Proliferative Lesions of the Prostate and Other Accessory Sex Glands in Male Rats


1NYU Medical Center, New York, NY
2Schering-Plough Research Institute, Lafayette, NJ
3Experimental Pathology Laboratories, Inc., Herndon, VA
4Nagoya City University Medical School, Nagoya, Japan
5National Cancer Institute, Frederick, MD
6Consultant Pathology Services, Flemington, NJ

INTRODUCTION

Spontaneously occurring proliferative lesions of the accessory sex glands of the male rat are infrequent, with the exception of atypical hyperplasia of the ventral prostate which has a variable incidence in many rat strains. Most macroscopic changes that could be indicative of a tumor, such as diffuse or nodular enlargements, are more likely to represent inflammation rather than proliferative lesions. Accessory sex gland tumors may be microscopic or grossly visible, sometimes they can be so large that it is difficult to determine from which accessory sex gland they originate. The main accessory sex glands in the male rat have intricate structural relationships and consist of the multilobulated prostate (including the coagulating glands), ampullary glands, and seminal vesicles (23). Other accessory sex glands, such as the bulbourethral gland and periurethral glands, are usually not examined routinely in toxicity or carcinogenicity studies and are not discussed here. The preputial glands are not accessory sex glands in a strict sense; proliferative lesions in these glands are discussed elsewhere (19, 32).

Detection of male accessory sex gland lesions is subject to several sources of potential bias. First, tissue sampling and processing may introduce inaccuracy and variation. Second, the anatomical complexity of the accessory sex glands discourages a uniform histopathological evaluation and may thereby introduce inter- and intra-observer bias. To reduce both types of bias, anatomical considerations and recommendations for standardizing sampling methods and examination of the male rat accessory sex glands are discussed first.

ANATOMICAL CONSIDERATIONS AND METHODS OF EXAMINATION

ANATOMICAL CONSIDERATIONS

The nomenclature presented here is based upon that of Jesik et al. (23) who have described in detail the anatomy and histology of the male accessory sex glands of rats. The rat prostate consists of four paired lobes (23, 27, 37): the ventral, dorsal, lateral, and anterior lobes, which are of urogenital sinus origin (37). The anterior prostate is commonly referred to as the coagulating gland and, unlike
the other prostate lobes, extends parallel along the paired seminal vesicles, which are derived from the Wolffian duct (37, 46). The dorsal and lateral prostate lobes are often referred to as the dorsolateral prostate because it is difficult and usually unnecessary to distinguish between these lobes on a routine basis. The dorsal and lateral prostate lobes are morphologically similar (23, 27) and often display a comparable spectrum of pathological changes in the aging rat (7-9, 11). Nevertheless, it is possible to grossly and microscopically distinguish the lateral from the dorsal prostate (23, 27). The ventral prostate is anatomically distinct from the dorsolateral prostate (23, 27) and shows a spectrum of lesions that may differ from that found in the dorsolateral prostate (7-9). The tissues that directly surround the prostatic urethra and prostatic utricle are structurally very complex. They consist of the ducts of all four prostate lobes and the seminal vesicles, as well as the deferent ducts and ampullary glands which are both derived from the Wolffian duct (47).

METHODS OF TISSUE SAMPLING AND TRIMMING FOR HISTOLOGIC EXAMINATION

Because of the extraordinary complexity of the male accessory sex gland structures, knowledge of their anatomical relationships and incorporation of this information in tissue sampling and processing protocols is imperative. Vigorous standardization of tissue sampling and trimming methods will consistently provide the pathologist with the same orientation of the tissue and thereby reduce inter- and intra-observer variability.

The accessory sex glands (prostate lobes, coagulating glands, and seminal vesicles) are best removed and fixed in toto, together with the urethra and urinary bladder; the bladder can be removed for separate fixation, if required. This approach interferes with weighing individual accessory sex glands. However, there is an easy and accurate way to obtain a measure of accessory sex gland weight. Following removal of these glands in toto (prior to fixation), the urinary bladder and all but the intraprostatic part of the urethra are removed and connective tissue is cleaned away. An aggregate accessory sex gland weight can then be obtained. The ventral lobes can be removed by blunt dissection for separate weighing without affecting the structural integrity of the remaining glands; however, this requires knowledge of the anatomical relations and skillful dissection. Removal of the intraprostatic urethra, on the other hand, will interfere with further histological examination of the dorsolateral prostate-ampullary gland complex. In addition, it could cause considerable spillage of seminal vesicle secretion. The latter problem also arises when the seminal vesicles and coagulating glands are dissected away before fixation and leads to high variability in seminal vesicle weights and histological appearance of the tissues.

Correct tissue trimming is critical in the microscopic evaluation of rat accessory sex glands. One such trimming method has been described previously (9, 11). Briefly, it consists of trimming off the urinary bladder, ventral prostate, and seminal vesicle/coagulating gland complexes, and cutting the dorsolateral prostate complex in halves at a right angle to the prostatic urethra. These dorsolateral prostate lobe halves are both embedded and sections will include dorsal and lateral prostate, ampullary glands, prostatic urethra, and prostatic utricle, as well as ducts of the prostate lobes, coagulating glands, and seminal vesicles; the ventral lobes are separately embedded. Alternatively, it is possible to make sections in a para-sagittal plane including dorsolateral lobes and some of the ampullary glands, and, in smaller rats, the ventral lobes; the perirethral region cannot easily be viewed in this approach. It is often neither necessary nor practical to distinguish between ventral and dorsolateral prostate on a routine basis in toxicologic pathology. Nevertheless, it is important to include both ventral and dorsolateral prostate in microscopic examination of the male genital tract, regardless of which tissue trimming method is used, so that the exact lobe localization of lesions can be determined when required. The coagulating glands and seminal vesicles are best examined together. A longitudinal section is preferable to a cross-section because there may be proximal-distal differences in the occurrence of proliferative lesions in these glands. However, one or two cross sections may be sufficient for routine examination. Vigorous standardization of tissue trimming and embedding is more important than the actual methods selected, as long as all prostate lobes (including the coagulating gland) and the seminal vesicles are included for evaluation.

MORPHOLOGY

The rat prostate has four distinct paired lobes. It is neither practical nor necessary in routine toxicologic pathology to discriminate between these lobes when recording prostate lesions, with the exception of the coagulating gland. However, when there appear to be treatment-related effects in a study, it may be important to determine the exact lobe localization of lesions. The ventral and dorsolateral prostate lobes are considered separately here because their spectrum of spontaneous and induced pathological changes differs. The coagulating gland is easily distinguishable from the other prostate lobes and is usually embedded together with the seminal vesicle. The structure of this gland and its spectrum of pathological changes is distinctly different from that of the other prostate lobes. Therefore, it is recommended that the coagulating gland be considered a separate tissue for routine evaluation.
VENTRAL PROSTATE

Hyperplasia (Figures 1-6)

There are basically three morphologically distinct types of hyperplasia that may be found in the ventral prostate of the rat—reactive hyperplasia and two types of non-reactive hyperplastic lesions (functional and atypical). Reactive hyperplasia (9, 11) occurs in association with inflammatory cell infiltrate. It consists of a simple thickening of the epithelium to two or more cell layers, but pseudoglandular structures can also be found. The hyperplastic cells may be slightly atypical but are mostly uniform. The occurrence of this lesion parallels that of prostatitis and is usually not separately recorded.

Physiologic or functional hyperplasia (9,11) can be focal, multifocal, or diffuse in the ventral prostate, and is usually found at the periphery of the lobe. It is characterized by crowded tall columnar epithelial cells and by infolding of the lining epithelium into the alveolar lumen. The cells are hyperbasophilic but are otherwise normal in morphology. The amount of intra-alveolar secretion is often decreased. In contrast to reactive or atypical hyperplasia, the epithelium is not multilayered, but the increased infolding can be mistaken for cribriform growth. Functional hyperplasia may be accompanied by diffuse enlargement of the gland, but glandular enlargement is most often the result of hypersecretion or obstruction of the outflow of secretum rather than hyperplasia. A mild degree of functional hyperplasia is frequently present in adult rats, but its presence is rarely recorded as a spontaneous finding.

Pathologic or atypical hyperplasia (9, 11, 29, 38, 50) is a focal, often multifocal, lesion that involves single or sometimes two or three adjacent alveoli; prostatic ducts are occasionally affected. It does not occur at a specific localization within the lobe. The lesion consists of epithelial proliferations that follow the alveolar lining and do not obliterate the alveolar lumen, although papillary formations can be found. The epithelium is two or more cells layers thick and a cribriform pattern is common. Normal alveolar architecture is not disturbed and there is no fibrous capsule formation or compression of surrounding tissue. The affected alveoli have a normal amount of secretion.

In comparison with normal epithelial cells, atypical cells have lost their polarity, often have an increased cytoplasmic/nuclear ratio, and may be somewhat enlarged. The cytoplasm is usually slightly eosinophilic to hypochromatic and the nucleus is usually slightly hyperchromatic, sometimes with prominent nucleoli. Cellular and nuclear pleomorphism is minimal and mitotic figures are infrequent. The earliest change is a focal loss of cellular polarity and increase in cellularity, usually in an area where the epithelial cells are columnar and somewhat hyper eosinophilic. Abrupt changes from normal to atypical hyperplastic epithelium occur as well as more gradual transitions via increasingly tall columnar epithelial cells. In the latter case, the cells have hyper eosinophilic cytoplasm and basally-located hyperchromatic nuclei, and are sometimes arranged in two or three layers. Focal areas with increased cellular atypia (dysplasia) and focal squamous metaplasia can occur within areas of atypical hyperplasia. Grading of atypical hyperplasia can be done on the basis of a combination of the number of alveoli affected and the extent of the lesions.

Squamous Metaplasia

Focal squamous metaplastic changes of the ventral prostate are rare spontaneous lesions, but may be induced by certain carcinogens (35, 36). These types of lesions consist of multilayered squamous epithelium. There is reduced or no secretion in affected alveoli. Keratinization may be present but is rare. Diffuse squamous metaplasia has not been reported to occur spontaneously.

Adenoma (Figures 7-10)

Spontaneously occurring and chemically induced adenomas of the ventral prostate are usually not grossly visible. These tumors (8, 11, 29, 38, 50) are intra-alveolar epithelial proliferations that completely or almost completely fill the lumen of one to several adjacent alveoli. Distortion of normal alveolar architecture and compression of surrounding tissue are hallmark features, but they vary widely in severity. A thin fibrous capsule can completely or partly surround the lesion, particularly in the case of large adenomas. There is sometimes extension along the epithelial lining of adjacent alveolar lumina or ducts, but the lesions are not clearly invasive. Adenomas can occur multifocally at any location in the ventral prostate lobe. The epithelial cells in adenomas are characteristically arranged in a cribriform pattern, with some occasional comedo growth patterns and solid or microglandular-tubular areas. The cells have completely lost their normal polarity and they are mostly polygonal. Basal cells are absent. The cells are often enlarged and have an increased cytoplasmic/nuclear ratio and their cytoplasm is eosinophilic in comparison with normal epithelium. Their nuclei are round to oval, often slightly hyperchromatic, mildly to moderately pleomorphic, and usually enlarged. Focal areas with increased dysplasia and some squamous metaplasia may be present, particularly in larger adenomas. A few mitotic figures, sometimes in abnormal configurations, are common. There is usually no inflammation.

There is a single report of a cystadenoma of the ventral prostate (8, 36). This tumor consists of a conglomerate of cystic spaces that is sharply demarcated with a thin fibrous capsule and compresses surrounding tissue. There are many infoldings and papillary projections into the lumen of the cysts. The epithelial cells are flat to cuboidal and have hyperchromatic cytoplasmic and nuclei.
Adenocarcinoma (Figures 11 & 12)

Adenocarcinomas of the ventral prostate (7, 11, 38, 50) are epithelial proliferations that vary in size from approximately five alveoli to the entire ventral lobe. Large adenocarcinomas often markedly distort the architecture and gross shape of the ventral prostate. Hemorrhagic areas and focal necrosis are common. Macroscopically detectable adenocarcinomas have been described in aging ACI/HapBR rats as hemorrhagic and pigmented nodular areas (49). Small adenocarcinomas may be grossly apparent. Microscopically, a distinct fibrous capsule is often present and sharply demarcates the tumor from adjacent normal tissue. Fibrous stromal septa dividing the tumor into pseudolobules are common. Invasive growth is generally limited to invasion of the tumor capsule and surrounding alveoli and stroma, whereas perineural invasion, blood vessel infiltration, and penetration of the prostatic capsule are infrequent. Cribriform, comedo, and solid growth patterns predominate. Cellular atypia is increased in comparison with adenomas and atypical hyperplasias, and it tends to increase further with increasing tumor size (49). Poorly-differentiated epithelium can be found in solid areas (38). In comparison with normal ventral prostate epithelium, the cells are often clearly pleomorphic and enlarged, and have a higher cytoplasmic/nuclear ratio, hyperchromatic and eosinophilic cytoplasm, and larger nuclei with prominent and sometimes multiple nucleoli. There are often some mitotic figures and a mixed cell inflammatory infiltrate is frequently present. Metastases from adenocarcinomas of the ventral prostate have not been reported (38, 50).

Squamous Cell Carcinoma

Squamous cell carcinomas of the ventral prostate induced by some chemical carcinogens have been described in the literature (35, 36, 39). These lesions consist of epidermoid cells invading surrounding tissue. Blood vessel penetration and metastases give additional indication of malignancy. Keratinization is sometimes present.

DORSOLATERAL PROSTATE

Hyperplasia (Figure 13)

As with the ventral prostate, three morphologically distinct types of hyperplasia may be found in the dorsolateral prostate—reactive, functional, and atypical hyperplasia. Reactive hyperplasia (9, 11) is always combined with inflammatory cell infiltrate and consists of a simple thickening of the epithelium to two or more cell layers. Sometimes pseudoglandular structures are present which may have some cellular atypia. Reactive hyperplasia is usually not recorded separately from prostatitis.

Physiologic or functional hyperplasia (9, 11) can be found focally or multifocally at the periphery of the lobe. It is difficult to detect due to the variability in the morphology of the normal dorsolateral prostate. As in the ventral lobe, it is characterized by crowded, slightly hyperbasophilic, but otherwise normal, epithelial cells that are cuboidal to columnar. It is also characterized by increased infolding of the lining epithelium into the alveolar lumen. The amount of intra-alveolar secretion is usually decreased, and the increased infolding can be mistaken for cribriform growth. A mild degree of functional hyperplasia is occasionally present in adult rats, but its presence is rarely recorded as a spontaneous finding.

Pathologic or atypical hyperplasia of the dorsolateral prostate is very rare. There is considerable variation in the appearance of these lesions (9, 11, 25, 26, 31, 43, 44). The lesion may vary from intra-alveolar microgland formation with atypical cells arranged in a single layer to multilayered areas consisting of disorderly piled-up, enlarged, hypochromatous cells with large pale nuclei, sometimes with a cribriform growth pattern. Atypical hyperplasia can occur in the alveoli as well as in the ducts. It is difficult if not impossible to distinguish between atypical hyperplasia with a distinct inflammatory component and reactive hyperplasia, which occurs frequently in the dorsolateral prostate. Atypical hyperplasia may sometimes show continuous morphological progression into early stage adenocarcinomas (11, 43, 44).

Squamous Metaplasia (Figure 14)

Focal metaplastic changes of the prostate, occasionally with some keratinization, occur in aging rats often as a reaction to either the presence of large concretions in the dorsolateral prostate ducts or inflammatory processes (11). Diffusely occurring squamous metaplasia has not been reported to occur spontaneously in the rat dorsolateral prostate. There is a report of induction of diffuse squamous metaplasia by estrogen in rat dorsolateral prostate ducts (4).

Seminal Vesicle-Like Metaplasia

Seminal vesicle-like metaplasia (11) is a change that is characterized by the replacement of the normal epithelium of the dorsolateral prostate by epithelial cells that closely resemble seminal vesicle epithelium. The cells in this lesion are columnar with somewhat elongated, strongly basophilic, basal nuclei. There is a distinct increase in the number of cells per unit length of basement membrane as compared with normal epithelium, and there often is an increase in the number of glandular infoldings. Areas of atypical hyperplasia may also occur in this lesion.

Adenoma (Figure 15)

There is a single report of an adenoma of the dorsolateral prostate (8). It is described as a microglandular-tubular proliferation that does not invade surrounding tissues. It has a pronounced fibrous capsule, abnormal alveolar architecture, and slightly compressed surround-
ing prostatic tissue. The microglands are lined with one to three layers of cells, which are columnar and have hypochromatic, basophilic cytoplasm and hypochromatic, enlarged nuclei with sometimes conspicuous nucleoli. There are no basal cells. Cribriform adenomas, which are morphologically similar to those in the ventral prostate, may also occur in the dorsolateral prostate of carcinogen-treated rats, but these are very rare (11).

**Adenocarcinoma (Figures 16 - 19)**

Adenocarcinomas of the dorsolateral prostate have a glandular growth pattern with an often abundant amount of stromal tissue (7, 11, 29, 36, 43). Cribriform and comedo growth patterns are very rare (11) and have not been reported in the literature. The glandular-type carcinomas display a considerable variation in degree of differentiation, both from tumor to tumor and within a single carcinoma. Well-differentiated carcinomas are composed of small glandular and sometimes tubular structures, consisting of a single or occasionally double layer of neoplastic epithelial cells. Less differentiated tumors have sheets, solid fields, and often cords of neoplastic cells embedded in connective tissue stroma. There are no basal cells. The neoplastic cells vary in size, cytoplasmic/nuclear ratio, and staining properties of cytoplasm and nuclei. There are often prominent nucleoli and a moderate number of mitotic figures. Signs of secretory activity can be found in well-differentiated carcinomas. A mixed cell to predominantly polymorphonuclear inflammatory infiltrate is common in these tumors, and some central necrosis may be present. These carcinomas are invasive, infiltrating blood vessels and perineural spaces and invading into adjacent normal prostate, prostatic capsule, and surrounding tissues. They have often invaded into several accessory sex gland structures, impeding determination of the exact site of origin. Adenocarcinomas often metastasize, primarily to the regional lymph nodes and lungs, but also to the liver and other tissues (15, 16, 44), indicating that both hematogenic and lymphogenic dissemination can occur. Skeletal metastases have not been described. The histogenesis of these carcinomas has not been described in detail, but they arise de novo from atypical hyperplasias rather than via an adenoma stage (7, 11, 43, 44).

**Squamous Cell Carcinoma**

There is one report of a spontaneous squamous cell carcinoma in the rat dorsolateral prostate (29). This type of carcinoma can be induced by some chemical carcinogens or hormonal treatments (21, 35, 36, 39). These lesions are characterized by epidermoid cells invading surrounding tissue. Blood vessel penetration and metastases give additional indication of malignancy. Keratinization is sometimes present.

**COAGULATING GLAND**

**Hyperplasia (Figure 20)**

Two types of hyperplasia have been described in the coagulating gland of male rats—reactive hyperplasia and atypical hyperplasia. **Reactive hyperplasia** (11) is always combined with inflammatory cell infiltrate and consists of a simple thickening of the epithelium to two or more cell layers. Sometimes pseudoglandular structures are present which may have some cellular atypia. It is usually not recorded as a separate lesion from inflammation.

Pathologic or **atypical hyperplasia** (11, 21) of the coagulating gland is rare. It occurs in areas with increased glandular infolding and consists of cells that have lost normal polarity, are slightly enlarged, and have a hypochromatic cytoplasm. The nuclei are usually hypobasophilic but occasionally are hyperbasophilic. The cells are usually not piled-up and follow the lining of the glandular infoldings, but cribriform papillary hyperplasia can also occur (11). Atypical hyperplasia may sometimes show continuous morphological progression into early stage adenocarcinomas. (11, 43, 44).

**Squamous Metaplasia (Figure 21)**

Focal squamous metaplasia, occasionally with keratinization, can occur in the coagulating gland of aging rats. Usually, it occurs in reaction to inflammation. Diffuse squamous metaplasia of the coagulating gland has not been reported to occur spontaneously but has been induced by perinatal estrogen treatment (1, 2) and estrogen administration to adult rats (12), similar to what has been described for mice (3).

**Seminal Vesicle-Like Metaplasia (Figure 22)**

Seminal vesicle-like metaplasia (11) is characterized by the replacement of the normal epithelium of the coagulating gland by epithelial cells that closely resemble or are indistinguishable from seminal vesicle epithelium. The cells in this lesion are cylindrical with somewhat elongated, strongly basophilic, basal nuclei. The number of cells per unit basement membrane is distinctly increased in comparison with normal epithelium, and the amount of glandular infolding is usually increased. Atypical hyperplasia may occur within this lesion.

**Adenoma (Figure 23)**

Induced adenomas of the coagulating gland are cribriform lesions which are morphologically similar to those occurring in the ventral prostate (11). These adenomas are not grossly visible. Demarcation, compression of surrounding tissue, disruption of normal glandular architecture, and cellular atypia within the lesion can be used as criteria to distinguish between adenoma and hyperplasia. Spontaneous adenomas of the coagulating gland have not been reported in the literature.
Adenocarcinoma (Figures 24–26)

Induced adenocarcinomas of the coagulating gland have a glandular growth pattern with often abundant scirrhous stromal tissue and desmoplastia. These tumors are morphologically similar to those found in the dorsolateral prostate (11, 21, 43, 44). Cribriform and comedo growth patterns, similar to those occurring in the ventral prostate, can also occur in the coagulating gland, but are extremely rare (11). Spontaneous coagulating gland adenocarcinomas have not been reported in the literature.

Glandular pattern tumors probably develop de novo from areas of atypical hyperplasia rather than from adenomas (11, 21, 43, 44). They invade into the coagulating gland capsule and surrounding tissues rather than into the glandular lumen. These adenocarcinomas vary considerably in degree of differentiation from well-differentiated to poorly-differentiated or anaplastic, and they can metastasize. They may develop from the glandular portion of the coagulating gland as well as from its ducts. When these tumors are larger and have invaded into other accessory sex gland structures, it may be difficult or impossible to determine the exact site of origin.

Squamous Papilloma

Squamous papillomas can occur in the ducts of the coagulating gland but are extremely rare, even in carcinogen-treated rats (11). These lesions consist of hyperplastic, keratinizing squamous epithelium arranged on stalks protruding into the lumen of the ducts. The ducts are often distended due to the presence of the papilloma and abundant keratin production.

SEMINAL VESICLE

Hyperplasia (Figures 27 & 28)

Two types of hyperplasia may be found in the seminal vesicle of the rat—reactive hyperplasia and atypical hyperplasia. Reactive hyperplasia is always combined with inflammatory cell infiltrate and often consists of a thickening of the epithelium with or without pseudoglandular structures. The hyperplastic cells are often slightly atypical. It is usually not recorded separate from vesiculitis.

Atypical hyperplasia of the seminal vesicle consists of pale cells that have lost normal cellular polarity and display moderate nuclear and cellular atypia with considerable variation in size and shape of the cells (10, 15, 18, 40, 41). The cells are disorderly arranged and are often piled-up in solid, microgland, and/or cribriform patterns. Atypical hyperplasia may sometimes show continuous morphological progression into early stage adenocarcinomas (11, 43, 44).

A variation of this focal lesion is a combination of hyperplasia and cellular hypertrophy, where the hypertrophy is more prominent than the hyperplasia, particularly in small lesions. In this variation, there is only slight cellular atypia and no pleomorphism, and the cells follow the normal glandular contours. The lesion is well-demarcated with abrupt transitions from normal to affected epithelium. It sometimes contains areas with increased cellular atypia but it has not been found to be associated with carcinoma (10, 11, 40, 41).

Squamous Metaplasia

Squamous metaplasia may be focal or diffuse. It consists of multilayered squamous epithelium, and there is reduced or no secretion in affected alveoli. Keratinization may be present, but is rare. If the lesion is focal, it is often associated with large intra-alveolar or intraductal concretions or inflammation. Focal squamous metaplasia can occur as a reaction to the presence of inflammation in the seminal vesicle. Spontaneous diffuse squamous metaplasia has not been reported, but it may be induced by estrogen in the rat seminal vesicle (4).

Adenoma

There is one report that describes the morphology of a spontaneous seminal vesicle adenoma (6, 46). This lesion consists of epithelium arranged in a papillary and glandular pattern that compresses surrounding tissue. There is some nuclear atypia and cellular crowding, but the cells otherwise closely resemble normal epithelium. Demarcation, compression of surrounding tissue, disruption of normal glandular architecture, and cellular atypia within the lesion may serve as criteria to distinguish adenoma from hyperplasia.

Adenocarcinoma (Figures 29 & 30)

Adenocarcinomas of the seminal vesicle usually have a glandular growth pattern with an abundant amount of desmoplastia of scirrhous stromal tissue. These carcinomas have a morphology similar to that found in the dorsolateral prostate, and vary considerably in degree of differentiation (10, 11, 18, 21, 43, 44). Adenocarcinomas invade into the glandular capsule and surrounding tissues but not into the glandular lumen, and they can metastasize. These tumors are more likely to develop from the glandular portion of the gland than from its ducts. When these tumors are larger and have invaded into other accessory sex gland structures, it may be difficult or impossible to determine the exact site of origin.

Squamous Papilloma

Squamous papillomas can occur in the ducts of the seminal vesicle of carcinogen-treated rats but are rare (11). These lesions consist of hyperplastic, keratinizing squamous epithelium arranged on stalks protruding into the lumen of the ducts. The ducts are often distended due to the presence of the papilloma and abundant keratin production.
AMPULLARY GLAND

Little is known about spontaneous or induced lesions in the rat ampullary gland. Spontaneously occurring neoplastic lesions of the ampullary gland have not been described, nor have they been observed in carcinogen-treated rats (11).

Hyperplasia (Figures 31 & 32)

Two types of hyperplasia have been described in the ampullary gland of male rats—reactive hyperplasia and atypical hyperplasia. Reactive hyperplasia associated with chronic inflammation probably results from sperm reflux and can ultimately develop into sperm granulomas (11, 15, 28). Atypical hyperplasia may also occur in the ampullary gland, but this lesion has only been found in carcinogen-treated rats (11). Such atypical hyperplasia of the ampullary gland consists of proliferation of small cells. Some of these cells may be similar to normal ampullary epithelium but some cellular atypia is always present. These cells are enlarged and have a pale cytoplasm and nuclei. The epithelium is thickened to up to seven cell layers and intra-epithelial microglandular formations are frequent in severe cases.

MESENCHYMAL ACCESSORY SEX GLAND TUMORS

A variety of spontaneous and chemically induced mesenchymal tumors can occur in the rat accessory sex glands: leiomyosarcoma, fibroma, fibrosarcoma, paraganglioma, neurofibroma, neurofibrosarcoma, histiocytic sarcoma, malignant fibrous histiocytoma, hibernoma, mesothelioma, undifferentiated sarcoma, and metastases such as from generalized lymphomas (11, 15, 20, 29, 45). Their morphology does not differ from similar tumors found in other organs.

DISCUSSION

Spontaneous proliferative lesions of most male accessory sex glands occur in low frequency in aging rats (11). The only gland that shows a significant and, in some rat strains, considerable incidence of proliferative changes is the ventral prostate lobe (11, 22, 38, 50). Little doubt has been expressed that adenoma of the ventral prostate develops from atypical hyperplasia and that carcinoma develops from adenoma (11, 38, 50). While it is relatively easy to make a distinction between hyperplasia, adenoma, and carcinoma in most accessory sex glands, such distinction is difficult in the ventral prostate. All stages, from very early hyperplasia to adenoma or invasive carcinoma, have been observed in aged rats without a clear separation between these lesions (11, 38, 50). This suggests that the development of carcinomas in the ventral prostate is a continuum without discrete steps. Nevertheless, with increasing size of the proliferative lesions, there is an increase in the occurrence of focal dysplastic areas and of comedo and solid growth patterns, while cellular atypia increases slightly (11, 38, 50).

Distinction between atypical hyperplasia, adenoma, and carcinoma in the ventral prostate is thus to some extent arbitrary, particularly in borderline cases. Hyperplasia of the ventral prostate has therefore sometimes been classified as early carcinoma in situ, and adenoma as advanced carcinoma in situ or early carcinoma (24, 40). These small lesions may have all the morphological characteristics of carcinoma except clear invasive growth, which might justify classifying them as early carcinoma. This may be appropriate in a basic research setting, but for the proper interpretation of long-term toxicologic studies it is essential to distinguish between atypical hyperplasia, benign tumors, and malignant tumors. Furthermore, there is no evidence that all atypical hyperplasias will eventually progress to larger and expansive tumors (adenoma), or that all adenomas will ultimately progress to more bulky and invasive neoplasms (carcinoma), or that these lesions even have the capability to do so. Therefore, it is proposed to apply a distinction between hyperplasia, adenoma, and carcinoma using standard morphological criteria that are used for other organs in rodent species until there is more insight into the biologic behavior of these proliferative lesions (5).

The proposed practical approach to distinguish adenoma from atypical hyperplasia and adenoma from carcinoma in the rat ventral prostate is presented in Table 1. A combination of several criteria listed in this table should be used to make these distinctions. It is recommended that the term adenoma be reserved for proliferations that completely obliterate the lumen of one or more alveoli, compress surrounding tissue, and disturb normal alveolar architecture. Capsule formation and the presence of comedo growth patterns provide further indications for the diagnosis of neoplasia. Invasive growth and metastases are conclusive criteria for separating adenomas from carcinomas. However, since most ventral prostate adenocarcinomas are of low grade malignancy, size may be an additional criterion. It is likely that large tumors involving more than ten alveoli have invaded through alveolar walls, although there may be no clear-cut histologic evidence for invasive growth other than fibroplasia. In addition, distinct cellular and nuclear pleomorphism and comedo growth patterns should also be present for the diagnosis of carcinoma.

Discrete precursor lesions of carcinomas of the dorsolateral prostate, coagulating gland, and seminal vesicle are not well defined because atypical hyperplasia in these structures is not as well delineated as atypical hyperplasia of the ventral prostate. Current evidence indicates that adenocarcinomas in these structures develop
de novo and do not involve a benign (adenoma) stage. Carcinomas have been observed to develop early from small areas of atypical hyperplasia in all these glands (11, 12, 43, 44). Although classification as carcinoma in situ (6, 17) is perhaps suitable for some intra-epithelial hyperplastic lesions with a high degree of atypia and pleomorphism, the use of this term is not recommended because it leads to confusion about the distinction between hyperplasia and neoplasia.

The localization and the site of origin of prostatic carcinomas is usually easy to determine for tumors of the ventral prostate. However, the site of origin of carcinomas in the dorsolateral prostate is often unclear. Tumors in this region can potentially arise from any of the following epithelial structures: dorsolateral prostate, ampullary gland, seminal vesicle, coagulating gland, the ducts of these glands and of the ventral prostate, prostatic utricle, urethra, and periurethral glands. For practical purposes, only tumors that are clearly located in a specific gland should be classified as prostatic, coagulating gland, or seminal vesicle tumors. Tumors that are larger and whose site of origin and localization is not clear could be classified as accessory sex gland tumors or as seminal vesicle/coagulating gland tumors if they only involve these glands. Very large tumors that involve other pelvic structures as well as the accessory sex glands could be classified as pelvic (cavity) tumors or accessory sex gland tumors.

Observer bias probably plays an important role in the detection of small proliferative lesions in the rat prostate and may lead to under diagnosis due to missed lesions from improper trimming. Correct tissue trimming and processing methods are critical in the microscopic evaluation of rat accessory sex glands. Regardless of the tissue trimming method used, it is important to include both the ventral and dorsolateral prostate in microscopic examination of the male genital tract of rats. This allows for the determination of the exact lobe localization of proliferative lesions when treatment-related effects are suspected.

Information about the spontaneous occurrence of proliferative lesions of the rat male accessory sex glands and about possible etiology and experimental induction of these lesions can be found elsewhere (7, 11, 13, 39). The incidence of spontaneous proliferative lesions of the ventral prostate varies widely among rat strains (7-9, 11, 22, 29, 38, 50). This may point to genetic factors being involved in their etiology (13). There may be genetically controlled differences between strains in susceptibility to background levels of environmental carcinogens, but there are also data suggesting that genetically determined high plasma testosterone levels or high testosterone/estrogen ratios are related to high risk (22). Spontaneous proliferative lesions of the dorsolateral prostate, coagulating gland, seminal vesicle, and ampullary gland are rare (11). However, the following lesions can be induced by hormones, (1, 25, 26, 30, 31, 34) chemical carcinogens, and combined treatment with these types of compounds (4, 14-18, 21, 33, 36, 40-44, 46); physiological/functional hyperplasia of ventral and dorsolateral prostate; seminal vesicle-like metaplasia of the coagulating gland and dorsolateral prostate; squamous metaplasia, atypical hyperplasia, adenoma, and carcinoma of all glands; squamous papilloma of the coagulating gland and seminal vesicle; and squamous cell carcinoma of the ventral and dorsolateral prostate (7-9, 11-13, 48). The rate of occurrence of these lesions depends in part on the rate of epithelial cell proliferation during carcinogen exposure and on the levels of circulating androgens (14, 15, 33, 36, 43).

**RECOMMENDED NOMENCLATURE AND DIAGNOSTIC CRITERIA**

**HYPERPLASIA**

**Reactive Hyperplasia**

Occurs in all accessory sex glands

1. Occurs in association with inflammatory cell infiltrate
2. Simple thickening of the epithelium to two or more cell layers
3. Pseudoglandular structures may be present
4. May have some cellular atypia
5. Usually not recorded separately from inflammation

**Functional Hyperplasia**

Occurs in ventral and dorsolateral prostate

1. Focal, multifocal, or diffuse, usually located in periphery of the gland
2. Increased infolding in alveolar lumen
3. Epithelial cell height is increased and cytoplasm is hyperbasophilic, but cells are otherwise normal
4. Epithelium is not multilayered
5. Amount of intra-alveolar secretum is often decreased

**Atypical Hyperplasia**

Occurs in all accessory sex glands

1. Is characteristically a focal or multifocal lesion occurring in single or 2-3 adjacent alveoli
2. Does not disturb normal glandular architecture and does not compress surrounding tissue
3. Atypical cells follow alveolar lining in one or more growth patterns (see 7) and they do not obliterate alveolar lumen, but papillary growth occurs
4. Atypical cells may merge abruptly with normal epithelium or merge gradually without clear demarcation
5. Cells characteristically have lost normal cellular polarity, are often enlarged, and have a pale (or in the case of the ventral prostate, pale to eosinophilic) cytoplasm and often hyperchromatic nuclei
6. Mitotic index is very low
7. Cells in the lesion are disorderly arranged; their growth pattern differs for the different accessory sex glands:
   • ventral prostate: multilayered, often cribriform pattern
   • dorsolateral prostate: multilayered intra-epithelial microgland pattern
   • coagulating gland: cells are not piled-up but follow normal alveolar contours, or in cribriform pattern (rare)
   • seminal vesicle: multilayered, solid field, intra-epithelial microgland, and/or cribriform pattern; alternatively, cells are enlarged and pale with minimal atypia, are not piled-up but follow normal glandular contours, and are sharply demarcated from normal tissue
   • ampullary gland: multilayered, intra-epithelial microgland, and/or cribriform pattern

SQUAMOUS METAPLASIA
Occurs in all accessory sex glands except the ampullary gland
1. Focal or diffuse
2. Consists of multilayered squamous epithelium
3. There may be keratinization (rare)
4. There is less than normal or no secretion left in affected alveoli
5. If focal, often associated with large intra-alveolar or intraductal concretions or inflammation

SEMINAL VESICLE-LIKE METAPLASIA
Occurs in coagulating gland and dorsolateral prostate
1. Focal lesion
2. Increased number of glandular infoldings lined with a single layer of crowding cells
3. Cells characteristically resemble seminal vesicle epithelium and are high cylindrical with basally located, oval to elongated nuclei and basophilic cytoplasm
4. Affected epithelium abruptly merges with normal epithelium
5. No compression of surrounding tissue

ADENOMA
Occurs in all accessory sex glands except the ampullary gland
1. Well-demarcated lesion that completely obliterates the lumen of one to several (<10) alveoli, compresses surrounding tissue, and distorts normal alveolar architecture
2. May have a fibrous capsule and fibrous septa
3. Cells that have lost normal polarity are disorderly arranged in one of the following growth patterns:
   • cribriform pattern with occasional comedo pattern (ventral prostate, occasionally dorsolateral prostate and coagulating gland)
   • microglandular pattern (dorsolateral prostate)
   • cystadenoma pattern (ventral prostate, rare)
4. The cells in cribriform areas are mildly pleomorphic, are enlarged with eosinophilic cytoplasm, and have round to oval, hyperchromatic nuclei; mitotic index is low
5. The cells in microglandular areas are moderately pleomorphic, are flat to cuboidal, and have pale cytoplasm and enlarged nuclei
6. Basal cells are absent

ADENOCARCINOMA
Occurs in all accessory sex glands except the ampullary gland
1. Local invasion, penetration of glandular capsule, fibroplasia, and metastasis indicate malignancy
2. The growth pattern is either predominantly cribriform or glandular
3. Cribriform adenocarcinomas (ventral prostate and rarely, coagulating gland):
   • May lack clear invasive growth and metastases; size (>10 alveoli) in combination with the presence of comedo patterns and marked cellular and nuclear pleomorphism can be used as additional diagnostic criteria
   • Have a distinct fibrous capsule with fibroplasia, some mononuclear inflammatory cell infiltrate, and a variable mitotic index
4. Glandular type adenocarcinomas (dorsolateral prostate, coagulating gland, and seminal vesicle):
   • Have variable, sometimes marked, amount of scirrhus stroma and often show fibroplasia
   • Vary in degree of differentiation from forming well-differentiated glands with low mitotic index to strands and sheets of poorly-differentiated cells with moderate mitotic activity
5. Basal cells are absent

SQUAMOUS PAPILLOMA
Occurs in ducts of coagulating gland and seminal vesicle
1. Consists of hyperplastic, keratinizing squamous epithelium arranged on stalks protruding in lumen of duct
2. Duct often distended due to presence of papilloma and abundant keratin production

SQUAMOUS CELL CARCINOMA
Occurs in ventral and dorsolateral prostate
1. Consists of epidermoid cells invading surrounding tissue
2. Blood vessel penetration and metastases give additional indication for malignancy
3. Keratinization may be present
MESENCHYMAL TUMORS
The morphology of the following tumors is similar to that found in other organs:
- Fibroma
- Hibernoma
- Histiocytic sarcoma
- Fibrosarcoma
- Leiomyosarcoma
- Mesothelioma
- Metastases of lymphoma/leukemia
- Neurofibroma
- Neurofibrosarcoma
- Paraganglioma
- Undifferentiated sarcoma

ACKNOWLEDGMENTS
This work was supported in part by the following grants to MCB: PHS Grants No. CA43151 and CA48084 from the National Cancer Institute.

Table 1
Morphologic Criteria to Distinguish Atypical Hyperplasia, Adenoma, and Adenocarcinoma of the Ventral Prostate in Male Rats*

<table>
<thead>
<tr>
<th>Morphologic Feature</th>
<th>Atypical Hyperplasia</th>
<th>Adenoma</th>
<th>Adenocarcinoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Obliterated alveolar lumen</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Distorted normal architecture</td>
<td>No</td>
<td>Yes</td>
<td>Yes (marked)</td>
</tr>
<tr>
<td>Compressed surrounding tissue</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Invasive growth</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Capsule formation</td>
<td>No</td>
<td>Sometimes</td>
<td>Often with marked fibroplasia</td>
</tr>
<tr>
<td>Growth pattern</td>
<td>Cribriform</td>
<td>Predominantly cribriform, also solid and comedo</td>
<td>Cribriform, solid, and (always) comedo</td>
</tr>
<tr>
<td>Degree of pleomorphism (atypia)</td>
<td>Mild</td>
<td>Mild to moderate</td>
<td>Mild to marked</td>
</tr>
<tr>
<td>Size</td>
<td>One to a few adjacent alveoli</td>
<td>One to several adjacent alveoli</td>
<td>Several to many adjacent alveoli</td>
</tr>
<tr>
<td>Central necrosis</td>
<td>No</td>
<td>No</td>
<td>Frequently</td>
</tr>
<tr>
<td>Inflammatory infiltrate</td>
<td>No</td>
<td>Occasionally</td>
<td>Frequently</td>
</tr>
</tbody>
</table>

*Adapted in part from Mitsumori and Elwell (29) and Bosland (7, 8, 11)
REFERENCES


**Fig. 1 -** Reactive hyperplasia, ventral prostate, 1.5 year-old Wistar (Cpb:WU) rat (H&E).

**Fig. 2 -** Functional hyperplasia, ventral prostate, 1.5 year-old Wistar (Cpb:WU) rat (H&E).

**Fig. 3 -** Small focal atypical hyperplasia, (normal epithelium at bottom of figure), ventral prostate, 2 year-old Wistar (Cpb:WU) rat; (H&E).

**Fig. 4 -** Focal atypical hyperplasia, ventral prostate, 2 year-old Wistar (Cpb:WU) rat; high power of figure 3 (H&E).
Fig. 5 - Focal atypical hyperplasia, ventral prostate, 2 year-old Wistar (Cpb:WU) rat; high power of figure 4 (H&E).

Fig. 6 - Large focal atypical hyperplasia, ventral prostate, aged ACI/SegHapBR rat. This lesion almost completely fills the alveolar lumen, while it does not distort normal architecture or compress surrounding tissue (H&E).

Fig. 7 - Small adenoma, ventral prostate, 2-year-old Wistar (Cpb:WU) rat. This lesion completely fills at least one alveolar lumen, and it slightly distorts the tissue architecture and compresses surrounding tissue (H&E).

Fig. 8 - Large adenoma, ventral prostate, aged ACI/SegHapBR rat. (H&E).
Fig. 9 - Adenoma, ventral prostate, aged ACI/SegHapBR rat; high power of figure 8 (H&E).

Fig. 10 - Cystadenoma, ventral prostate, 1.5 year-old Wistar (Cpb:WU) rat treated with a carcinogen and testosterone (H&E).

Fig. 11 - Adenocarcinoma, ventral prostate, aged ACI/SegHapBR rat (H&E).

Fig. 12 - Adenocarcinoma, ventral prostate, aged ACI/SegHapBR rat; high power of figure 11 (H&E).
Fig. 13 - Atypical hyperplasia (normal epithelium at bottom and left hand side of figure), dorsolateral prostate, 1.5 year-old Wistar (HsdCpb:WU) rat treated with a carcinogen and testosterone (H&E).

Fig. 14 - Focal squamous metaplasia (in association with an intraductal concretion), dorsolateral prostate, 2-year-old Wistar (Cpb:WU) rat (H&E).

Fig. 15 - Adenoma, dorsolateral prostate, 1.5 year-old Wistar (HsdCpb:WU) rat treated with a carcinogen and testosterone (H&E).

Fig. 16 - Multiple adenocarcinoma closely associated with area of atypical hyperplasia, 1.5 year-old Wistar (Cpb:WU) rat treated with a carcinogen and testosterone (H&E).
**Fig. 17** - Small adenocarcinoma, dorsolateral prostate duct, 1.5 year-old Noble (NBL/Cr) rat treated with testosterone and estradiol-17β (H&E).

**Fig. 18** - Adenocarcinoma, dorsolateral prostate region (exact origin of this large tumor is not clear), 1.5 year-old Wistar (Cpb:WU) rat treated with a carcinogen and testosterone (H&E).

**Fig. 19** - Adenocarcinoma, dorsolateral prostate, 1.5 year-old Wistar (Cpb:WU) rat treated with a carcinogen and testosterone; high power of the tumor depicted in figure 11 (H&E).

**Fig. 20** - Atypical hyperplasia, coagulating gland, 1.5 year-old Wistar (HsdCpbLWU) rat treated with a carcinogen and testosterone (H&E).
Fig. 21 - Diffuse squamous metaplasia, coagulating gland. 1 year-old Wistar (HsdCpb:WU) rat treated with estradiol-17β (H&E).

Fig. 22 - Seminal vesicle-like metaplasia, coagulating gland. 1.5 year-old Wistar (HsdCpb:WU) rat treated with a carcinogen and testosterone (H&E).

Fig. 23 - Adenoma, coagulating gland. 1.5 year-old Wistar (HsdCpb:WU) rat treated with a carcinogen and testosterone (H&E).

Fig. 24 - Multiple adenocarcinomas closely associated with areas of atypical hyperplasia. 1.5 year-old Wistar (HsdCpb:WU) rat treated with a carcinogen and testosterone (H&E).
Fig. 25 - Adenocarcinoma (and atypical hyperplasia), coagulating gland, 1.5 year-old Wistar (HsdCpb:WU) rat treated with a carcinogen and testosterone (H&E).

Fig. 26 - Adenocarcinoma with areas of squamous differentiation, coagulating gland, 1.5 year-old Wistar (HsdCpb:WU) rat treated with a carcinogen and testosterone (H&E).

Fig. 27 - Atypical hyperplasia, seminal vesicle, 1.5 year-old Wistar (HsdCpb:WU) rat treated with a carcinogen and testosterone (H&E).

Fig. 28 - Atypical hyperplasia, (combined with hypertrophy), seminal vesicle, 1.5 year-old Wistar (HsdCpb:WU) rat treated with a carcinogen and testosterone (H&E).
Fig. 29 - Adenocarcinoma, seminal vesicle, 1.5 year-old Wistar (HsdCpb:WU) rat treated with a carcinogen and testosterone (H&E).

Fig. 30 - Small adenocarcinoma and atypical hyperplasia, seminal vesicle, 1.5 year-old Wistar (HsdCpb:WU) rat treated with a carcinogen and testosterone (H&E).

Fig. 31 - Reactive hyperplasia in association with the presence of sperm, ampullary gland, 1.5 year-old Wistar (HsdCpb:WU) rat (H&E).

Fig. 32 - Atypical hyperplasia, ampullary gland, 1.5 year-old Wistar (HsdCpb:WU) rat treated with a carcinogen and testosterone (H&E).