distinguishing benign and malignant categories remains uncertain. Any information relative to the biological behavior of these neoplasms should prove useful in establishing helpful diagnostic

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criteria for classifying preputial gland neoplasms based on morphologic features. The occurrence of several preputial gland neoplasms in F344 rats during the course of conducting 2-year toxicity/carcinogenicity studies afforded the opportunity to perform transplantation studies on these neoplasms. The results of those transplantation studies are presented here along with suggested histologic features, which should help in classifying proliferative lesions of the rat preputial gland.

Materials and Methods

Male Fischer 344 rats used as recipients for transplantation studies were obtained as weanlings from the Frederick Cancer Research Facility, Frederick, MD, and housed five per polycarbonate cage. Rats were quarantined a minimum of 10 days and were subsequently randomly assigned to transplant tumor lines for up to 4 months after quarantine. All rats were fed NIH-07 diet and given water *ad libitum*. Animal care and handling was done according to currently accepted guidelines (*13*).

Ten preputial gland neoplasms were obtained from terminal or moribund sacrificed rats of the carcinogenicity studies of C.I. Direct Blue 15, C.I. Acid Red 114, or 3,3′-dimethoxybenzidine sponsored by the National Toxicology Program (NTP). Selection of neoplasms for transplantation was based on the presence of sufficient viable tissue to provide material for passage and for histologic examination. Two of the preputial gland neoplasms selected for transplantation were obtained from control rats, and the remaining neoplasms were obtained from treated rats. All tumors were bisected; one-half of each tumor was preserved in formalin, and the remaining half was used for transplantation. Material for each transplant was debrided of necrotic material and cut into 2 × 2 × 10-mm strips. Strips were implanted into the left mammary fat pad of recipients using a 10-gauge Silverman bone marrow biopsy needle. Each of the original 10 preputial gland neoplasms was implanted into 10 rats (passage 1). Of those successful transplants, 2 were randomly selected for implantation into 5 recipients each (passage 2). Passages 3 and 4 were done similarly, with two previously successfully transplanted neoplasms each being implanted into 5 recipients.

Animals were observed daily for abnormal clinical signs and mortality. Body weights were recorded weekly. Implantation sites were palpated weekly, and the size of developing masses was recorded. Recipients that became moribund or developed large ulcerated neoplasms were euthanatized for humane reasons and to preclude loss from autolysis or cannibalism. Any recipient that did not have evidence of transplant growth by 6 months was euthanatized and necropsied, and the implantation site was observed grossly. Histopathologic examination was made on all original neoplasms retrospectively and on all successful transplants. In addition, the lungs, regional lymph nodes, and suspected metastases from all animals were microscopically examined.

Results

Table 1 summarizes some of the characteristics of the 10 original preputial gland neoplasms used for transplantation. All 10 primary preputial gland neoplasms were diagnosed as well or moderately well-differentiated carcinomas. Four of the original donors with preputial gland carcinomas also had mononuclear cell leukemia with infiltrates of leukemia cells in the preputial gland neoplasms.

The 10 original preputial gland carcinomas were observed grossly as swellings in the inguinal areas. As these neoplasms grew, the overlying skin ulcerated and the necrotic contents of the neoplasm exuded through the ulceration. At this time the neoplasms often became everted and assumed a verrucose pattern. Microscopically there was loss of normal architecture and a disorganized growth pattern. Frequently, the tumors consisted of sebaceous cells with foamy, pink cytoplasm admixed with smaller basophilic epithelial cells compatible with basal cells. All primary carcinomas tended to grow by expansion, with limited extension into the surrounding stroma by papillary or podlike extensions. There were no metastases observed.

Table 2 summarizes biologically relevant characteristics of the 10 transplanted preputial gland tumor lines. All 10 primary carcinomas grew in recipients through 4 successive serial passages. Only 90 recipients were used in the fourth passage because 1 subgroup of recipients each from 2 tumor lines expired from fulminating leukemia before the transplanted preputial gland tumors were large enough for passage. The rate of transplantation success was high in the first 2 passages but declined in the third and fourth passages due in large part to complications from the mononuclear cell leukemia. Leukemia was apparently introduced into recipients by leukemic cells that were present in the stroma of the original neoplastic tissue used for transplantation.

The transplants grew rapidly, became palpable in 3.3 to 4.5 weeks over the four serial passages, and reached 30 mm in greatest dimension in 7.1 to 9.7 weeks (Table

Table 1. Preputial gland neoplasms selected for transplantation.

Donor	Diagnosis	Week of appearance	Initial size, mm	Presence of leukemia at necropsy
DO-044[a]	Carcinoma	52	65	–
DO-063[a]	Carcinoma	60	68	–
DO-152	Carcinoma	56	45	–
DO-155	Carcinoma	76	45	+
DB-170	Carcinoma	60	60	+
CB-289	Carcinoma	68	37	–
AR-159	Carcinoma	52	32	+
AR-267	Carcinoma	28	28	–
AR-418	Carcinoma	56	33	+
AR-440	Carcinoma	52	38	–

[a]Untreated control.

Table 2. Transplantation characteristics of preputial gland tumors in F344 rats.

	Passage number			
	1	2	3	4
No. of tumor lines transplanted	10	10	10	10
No. of recipients	100	100	100	100
No. of recipients with growing transplants	95	96	79	64
No. of tumor lines with metastases	0	1	1	2
Mean values				
Weeks to palpable tumor[a]	3.3	3.5	3.4	4.5
Weeks to 30 mm	8.0	7.1	9.7	9.1
Weeks to death[b]	17.4	23.7	25.3	23.5

[a]Tumors ≥ 10 mm.
[b]Excluding recipients with leukemia.

2). The gross appearance was typical of preputial gland neoplasms in that the transplanted growth often rapidly ulcerated, discharged the central necrotic contents of the growing neoplasm, and continued as an everted growth. At necropsy, the transplanted neoplasms were often large, reaching an average of 50 mm in greatest dimension. The average time to death or sacrifice of recipients was 19 to 24 weeks.

Histologic features of the transplants were similar to those of the original neoplasms in each of the 10 cases. There was no tendency to become less differentiated or anaplastic over the course of the 4 serial passages. There were no morphologic or growth differences between the tumor lines originating from untreated control and dosed rats. Pulmonary metastases of transplanted neoplasms occurred in a total of 3 tumor lines (Table 2). The DO-044 tumor line had 1 animal each in the second and fourth passage with pulmonary metastases; the AR-267 line had one metastasis in the third passage; the DO-063 line had two animals with metastases in the fourth passage.

Results of a retrospective computer search of the National Toxicology Program tumor incidence data base to determine the prevalence of proliferative lesions of the preputial gland in male F344 rats are summarized in Table 3. There was a modest treatment-associated increase in preputial gland tumors in the rat in only one study (2,4-diaminoanisole sulfate; NTP Technical Report No. 84). It should be noted that in many of the early studies that comprise this data base, preputial glands were examined histologically only when grossly abnormal. Thus, the data base may underestimate lesions such as hyperplasias and small adenomas not associated with an enlargement of the preputial glands. The published technical reports from the 48 most recent National Toxicology Program 2-year toxicity/carcinogenicity studies showed that the average age at death among 53 control rats with preputial adenomas was 101 weeks, whereas the average age at death among the 76 controls with preputial carcinomas was 100 weeks. This clearly indicates that spontaneous preputial gland neoplasms in the rat are incidental neoplasms that occur late in life and/or grow slowly.

Table 3. Frequency of preputial gland proliferative lesions in the NTP data base as of August 1986.[a]

Hyperplasia	279/51230 (0.5%)
Adenoma	618/51230 (1.2%)
Carcinoma	896/51230 (1.7%)

[a]Includes treated and control male F344 rats.

Discussion

All 10 original preputial gland tumors were successfully transplanted into syngeneic hosts and grew rapidly. The morphologic features and growth characteristics of all 10 tumor lines were similar, with no apparent distinction between preputial gland carcinomas obtained from control or chemically treated rats. There was a total of 5 recipients from 3 of the tumor lines that developed pulmonary metastases, giving an overall incidence of metastasis of 5/334 (1.5%). Two of the lines that ultimately had metastases were derived from untreated control animals. The histologic appearance of the tranplants remained similar to the original well-differentiated preputial gland carcinomas, with no tendency to become anaplastic or less differentiated over the four serial passages.

Complications from leukemia inadvertently introduced into recipients via tissue implants influenced the third and fourth passages of some tumors in the present study. This phenomenon has been noted previously (*14*), and it is well established that mononuclear cell leukemia in F344 rats rapidly becomes fulminating upon serial passage (*15–16*). The aggressive growth of the leukemic cells can be contrasted with the less rapid growth of the neoplastic preputial gland cells to provide a relative measure of degree of malignancy. The leukemia clearly behaved in a more malignant fashion than did the preputial gland carcinomas upon serial transplantation.

It is unfortunate that none of the original preputial gland tumors available for the present study were adenomas; however, this was unavoidable in that the nature of each neoplasm used for transplantation was unknown at the time of transplantation. Limited transplantation studies on preputial/clitoral gland adenomas have been previously reported (*14*). Based on this previous documentation, the latency period for adenoma was approximately 17 weeks versus 3 to 5 weeks for carcinomas in the present study. Also, the adenomas reached 20 mm in approximately 22 weeks (*14*) versus the carcinomas reaching 30 mm in 7 to 9 weeks in the present study. In addition, the time-to-death for animals receiving adenoma transplants was approximately 36 weeks (*14*) versus 18 to 24 weeks for carcinomas in the present study.

Available published information relative to transplantation of leukemia and preputial gland adenomas, when contrasted to the results of the present study, indicate that the 10 preputial gland carcinomas behaved biologically as neoplasms with a low degree of malignancy. There were few metastases even after multiple

Table 4. Histologic features that help to classify proliferative lesions of the rat preputial gland.

Hyperplasia
Focal circumscribed proliferation of glandular cells, sometimes including basal cells
Minimal distortion of normal glandular structure
Acini present in lesion and frequently larger than normal
May be associated with minimal compression, particularly if the lesion contains dilated ducts
Adenoma
Lesion is generally a well-circumscribed single nodular proliferation, frequently causes compression
Some semblance of acinar and duct structures and some evidence of holocrine secretion into ducts
Acini may not be as discrete as in normal glands, vary in size, and may be spherical to elongated
Sometimes an increase in the number of basal cells in the acini
Growth is by expansion; rarely, papillary fronds extend into surrounding tissue
Carcinoma
Lesions are frequently multilobular
Although lesions may be circumscribed, there are usually areas where proliferating cells extend into the surrounding tissues
Often active growth where the neoplasm is exposed to skin or mucous membranes
Extension of the neoplasm is by relatively nonaggressive local infiltration with a minimal schirrous reaction
Moderate to extensive necrosis may be present
Acinar structure is absent; neoplastic cells are arranged in irregular masses or sheets
Usually an increase in the number of basal cells, and portions of the neoplasm may differentiate into squamous cells
Variation in nuclear size and frequent mitoses are present in some carcinomas

transplantations. We thus consider the original diagnoses of carcinomas to be supported by the transplantation study results. Based on the morphologic features of the carcinomas used in the present study, available published information relative to preputial gland neoplasia (*17*), and a retrospective review of proliferative preputial gland lesions obtained from the NTP data base, several histologic features are frequently associated with each type of proliferative preputial gland lesion. These histologic features are summarized in Table 4. It should be emphasized that both benign and malignant preputial gland proliferative lesions are comprised of well-differentiated cells and that diagnosis will depend upon the entire spectrum of changes present in each tumor and will be influenced by the experience of the pathologist making the diagnosis.

Because of the difficulty of clearly separating proliferative preputial gland lesions into diagnostic categories of hyperplasia, adenoma, and carcinoma, it is considered appropriate to combine adenomas and carcinomas for purposes of assessing carcinogenicity (*18*). The presence of hyperplasia would support the biological significance of either adenomas or carcinomas when present, as regenerative preputial gland hyperplasia does not occur as a normal mechanism of repair following damage or surgical removal of portions of this tissue (*8*).

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