Prostate Pathology of Genetically Engineered Mice: Definitions and Classification. The Consensus Report from the Bar Harbor Meeting of the Mouse Models of Human Cancer Consortium Prostate Pathology Committee

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Abstract

The Pathological Classification of Prostate Lesions in Genetically Engineered Mice (GEM) is the result of a directive from the National Cancer Institute Mouse Models of Human Cancer Consortium Prostate Steering Committee to provide a hierarchical taxonomy of disorders of the mouse prostate to facilitate classification of existing and newly created mouse models and the translation to human prostate pathology. The proposed Bar Harbor Classification system is the culmination of three meetings and workshops attended by various members of the Prostate Pathology Committee of the Mouse Models of Human Cancer Consortium. A 2-day Pathology Workshop was held at The Jackson Laboratory in Bar Harbor, Maine, in October 2001, in which study sets of 93 slides from 22 GEM models were provided to individual panel members. The comparison of mouse and human prostate anatomy and disease demonstrates significant differences and considerable similarities that bear on the interpretation of the origin and natural history of their diseases. The recommended classification of mouse prostate pathology is hierarchical, and includes developmental, inflammatory, benign proliferative, and neoplastic disorders. Among the neoplastic disorders, preinvasive, microinvasive, and poorly differentiated neoplasms received the most attention. Specific criteria were recommended and will be discussed. Transitions between neoplastic states were of particular concern. Preinvasive neoplasias of the mouse prostate were recognized as focal, atypical, and progressive lesions. These lesions were designated as mouse prostatic intraepithelial neoplasia (mPIN). Some atypical lesions were identified in mouse models without evidence of progression to malignancy. The panel recommended that mPIN lesions not be given histological grades, but that mPIN be further classified as to the absence or presence of documented associated progression to invasive carcinoma. Criteria for recognizing microinvasion, for classification of invasive gland-forming adenocarcinomas, and for characterizing poorly differentiated tumors, including neuroendocrine carcinomas, were developed and are discussed. The uniform application of defined terminology is essential for correlating results between different laboratories and models. It is recommended that investigators use the Bar Harbor Classification system when characterizing new GEM models or when conducting experimental interventions that may alter the phenotype or natural history of lesion progression in existing models.

Introduction and Objectives

The increased generation of potential models of prostate neoplasia in genetically engineered mice (GEM) and their use in investigations of possible cancer therapies in prostate carcinoma (Pca) mandate the development of a standardized pathology classification scheme. Because mice and other rodents do not spontaneously develop Pca, histological criteria have been developed based on the disorders observed in newly created GEM models and by efforts to translate these lesions to the familiar histopathology of human Pca and its precursor lesions. Because the goal of the Mouse Models of Human Cancer Consortium (MMHCC) is to model human neoplasia, use of criteria and terminology applied to human prostate pathology is logical. However, as detailed herein, there are anatomical and natural history issues that impact on the ability to make straightforward analogies between GEM models of Pca and the human disease being modeled. Furthermore, in addition to pathological criteria, other criteria that can be incorporated into characterization and validation of GEM models include genetic and other molecular alterations and the natural history of the prostate lesions, and the similarity of these aspects to human Pca.

GEM models will be useful for delineating novel causative molecular alterations in the development and/or progression of Pca and useful in testing interventions that will translate to treatments in human Pca patients if such models are similar, at least in some regards, to this heterogeneous human neoplasia at initiating or secondary molecular alterations. Because histopathologic features are a phenotypic consequence of these underlying molecular alterations, pathology assessment will be useful for characterizing new models and for detecting potentially meaningful changes as a consequence of genetic crosses or therapeutic interventions.

Protocols for proper tissue submission are necessary for characterizing the pathology of the prostate and other organs in new GEM models. Tissue-based analysis of biological parameters including proliferation, apoptosis, and microvessel density can contribute to model characterization and provide mechanistic insight into effects of genetic manipulations and therapeutic interventions.

Hence, the specific objectives of the MMHCC Prostate Pathology Committee to facilitate characterization and application of GEM models of prostatic disease were as follows: (a) development of a classification scheme for disorders of the prostate and related organs in GEM; (b) provision of histopathologic definitions for these disorders; (c) collection and annotation of images illustrating these disorders; and (d) collection, organization, and distribution.
of pathology protocols useful in characterization of prostate disorders in GEM.

Because GEM are being used to model human neoplasms for investigational purposes, the pathological classifications of the disorders in various organ sites in GEM are intended to facilitate translation of GEM research to critical issues in understanding the causes and discovering more effective treatments for human malignancies. For GEM models to have application to specific or broad subsets of human Pca patients, it is fundamental to understand GEM prostate pathology in the context of human prostate pathology. This is a driving principle in the development of the classification scheme presented herein. Knowledge of the basic anatomical and histological similarities and differences between the mouse and human prostate is necessary. As such, this report includes considerations of prostate anatomy, and clinical and pathological features of the full spectrum of both benign and malignant human prostate disorders, similarities to which have been or can potentially be encountered in GEM models. Each section for the classification scheme is in general divided into definition of that disorder, criteria for recognition of the disorder in the GEM prostate, clinical considerations and morphological features in human prostate pathology, and the pathological and biological features of that entity in GEM models.

The images illustrating the various lesions in the mouse pathology classification were taken from the slides of the models provided to the MMHCC Prostate Pathology Committee (Table 1) or additional materials made available to the authors. Where possible, multiple models are illustrated for a specific lesion, to emphasize the common features of the disorder and to allow visualization of the process against different backgrounds, and so forth. The inclusion of a model or the reference to a model regarding a specific lesion is not intended as a potential endorsement or criticism of that specific model. Similarly, the illustration of a specific lesion in a single slide is not intended to be taken as a generalization regarding the natural history of that model. Characterization of GEM models is an integrated endeavor incorporating pathology, natural history, and molecular characterizations. This classification scheme and accompanying images illustrate how pathology characterization can be applied to existing models, and, hence, provides guidelines for characterization of future models as well.

A brief protocols section is included, primarily to address issues of tissue submission and immunohistochemical assays to support model classification or utilization.

General Considerations

Several general principles regarding characterization of GEM models for Pca were elaborated at the Bar Harbor Pathology Workshop (Table 2).

Table 1 Genetically engineered mouse (GEM) models of prostatic neoplasia reviewed by the Bar Harbor Pathology Workshop

<table>
<thead>
<tr>
<th>Category</th>
<th>Model</th>
<th>Transgene or knockout</th>
<th>Background</th>
<th>Promoter or selectivity of knockout</th>
<th>Reference</th>
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<td>SV40 early region</td>
<td>C57Bl/6 x FVB</td>
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<td>C57Bl/6</td>
<td>Short PB</td>
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<td>CD1</td>
<td>Long PB</td>
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<tr>
<td></td>
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<td>SV40 early region p27 knockout</td>
<td>FVB</td>
<td>CR2 Genomic knockout, heterozygous</td>
<td>(33) 12</td>
</tr>
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</table>

Growth factors/signal pathways

| MT-TGFlα                        | Rat TGFα                     | B6D2F1                | MT                 | 13                     |
| PB-ras                          | H-ras                        | B6D2F1                | PB                 | 14                     |
| PB-FFG8b                        | FGF, isofrom b               | B6D2F1                | ARR-PB             | (102) (103)            |
| MT-DNIIR                        | TGFβRI/II dominant-negative  | B6D2F1                | MT                 | 15                     |

Receptors

| PTEN +/−                        | PTEN knockout                | Balb/c x 129         | Genomic knockout, heterozygous | 16                     |
| PTEN +/−                        | PTEN knockout                | SV1129                | Genomic knockout, heterozygous | (35) (12)             |
| Men1 TSM-1                      | Men1 knockout                | NIH Black Swiss       | Genomic knockout, heterozygous | (104)                 |

Homeobox genes

| Nkx 3.1 +/−                     | Nkx 3.1 knockout             | 129/SvEvTacFBR        | Genomic knockout, homozogous     | (54)                 |
| Bigenics                        | LPB-Tag 12T7f                | See above             | (105)                |
| MT-DNIIR                        | See above                    | (17)                  |
| MT-TGFflαα × MT-DNIIR           | See above                    | (17)                  |
| Nkx +/− × Pten +/−              | See above                    | (35) (12)             |
| Nkx +/− × Pten +/−              | See above                    | (12)                  |
| Pb-ras +/− × mxz1 +/−           | See above                    | (106) (14)            |

11 R. Herbert, unpublished observations.
12 C. Abate-Shen, unpublished observations.
13 R. J. Coffey, unpublished observations.
14 N. Schreiber-Agus, unpublished observations.
15 H. Moses, unpublished observations.
16 H. Wu, unpublished observations.
17 S. Cutler and R. J. Coffey, unpublished observations.
Lesions in Mice

The low incidence of spontaneous pathological lesions in the wild-type mouse prostate was emphasized. This is true not only for neoplastic proliferations, but also for non-neoplastic disorders, and includes aged mice and mice of different genetic backgrounds. In a recent survey of 612 control B6C3F1 mice for 2-year toxicology and carcinogenicity studies conducted in the National Toxicology Program, not a single example of a spontaneous carcinoma was observed in the prostate of these wild-type mice, with fairly uniform sampling of anterior prostates (APs), dorsolateral prostates (DLPs), and ventral prostates (VPs; Ref. 1). Epithelial hyperplasia was rare, noted in 0.7% of VPs, and 0.9% of APs and DLPs (1). Adenocarcinoma of the seminal vesicles was noted in 2 mice (0.3%). Other pathological diagnoses were also uncommon in 2-year-old control B6C3F1 mice. Whereas lymphocytic infiltration in the prostate was fairly common (~30% of DLPs and VPs, and 20% of APs), more pronounced degrees of inflammation, including neutrophilic infiltrates within prostate acini that may constitute a designation of prostatitis, were uncommon (~1, 3, and 5% for AP, VP, and DLP, respectively; Ref. 1). Atrophy was noted in only the AP of 2 of 612 mice and possible mucinous metaplasia in only 1 DLP (1).

The low incidence of pathology in the prostate of wild-type mice suggests that any of the lesions described in GEM, including inflammatory and other non-neoplastic disorders, could be a consequence of the genetic manipulation involved. These lesions could be due to systemic effects (e.g., immunological or endocrinologic effects) or be a direct consequence of a genetic alteration in the prostate. Some differences in the frequency of spontaneous lesions could also exist between different genetic strains of mice. These factors should be borne in mind when evaluating mild phenotypes occurring in a small percentage of examined animals.

Importance of Adequate Controls and of Blinded Histopathologic Assessment

The characterization of new GEM models should include comparison with appropriate controls, particularly aged-matched mice of identical genetic background. The importance of aged-matched control mice is especially true for aged GEM mice and those with only mild phenotypes. Blinded histopathologic analysis is highly desirable in all of the studies that use histopathology as an end point. Blinded histopathologic analysis for data collection can be performed in a blinded fashion after initial review of possible pathology in GEM mice. Characterization of GEM models for Pca should, when at all possible, include the analysis by an experienced pathologist, and preferably one with experience in both human and mouse prostate pathology. Members of the MMHCC Prostate Pathology Committee are available for review of pathology material generated by investigators engaged in GEM research, both within and outside the MMHCC. Centralized review of particularly promising models, including in future Pathology Workshops, is desirable.

Importance of Genetic Background

The genetic background could have modifying influences on lesion development and/or progression in GEM models of prostate disorders. Some examples of the possible influence of different genetic backgrounds on neoplastic progression have already been observed (2, 3). Therefore, great care must be taken in describing the genetic background and breeding strategies involved in the creation of new GEM models and in the production of mice obtained from other laboratories.

The Role of Natural History in Model Characterization

The importance of the natural history of neoplastic progression was stressed as fundamental to the characterization of any given model. The time course of lesion development and progression, and any defined accompanying molecular alterations are important characteristics of a model. Individual GEM models may show different histopathologic features at different ages, and a careful description of the frequency of specific lesions at specific time points is vital. These may be the attributes used to validate a model for relevance to a particular aspect of the biology of human Pca.

Certain clinically relevant general features of the natural history of human Pca are well known. These features, such as development of invasive gland-forming cancer from in situ precursor lesions, progression to locally advanced disease, metastases to lymph nodes and bone, and progression to hormone refractory disease, may be desirable in a mouse model. It is unlikely that any given model will faithfully mimic these general attributes. Accurate descriptions of the temporal progression of histopathologic alterations and of the molecular changes detected with progression are important parameters of model characterization. Identification of molecular alterations in GEM models that are already known in some human Pcas will identify models that may be useful for testing targeted therapeutic strategies. Therefore, the histopathology, natural history, and accompanying molecular and genetic alterations will be part of the information available on National Cancer Institute websites for GEM models of Pca.

Anatomical Considerations

Anatomy of the Human Prostate and the Zonal Origin of Pca

Some anatomical similarities between the mouse and human prostate help support the application of GEM models for the study of molecular alterations that accompany the development and progression of Pca. Both species have male accessory organs that develop from the Wolffian ducts and the urogenital sinuses. Both species have androgen-sensitive organs and form lobular glands that have a similar triad of distinctly differentiated epithelial cells and similar functions. However, there are also some crucial differences between the prostatic glands in the two species. These include differences in the gross and microanatomy that have implications for pathological interpretation in mouse models and for the use of the mouse for modeling some clinicopathologic characteristics of human Pca.
Although distinct lobes can be recognized in the developing human prostate, the adult human prostate is not divided into discrete lobar structures. The past use of terms, such as lateral and posterior “lobes” has been supplanted by the term “zones” based on the concept of specific zones within the human prostate. These zones are anatomically recognizable, have characteristic histological features, and, importantly, have specific predisposition to benign or malignant neoplastic diseases. As described by McNeal (4–6), the human prostate is composed of the anterior fibromuscular stroma, the periurethral transition zone (TZ), the peripheral zone (PZ), and the central zone (CZ; Fig. 1). The TZ is particularly associated with benign prostatic hyperplasia (BPH) and the PZ with Pca. The TZ surrounds the ejaculatory ducts (Fig. 1C) and comprises an increasing portion of the prostate from where the ejaculatory ducts enter the urethra, near the prostate urethral at the verumontanum, to the base. The CZ glands have characteristic morphology, with large complex glands showing a more irregular luminal border, with epithelial tufting, papillary formations, and frequent Roman arches or even cribriforming (Fig. 1, C–E). The histological characteristics of the CZ and its spatial relationship to the ejaculatory ducts have lead to a suggested origin from the Wolffian duct (7). However, there is currently insufficient data to support a Wolffian duct rather than urogenital sinus origin of the CZ. The CZ is rarely the site of origin for Pca, although it can be secondarily involved by extension from a PZ tumor.

The TZ is located interiorly between the urethra and the surrounding PZ and CZ. In the young postpubertal adult, architectural and histological differences in the glands of the TZ and the PZ are not well defined. Therefore, the morphological distinction between TZ and PZ is made primarily by the “after the fact” involvement of the TZ by BPH (Fig. 1, A, B, H, and J). The TZ is the exclusive site of BPH in the human (Fig. 1, A and H; Fig. 4). BPH histological alterations in the TZ are increasingly common with age in the human prostate, and are present in as many as 80–90% of radical prostatectomy (RP) specimens (removed for Pca; Ref. 6). In contrast, only ~20% of clinically significant Pcas originate in the TZ (5, 8, 9). TZ tumors often have characteristic, if not specific, histology (Refs. 10, 11; Fig. 1, H and I), and may arise as a result of genetic alterations and precursor lesions differing from those Pcas occurring in the PZ (12). In contrast to its common occurrence in the PZ, prostatic intraepithelial neoplasia (PIN), including high-grade PIN (HGPIN), is rarely seen in the TZ. Rather, a lesion referred to as atypical adenomatous hyperplasia (or adenosis) is thought to be a precursor lesion for usual TZ tumors (12). TZ tumors are often composed extensively of low grade Gleason pattern 2 carcinoma (Fig. 1, H and I). Hence, many TZ tumors detected clinically are Gleason score 4 or 5, although TZ tumors not uncommonly contain higher-grade foci. TZ tumors detected clinically appear to have a better prognosis than clinically detected PZ tumors (13, 14).

The PZ contains ~75% of the glandular tissue in the normal human prostate and is the most frequent site of Pca origin (8–10). The PZ is located particularly on the posterior and lateral aspects of the prostate (Fig. 1, A, B, H, and J). This area includes most palpable tumors (i.e., clinical stage T2 versus T1c) and is located in the PZ (14, 15) and why transrectal biopsies are typically targeted to the PZ rather than the TZ. The PZ origin of most Pcas also dictates important anatomical relationships for prostate capsule penetration, or extracapsular extension (ECE), by Pca. In the human prostate, the glands are surrounded by a prominent stroma of contractile spindle cells and collagen (6, 16). This fibromuscular stroma, which is much more abundant in the human compared with the rodent, extends beyond the outer perimeter of the glands and forms a more or less distinct “capsule,” separating the prostate from periprostatic fat. The capsule is best defined histologically in the posterior and lateral portions of the human prostate (Fig. 1, H and J; Refs. 6, 16). Standard pathological staging of RP specimens addresses the absence or presence of ECE (i.e., stage pT2 or pT3 tumors, respectively), a major prognostically significant cutoff for increased risk of progression after surgical treatment (8, 9, 17, 18). Nerve bundles, which facilitate ECE, are located particularly in the posterolateral aspect of the gland, with the largest nerve plexus at the base and a smaller one at the apex (5). The typical site of ECE is, thus, at the posterolateral aspect of the human prostate gland, particularly at the base, which is also a common route for invasion of seminal vesicles, which are at the superior posterior aspect of the prostate (5, 8, 9).

The PZ is also the predominant, essentially exclusive, site of PIN in the human prostate (8, 9, 12, 18). Epithelial hyperplasia analogous to that seen in TZ BPH does not occur in the PZ. Instead epithelial proliferation occurs within the confines of pre-existing normal gland profiles, and is designated as low- or high-grade PIN based predominantly on nuclear features as described below (12, 19).

**Histology and Phenotype of Human Prostate Glands**

In the human prostate, benign glands are composed of a basal epithelial cell layer and differentiated secretory luminal cells (i.e., two cell types), with some immunophenotypically defined transitional or intermediate forms and a small subpopulation of cells showing neuroendocrine (NE) differentiation (6, 20, 21). Benign glands are larger than typical cancer glands, and have an undulating or slightly tufting luminal contour due in part to stratification or pseudostratification of secretory cells and the mechanisms of cellular secretion (Refs. 6, 22; Fig. 1F). Basal cells in benign human prostate glands are the dividing or progenitor cell (a subset of which may be the true prostatic “stem cells”), giving rise to the differentiated secretory cells lining the gland lumens and, most likely, to NE cells as well (21, 23). Basal cells tend to be oriented parallel to the basement membrane, are not always conspicuous by light microscopy, and can be difficult to distinguish from underlying spindle stromal cells (6). They are routinely recognized by immunostaining with antibodies to high molecular weight cytokeratin (HMWCK; Fig. 1G). Malignant prostate glands do not possess such a basal cell layer, with the atypical cells presumably representing aberrantly differentiated or neoplastic counterparts of secretory cells. Pragmatically, the absence of an immunophenotypically defined basal cell layer is a useful adjunct for recognizing malignant glands and distinguishing them (particularly in biopsies) from small gland profiles of benign glands or certain well-described mimics of Pca, such as atrophy, partial atrophy, and atypical adenomatous hyperplasia (adenosis; Refs. 8, 9, 18, 24–26).

**Anatomy and Histology of the Mouse Prostate**

In contrast to the human, the rodent prostate is divided into anatomically distinct lobes. The mouse prostate can be separated into the AP or coagulating gland, the VP, and dorsal and lateral lobes, often grouped together as the DLP (Figs. 2 and 12; Ref. 27). The lobes are generally invested by a thin mesothelial-lined capsule that separates the various lobes from each other. This capsule may not always be appreciated grossly, but can often be seen in microscopic sections. The individual mouse prostate lobes are composed of a series of branching ducts or tubules that end blindly (Fig. 2). The glandular prostate is separated from the mesothelial-lined capsule by various amounts of loose fibro adipose connective tissue that contains the major vascular channels, nerves, and ganglia. The individual ductules making up each lobe of the mouse prostate are surrounded by a thin fibromuscular tunica that is composed of only a few layers of bland spindle cells that are smooth muscle actin immunopositive and interspersed in eosinophilic collagen (Fig. 2, A–F). The abundant intervening dense fibromuscular stroma surrounding the glands and their immediate stroma of adjacent “lobules” found in the human prostate is not present in the mouse (compare to Fig. 1, A, B, H, and J; Ref. 16). Hence, there are clear, fundamental differences in the
glands typically exhibit a clear to granular, faintly eosinophilic cytoplasm, which is variably disrupted at the luminal border due to ongoing apocrine-type secretion. Microscopy. Secretory cells may be variably stratified or pseudostratified but lack features of cytologic atypia that are characteristic of prostatic intraepithelial neoplasia. Secretory cells in benign distinct cell layers, the basal cells and the differentiated luminal secretory cells. Basal cells are not always discernible or distinguishable from adjacent underlying stromal cells by light microscopy. Secretory cells may be variably stratified or pseud stratified but lack features of cytologic atypia that are characteristic of prostatic intraepithelial neoplasia. Secretory cells in benign glands typically exhibit a clear to granular, faintly eosinophilic cytoplasm, which is variably disrupted at the luminal border due to ongoing apocrine-type secretion. Intermediate magnification of HMWCK immunostaining (CK 903) of benign prostate glands in radical prostatectomy specimen. The basal cell layer is circumferentially intact in multiple, adjacent gland profiles. Basal cell hyperplasia is evident focally (arrowhead). H and I, typical human TZ tumor. H, whole mount section, in which TZ and PZ are easily identified due to the expansion of the TZ by BPH nodules (arrowheads) composed of hyperplastic glandular and stromal elements. Tumor (+), a Gleason score 2 + 3 = 5 carcinoma, is outlined by ink dots and is clearly located within the TZ and extending into the anterior aspect of the prostate (black arrow). Urethra and periurethral region where prostatic ducts enter, shown by white arrow at level of verumontanum. I, high power photomicrograph, showing a common TZ tumor morphology, corresponding to Gleason pattern 2. Tumor is composed of intermediate to large glands, with ample, fairly clear cytoplasm. Nuclei are basally located and some are pyknotic. Scattered large more vesicular nuclei with prominent nucleoli were also present confirming the carcinoma diagnosis. Occasional intra luminal dense pink secretions (more typical of carcinoma than benign glands) are noted (arrowheads). J and K, typical human PZ tumor. J, whole mount section showing outlined PZ tumor (+), a Gleason score 3 + 4 = 7 carcinoma, in right posterior lateral aspect of the gland. Expansion of the TZ by nodules of glandular and stromal hyperplasia (BPH changes, arrowheads) is evident. Note the extension of the PZ laterally (arrow). K, intermediate power photomicrograph of tumor in J, showing stromal invasion by discrete, well-formed glands (arrow) in a Gleason pattern 3 component and the transition to a higher grade Gleason pattern 4 focus (+), where more solid-like growth of fused glands is evident. Note occasional crystalloids and dense pink secretions within lumens of carcinoma glands (arrowheads).

Fig. 1. Gross and microscopic anatomy, and zone of origin of prostate adenocarcinoma in the human prostate. A, gross photograph showing a cross-section of a prostatectomy specimen in which the transition zone (TZ) is markedly expanded by fleshy nodules of benign prostatic hyperplasia (BPH; black arrowheads). The TZ is demarcated from the posteri orly and laterally located peripheral zone (PZ) by fibrous tissue (black arrow) compressed by the expanding TZ. Medially, the urethra (white arrows) is slit-like due to compression by the BPH-expanded TZ. Lateral aspects of the PZ are indicated (white arrowheads). B, gross photograph showing a cross-section of a prostatectomy specimen in which the TZ and PZ appear somewhat spongy due, in part, to dilated, atrophic glands. Note the homogenous, tan-gray tumor nodule in right posterolateral PZ (arrowhead). This is a common area of involvement for usual PZ tumors. A tumor in this posterior location would likely be palpable as a discrete, firm nodule on digital rectal examination. Prostate carcinoma is not typically discernible grossly, especially with smaller tumors detected by PSA screening (urethra, arrow). C–E, low, intermediate, and high magnification photomicrographs of normal central zone (CZ) glands in a prostatectomy specimen. C, the CZ surrounds the ejaculatory ducts (arrowhead), which penetrate the prostate parenchyma and empty into the urethra at the verumontanum, a raised posterior ridge at approximately the junction of the mid and apical third of the prostate. The CZ is located in the posterior medial aspects of the prostate and occupies more tissue toward base. D and E, CZ glands are larger in diameter than usual PZ glands and have more irregular luminal contours due to papillary infoldings. Roman arches (arrowheads), imparting a cribriform architecture (arrowheads), are common in CZ glands. However, normal CZ glands lack cytologic atypia, a feature that helps to distinguish them from prostatic intraepithelial neoplasia on transrectal biopsy. F, high magnification of normal benign PZ glands in radical prostatectomy specimen. Compared with usual acinar prostate carcinoma, benign glands are larger and have a tufted or undulating luminal border. Benign glands have two distinct cell layers, the basal cells and the differentiated luminal secretory cells. Basal cells are not always discernible or distinguishable from adjacent underlying stromal cells by light microscopy. Secretory cells may be variably stratified or pseud stratified but lack features of cytologic atypia that are characteristic of prostatic intraepithelial neoplasia. Secretory cells in benign glands typically exhibit a clear to granular, faintly eosinophilic cytoplasm, which is variably disrupted at the luminal border due to ongoing apocrine-type secretion. G, intermediate magnification of HMWCK immunostaining (CK 903) of benign prostate glands in radical prostatectomy specimen. The basal cell layer is circumferentially intact in multiple, adjacent gland profiles. Basal cell hyperplasia is evident focally (arrowhead). H and I, typical human TZ tumor. H, whole mount section, in which TZ and PZ are easily identified due to the expansion of the TZ by BPH nodules (arrowheads) composed of hyperplastic glandular and stromal elements. Tumor (+), a Gleason score 2 + 3 = 5 carcinoma, is outlined by ink dots and is clearly located within the TZ and extending into the anterior aspect of the prostate (black arrow). Urethra and periurethral region where prostatic ducts enter, shown by white arrow at level of verumontanum. I, high power photomicrograph, showing a common TZ tumor morphology, corresponding to Gleason pattern 2. Tumor is composed of intermediate to large glands, with ample, fairly clear cytoplasm. Nuclei are basally located and some are pyknotic. Scattered large more vesicular nuclei with prominent nucleoli were also present confirming the carcinoma diagnosis. Occasional intra luminal dense pink secretions (more typical of carcinoma than benign glands) are noted (arrowheads). J and K, typical human PZ tumor. J, whole mount section showing outlined PZ tumor (+), a Gleason score 3 + 4 = 7 carcinoma, in right posterior lateral aspect of the gland. Expansion of the TZ by nodules of glandular and stromal hyperplasia (BPH changes, arrowheads) is evident. Note the extension of the PZ laterally (arrow). K, intermediate power photomicrograph of tumor in J, showing stromal invasion by discrete, well-formed glands (arrow) in a Gleason pattern 3 component and the transition to a higher grade Gleason pattern 4 focus (+), where more solid-like growth of fused glands is evident. Note occasional crystalloids and dense pink secretions within lumens of carcinoma glands (arrowheads).
anatomical organization of the prostate between the human and mouse. These different anatomical features also create potential differences regarding the biology of neoplastic extension outside of the prostate in the human (e.g., amount of stroma, location of nerves, presence of a "capsule" between prostate and periprostatic fat, seminal vesicle proximity to sites commonly involved by ECE in the human). Therefore, it was the opinion of the Bar Harbor Pathology Panel that mouse models may not be adequate or suited to address these particular clinicopathologic or staging issues related to ECE in human Pca.

The anatomically distinct lobes of the mouse prostate have distinctive histology and biochemistry. The mouse DP is lined by simple columnar and occasionally slightly stratified and tufting epithelium (Fig. 2, B and C). The moderate degree of infolding is intermediate between the AP and the flatter luminal borders of the LP and VP. The secretory cells of the DP have lightly eosinophilic granular cytoplasm, and the central to basally located small uniform nuclei contain inconspicuous or small nucleoli. Gland lumens contain homogenous eosinophilic secretions (Fig. 2C). The LP has flatter luminal edges, with only sparse infoldings, with the abundant luminal space containing more particulate eosinophilic secretions (Fig. 2B). The epithelium is cuboidal to low columnar, with more clear to lightly granular cytoplasm and small uniform basally located nuclei. The mouse DLP has sometimes been stated to be the most homologous to the human DZ (16, 28–30). The embryologic development of the mouse prostate has been examined in detail and reviewed previously (27–29). The specific developing lobes identifiable in the embryo remain recognizable in the postnatal and adult mouse prostate, as the lobes described above. However, these developing lobes are recognizable as such in the human only in the embryo, but not in the adult (28).

It was, therefore, the consensus opinion of the Bar Harbor Pathology Panel that there is no existing supporting evidence for a direct relationship between the specific mouse prostate lobes and the specific zones in the human prostate. It is possible in the human prostate that the zones described above are contributed to by more than one embryologically recognizable "lobe." Until the relationships of mouse prostate lobes and human prostate zones can be more precisely defined, no data currently exists that would defend an *a priori* conclusion that one lobe of the mouse prostate is more relevant to human Pca than another lobe.

The mouse AP is closely apposed to the seminal vesicles, along its entire curving length (1, 16, 27, 31). Histologically, it normally demonstrates a more papillary and cribriform growth pattern than the other lobes, with cuboidal to columnar epithelial cells containing typically central nuclei with inconspicuous to small nucleoli, and eosinophilic granular cytoplasm. The gland lumens contain abundant slightly eosinophilic secretions (Fig. 2A). Despite the complex growth pattern of the epithelium in the AP and its close spatial relationship to the Wolffian duct-derived seminal vesicles, the mouse AP is clearly derived from the urogenital sinus (27). The mouse VP has flatter luminal edges and only focal epithelial tufting or in-folding (Fig. 2, D and E). The abundant luminal spaces typically contain homogenous pale serous secretions. The nuclei are small, uniform, typically basally located, and have inconspicuous to small nucleoli (Fig. 2E).

The glands of each of the mouse prostate lobes appear to have normal cell populations homologous to the human prostate, including luminal secretory cells, a basal cell layer, and a minor population of NE cells. In the mouse, as in normal human prostate glands, a basal cell layer is not conspicuous by routine light microscopy, and ultrastructural studies had reported previously the lack of a continuous basal cell layer in normal mouse prostate glands (32). Antibodies to HMWCK (66 kDa and 57 kDa), which identify the basal cell layer in benign human glands, had been reported to not identify a similar phenotypic basal cell layer in normal mouse glands (33). However, a more recent study using a rabbit polyclonal antibody to mouse cytokeratin (CK) 5 showed staining of a basal cell layer in histologically normal prostate glands (Ref. 34; Fig. 2F). Similar results have been...
achieved with antibodies to CK14 (35), and even with a murine antibody against human HMWCK that is commonly used in human prostate pathology (36). Whether decreased immunostaining for HMWCK will be observed in PIN lesions in GEM models (34) and/or absence of immunostaining for HMWCK will have diagnostic utility in recognizing invasive adenocarcinoma in GEM models as in human Pca remains to be thoroughly addressed. Limited markers exist for secretory cell differentiation in the mouse prostate, such as antibodies to DLP protein (37). Immunostaining for chromogranin (CG) demonstrates a very minor population of immunophenotypically defined NE cells in the normal mouse prostate (33, 38). Such cells appear to represent <0.3% of the normal mouse prostate cell population.

**Ampullary Glands**

The ampullary glands in the mouse are androgen-dependent glandular outpouchings of the proximal ductus deferens, with one gland on each side. The single proximal ducts enter into the ductus deferens proximal to the seminal vesicles. The ampullary glands are of Wolffian duct origin, in contrast to the urogenital sinus origin of the prostate, and add secretions to the semen that contribute to fertility (39). They have no known human counterpart. However, familiarity with their gross and microscopic anatomy is important for the proper interpretation of lesions that may be noted in the male accessory glands of GEM (40). Although they may be separately dissected to facilitate their identification, they can also be identified by their characteristic location and their characteristic secretions when seen in sections of male reproductive organs submitted en bloc as described in the protocols section below (e.g., see Fig. 12). The epithelium is simple columnar and in comparison to the prostate ducts, is surrounded by a denser fibromuscular stroma. The secretions have a characteristic “swiss cheese” appearance, with holes noted in the dense eosinophilic secretions. Enlargement with epithelial hyperplasia was noted in the ampullary glands (and other Wolffian duct-derived tissues, such as seminal vesicle), but not the prostate lobes, in mice overexpressing int2/Fgf-3 under the control of the mouse mammary tumor virus long terminal repeat (40).

**Bulbourethral Glands**

As with the prostate, the bulbourethral glands (BUGs) and periurethral glands are androgen regulated derivatives of the urogenital sinus. The BUGs in the male mouse are analogous to Cowper’s glands in human males, which are located subjacent to the urethra in the suburolithal connective tissue at the membranous portion of the urethra, immediately distal to the prostate apex. Cowper’s glands can occasionally be seen in apical portions of RP specimens and are rarely sampled “accidentally” in transrectal biopsies, where they may cause diagnostic confusion. In the human, Cowper’s glands are rarely the site of origin of carcinoma. As some strategies for targeting transgenes to the mouse prostate have also resulted in transgene expression and methods to discriminate these tissues can express prostate-specific antigen (PSA). In the mouse, the periurethral glands are located in the suburolithal tissue distal to the portions of the urethra that have the openings of the prostate ducts (more proximal than the BUGs). They are not routinely dissected from the prostate and other male accessory tissues grossly, but are commonly seen in “en bloc” sections (e.g., see Fig. 12 in the “Protocols” section) or in sections of remaining tissue (including urethra, and proximal SV and prostate ducts) submitted after dissection and separate submission of individual prostate lobes.

The periurethral glands are composed of lobules of acini and short excretory ducts that open into the urethra (e.g., see Fig. 11). In wild-type mice, the acinar epithelium is cuboidal with oval nuclei and a denser eosinophilic granular cytoplasm compared with secretory cells of the BUGs (41). A variable outpouching, called the urethral diverticulum, is occasionally found in some mouse strains. It is lined by a urethral mucosa with associated periurethral glands. Unaware pathologists may confuse this diverticulum with the BUG, because it is frequently at the same anatomical level as the BUG, or with an abnormal urethra.

**The Bar Harbor Pathology Workshop: Materials and Methods**

The Pathological Classification of Prostate Lesions in GEM is the result of a directive from the MMHCC Prostate Steering Committee to provide a specific hierarchical taxonomy of disorders of the mouse prostate to facilitate classification of existing and newly created mouse models and their translation to human prostate pathology. The classification system described herein is the culmination of three meetings and workshops attended by various members of the Prostate Pathology Committee of the MMHCC. In April 2001, an initial 2-day meeting was held at Vanderbilt University Medical Center (organized by S. B. S. and attended by S. B. S., R. L. R., R. H., N. R., R. B., J. M. W., and R. D. C.), in which models were presented, slides were reviewed, and approaches to classification of mouse prostate disorders were discussed. These processes were continued and a hierarchical taxonomy of mouse prostate diseases was drafted with annotated images at the 2-day Pathology Committee meetings (attended by S. B. S., G. V. T., R. L. R., N. R., J. M. W., and R. D. C.) accompanying the MMHCC Steering Committee meeting in San Francisco in July 2001. Finally, a formal 2-day Pathology Workshop was held at The Jackson Laboratory in Bar Harbor, Maine, in October 2001, preceding the National Cancer Institute MMHCC-sponsored Conference on Modeling Human Pca in Mice at The Jackson Laboratory on October 18–21, 2001. This session was organized by S. B. S. and attended by members of the MMHCC Prostate Pathology Committee (S. B. S., G. V. T., R. L. R., R. H., J. M. W., and R. D. C.), a representative of The Jackson Laboratory (J. P. S.), and three invited “outside” prostate pathology experts (M. M. I., M. A. R., and P. A. H.), who were chosen by the Prostate Pathology Committee Chairman on the basis of their well-recognized expertise in human prostate pathology, their research interests in Pca, and their experience with characterization and utilization of GEM models of Pca. The combined efforts leading to the Bar Harbor Classification represent a balanced effort of investigative human (M.D. and/or M.D./Ph.D.) pathologists (S. B. S., R. L. R., G. V. T., M. M. I., M. A. R., P. A. H., and R. D. C.) and veterinary (D.V.M. and/or D.V.M./Ph.D.) pathologists (R. H., J. P. S., N. R., and J. M. W.), typically with specific research interests in Pca and in studies using GEM.

For the Bar Harbor meeting, paraffin blocks and/or glass slides from 24

19 P. A. Humphrey, unpublished observations.

18 R. D. Cardiff, unpublished observations.
models of GEM were made available through generous donation by investigators from across the country. A complete set of these slides was brought to the Bar Harbor meeting, and slides and images derived from them were shared with all of the pathologists. Provided paraffin blocks were sectioned, and H&E stained before hand and supplemented with sets of H&E-stained slides from individual investigators to allow for the creation of personal study sets of individual slides from 22 of these models (Table 1). Some models included different ages of mice and/or different tissues, such that study sets of 93 slides from these 22 models were provided to the 10 panelists at the Bar Harbor meeting. All of these slides were reviewed in detail at the meeting and the slide sets were retained by the individual pathologists for later review.

S. B. S. organized the meetings with input from R. D. C. S. B. S. prepared the entire text of this article, incorporating ideas and comments made at the time of the meeting and subsequently by the Panel members. The classification scheme was generated by combined opinions of all of the participants, and all of the listed authors were valuable contributors to the material of this report.

The photomicrographs in Figs. 1–11 were prepared from images of the slide sets of the Bar Harbor meeting obtained with a Nikon Professional Digital SLR D1 camera, resolution 2012 × 1324 pixels and 12 bits per color, attached to an Olympus BX50 5-headed microscope with U-PLAN objectives. Images were captured using Nikon Capture software and processed in Photoshop, with final figures generated as Jpegs at 200 d.p.i. In figure legends, images referred to as low, intermediate, and high magnification typically represent original magnifications of ×40, ×100, and ×400, respectively.

Pathological Classification of Disorders of the Prostate in GEM (The Bar Harbor Classification: Table 3)

Disorders of Development

Agenesis/Aplasia and Hypoplasia (Fig. 3)

Definitions

Agenesis [1]. Agenesis (aplasia) is a disorder of development in which there is the absence of an organ due to the lack of formation and development of its primordium in the embryo.

Hypoplasia [2]. Hypoplasia is a disorder of development that differs from aplasia in that the organ is not completely absent, but there is inadequate development or underdevelopment of the organ.

Criteria/Explanation. Agenesis is characterized by the lack of any identifiable prostate lobes on gross examination at necropsy. It should be confirmed by the absence of histologically recognizable prostate in appropriate microscopic sections. In hypoplasia, the prostate should be small for age and may or may not have associated histological abnormalities (Fig. 3A).

Discussion. Absence of the prostate should be distinguished from a small prostate due to developmental reasons, that is hypoplasia, and both disorders should be distinguished from secondary regression, as in atrophy. Developmental abnormalities of the prostate may be associated with more widespread abnormalities of the genitourinary tract and/or other organ systems. In humans, the prostate is not appropriately developed in patients with disorders of androgen synthesis or signaling. The prostate is small in patients who have autosomal recessive deficiency of type II 5α-reductase (42), due to absent or inadequate formation within the prostate of dihydrotestosterone from circulating testosterone. Dihydrotestosterone is necessary for normal prostate development as well as the development of BPH. 5α-Reductase occurs in two different isoyme forms, type 1 and type 2. Although individual studies differ slightly regarding tissue specific expression, the prostate predominantly expresses type 2, whereas type 1 is expressed primarily in liver and skin (43).

Examples of abnormal prostate development have been reported in GEM, including as part of a constellation of more widespread abnormalities. Regarding androgen metabolism defects, 5α-reductase type 2 knockout mice have abnormal development of the prostate, similar to human patients with autosomal recessive 5α-reductase type 2 deficiency, whereas 5α-reductase type 1 knockout mice have an apparently normal prostate phenotype (43). Examination of the 10% of p57Kip2-deficient mice that survived beyond...
weaning showed “immaturity” of the prostate (most likely compatible with hypoplasia), as well as of the seminal vesicles and testes (44).

p63 knockout mice also have abnormal prostate development. p63 is selectively expressed in the basal cell layer of a variety of epithelial tissues, including human and mouse prostate (45). In contrast, in human Pca, p63 immunostaining is absent (46), similar to the “loss” of a basal cell layer in malignant prostate glands as determined by immunostaining for HMWCK. p63 knockout (p63−/−) mice die at birth and show severe defects in the development of multiple epithelial organs (47). Histological analysis of the periurethral region in day
1 p63 −/− male mice demonstrated that the prostate does not develop in these animals (agenesis; Ref. 46).

Homeobox (HOX) genes are important in prostate development and cell determination within the developing male genitourinary tract (29, 48), and HOX genes have been implicated in the development and progression of human Pca (49, 50). Hoxd-13 is expressed in mesenchyme and epithelium of the lower genitourinary tract in the perinatal period, including focally in the budding nascent ducts of the developing mouse prostate (51). Transgenic Hoxd-13-deficient mice have multiple abnormalities in development of the male accessory glands, including agenesis of the BUGS, diminished seminal vesicle luminal folding, and decreased size and ductal branching in the DP and VP (hypoplasia; Ref. 51). Hoxa-13 is also widely expressed in the developing lower genitourinary tract. In animals with a spontaneously occurring heterogeneous mutation involving one Hoxa-13 allele, there is decreased size and branching of the DLP and VP (hypoplasia), as well as abnormal seminal vesicle morphology (52). GEM mutants for both Hoxb-13 and Hoxd-13 show hypoplasia of the VP duct tips, and in Hoxb-13 mutants, the VP epithelium is composed of simple cuboidal rather than tall columnar cells, and expression of VP-specific secretory proteins is lost (53). In mice homozygous for a null mutation in the androgen-regulated murine Nkx3.1 homeobox domain, Bhatia-Gaur et al. (54) reported abnormalities in prostate ductal morphogenesis and reduced development of secretory differentiation in both the prostate and BUGs, compatible with hypoplasia. PIN-like lesions are also described in this mouse (54).

**Metaplasia (Fig. 3)**

**Definition.** Metaplasia [3]. Metaplasia is the replacement of one adult cell type (epithelial or mesenchymal) by another adult cell type.

**Criteria/Explanation.** Prostatic epithelial metaplasia is recognized histologically by the replacement of normal prostatic epithelium (basal and luminal secretory cells) by non-neoplastic transitional (urothelial), squamous, or mucinous epithelium, with cytologic features similar to other tissues in which these epithelia are found. The histological type of metaplasia observed in GEM should be specified. Although regarded as non-neoplastic, if such metaplasia develops nuclear atypia (potentially constituting dysplasia), it too should be noted and described.

**Discussion.** In the human prostate, well-recognized forms of metaplasia include transitional (urothelial) metaplasia, mucinous metaplasia, and squamous metaplasia. In the human prostate, in addition to the prostatic urethra, urothelial or transitional epithelium typically extends a variable length along primary periurethral ducts (Fig. 3B). Urothelial metaplasia is often seen admixed with more typical secretory cells for variable lengths along secondary periurethral ducts and is not uncommonly seen in prostaticctomy specimens or biopsies to involve even more distal PZ glands (6). Transitional metaplasia is commonly observed after antiandrogen therapy (6, 8, 9). Transitional or urothelial metaplasia is recognized by its resemblance to normal bladder urothelium (Fig. 3C), and its cells commonly exhibit focal nuclear grooves characteristic of urothelial cells. Also, similar to normal urothelium, it stains strongly and uniformly with antibodies to HMWCK, such that this property cannot be used to distinguish basal cell hyperplasia and transitional metaplasia. Indeed the two may be seen together, for example, in response to antiandrogen therapy. The prostatic urethra not uncommonly shows squamous metaplasia, possibly in response to chronic irritation, and squamous metaplasia can be seen focally in more peripheral regions of the human prostate and adjacent to infarcts involving BPH nodules in the TZ (6). Squamous metaplasia can occur more extensively in the prostate in response to antiandrogen treatment or with estrogen treatment for Pca (6), and is in fact a well-documented response to estrogens in a variety of species, including in rodents. Mucinous metaplasia, in which prostate secretory cells show cytoplasmic mucin, often with basilar displacement of the nucleus similar to intestinal goblet cells, is usually a very focal process (6).

Metaplasia is rarely noted in wild-type mouse prostates. In a survey of 2-year-old B6C3F1 mice, a form of possible mucinous metaplasia (with less pronounced goblet cells compared with GEM examples described herein) was noted in only 1 of 612 mice (1). Metaplasia in the prostate of GEM may arise in response to similar initiating stimuli as described above for human prostate or may more uniquely arise as a consequence of the specific genetic manipulation. Foci compatible with transitional metaplasia have been observed in the partially atrophic or regressing PIN-like lesions in faster growing lines of LPB-large T-antigen (Tag) mice after castration (Refs. 29, 55; Fig. 3D). Intestinal or adenomatous metaplasia was noted in GEM created with H-ras expressed in the prostate with a probasin promoter (Pb-ras +/+), with or without crossing to mxil −/− mice (Fig. 3E).

**Atrophy (Fig. 3)**

**Definition.** Atrophy [4]. Atrophy is an adaptive or secondary response in a previously normally developed organ that is characterized by shrinkage due to loss of cellular substance, leading to diminished tissue or organ size if sufficient numbers of cells are involved.

**Criteria/Explanation.** Grossly, the presence of atrophy may be reflected by smaller prostatic lobes (compared with age-matched wild-type mice if due to genetic manipulation or compared with control mice if due to treatment effect). Histologically, atrophy is manifested by shrunken or dilated glands lined by epithelium with less secretory cytoplasm.

**Discussion.** In GEM, a smaller prostate due to atrophy needs to be distinguished from hypoplasia. In addition to the documentation of normal prostate development (e.g., at earlier time points or before an experimental manipulation) the atrophic prostate should contain an essentially normal number of ductular and glandular structures, whereas these should be perceptively reduced in hypoplasia.

Atrophy is a common “spontaneous” histological alteration in the human prostate, in which shrunken or dilated glands are lined by a flattened epithelial lining. In the TZ (e.g., in BPH nodules) and occasionally in the PZ, it can take the form of cystically dilated glands imparting a swiss cheese appearance. In the PZ, atrophy is more often seen as angulated dark-appearing glands in which a lobular configuration is maintained, and the flat luminal lining gives a high nuclear:cytoplasmic ratio and, hence, a hyperchromatic appearance (Fig. 3F). A possibly related entity is postatrophic hyperplasia, recognized histologically as a somewhat lobular configuration of small acini surrounding a larger, more angulated gland with flatter lining cells (56). The cells in the small gland profiles have more cytoplasm than usual atrophy and can have prominent nuclei, especially when associated with admixed inflammation, leading to diagnostic difficulties regarding distinction from Pca (57). A possible relationship to inflammation has been suggested for usual forms of atrophy, and possible preneoplastic potential has been suggested for inflammation-associated atrophy (so-called proliferative inflammatory atrophy) and postatrophic hyperplasia (18, 58, 59). Cell proliferation rates are actually higher in these “usual” forms of atrophy and postatrophic hyperplasia compared with normal benign glands (60). In contrast, atrophy seen with hormone deprivation in humans may be more of an “active” involution, possibly involving apoptosis.

In contrast to its common occurrence in prostates of middle aged and older humans, spontaneous atrophy in the wild-type mouse pros-

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22 S. B. Shappell, unpublished observations.

23 R. D. Cardiff, N. Schreiber-Agus, unpublished observations.
tate is apparently extremely uncommon. In the survey of control 2-year-old B6C3F1 mice, atrophy was noted only in the AP of 2 of 612 mice and ~1% of seminal vesicles (1). Atrophy, with dilated gland profiles and flattened epithelium, typically associated with prominent inflammation, has been observed in castrated GEM compared with the PIN lesions in intact similarly aged mice from these models (Refs. 29, 55, 61; Fig. 3I). Of note, HGPIN in the human also appears to be an androgen-responsive lesion (62).

**Inflammatory Disorders**

**Prostatitis, Active or Chronic Active (Fig. 3)**

**Definition.** Active prostatitis (also referred to as chronic active prostatitis or chronic prostatitis) is an inflammatory process characterized by infiltration of inflammatory cells into prostatic stroma and glandular elements.

**Criteria/Explanation.** Prostatitis is recognized histologically by the presence of neutrophils and/or mononuclear inflammatory cells within the prostatic glandular epithelium and/or gland lumens, not simply by the presence of increased inflammatory cells (including lymphocytes) within prostatic stroma. Reactive epithelial proliferation secondary to inflammation, which can have mild cytologic atypia, should be described and the lesion classified as inflammation (prostatitis). Active prostatitis can be secondary to infectious etiologies or occur without documentable infection.

**Discussion.** In the human prostate, the presence of neutrophils within prostatic parenchyma is not necessarily indicative of an acute process, and typically reflects an ongoing (“active”) more chronic process. This is the rationale for the application of the terms active or chronic active for this entity in both the human and mouse prostate. When the term prostatitis is used without additional qualification, it is regarded as synonymous with active or chronic active prostatitis.

Clinicopathologic entities involving prostatic inflammation or related disorders in the human include acute prostatitis, chronic bacterial prostatitis, chronic abacterial prostatitis, and prostatodynia (63). These disorders are typically diagnosed by clinical features and laboratory procedures, although the histology when seen has characteristic features (6, 8, 63). Chronic prostatitis is common and is usually caused by organisms typically associated with urinary tract infections. Histological features of chronic active prostatitis are not uncommonly seen in transrectal biopsy (performed for abnormal digital rectal examination or elevated serum PSA), and may or may not correlate with symptoms.

Importantly, chronic prostatitis is not diagnosed in human or mouse by just the presence of possibly increased lymphocytes within the prostate stroma. In the human prostate, lymphocytes, including small lymphoid aggregates, are common in the prostate stroma. Similarly, in mouse prostates, scattered lymphocytes in the stroma and small lymphoid aggregates in the fibromuscular stroma or surrounding loose connective tissue can be observed. In the pathology survey of 2-year-old control B6C3F1 mice, such lymphocytic infiltration in the prostatic stroma was noted in ~30% of DLPs and VPs, and 20% of APs (1). Although this feature can be assessed in GEM (as it could be variable depending on genetic manipulation), it should not be classified as chronic prostatitis or active prostatitis. In the mouse prostate, chronic active prostatitis is characterized by neutrophils and monocytes (not readily distinguishable from lymphocytes in routine histology sections) within the prostatic epithelium and gland lumens (Fig. 3G). This type of active inflammation, including with neutrophils and cellular debris within gland lumens, was noted in only approximately 3–5% of DLPs and VPs, and 1% of APs in 2-year-old control wild-type B6C3F1 mice (1). As in the human prostate, inflammation in the mouse prostate can be accompanied by reactive epithelial proliferation and nuclear atypia, including the presence of nucleoli, although such alterations are expected to be relatively mild (1). Such lesions in the GEM prostate should be classified as inflammatory, with specification of reactive atypia, rather than proliferative, as in hyperplasia or PIN. Although the distinction could at times be difficult, attention should be given to areas relatively free or devoid of inflammation, where recognition of epithelial hyperplasia can be made with more confidence.

In addition to its importance in producing symptoms, recent attention has begun to focus on inflammation-associated atrophy as a predisposing condition or precursor for Pca in the human (18, 58, 59, 64). Although little attention has focused thus far on inflammatory disorders in the GEM prostate, similar inflammatory infiltrates can occur in the GEM prostate (Fig. 3I). Regarding possible etiologies of active inflammation in the GEM prostate, urinary stasis complicating obstruction due to large size of the prostate as a consequence of the genetic manipulation can likely contribute to and be complicated by infection. Consideration should also be given to modulation of the immune or inflammatory response as a consequence of nonprostate selective genetic manipulations.

**Granulomatous Prostatitis**

**Definition.** Granulomatous prostatitis [6]. Granulomatous prostatitis is a type of chronic prostatic inflammation, characterized microscopically by a dominant component of granulomas or cell types characteristic of granulomas, that is macrophages (histiocytes or tissue macrophages).

**Granuloma** [7]. A granuloma is a focal lesion composed of circumscribed accumulations of histiocytes/macrophages, often with multinucleated giant cells.

**Criteria/Explanation.** Granulomatous prostatitis is recognized when the inflammatory process within the prostatic parenchyma has an extensive component of granulomatous inflammation as defined above. In the prostate, such granulomas may be centered around disrupted glandular elements or within the stroma and may be accompanied by central necrosis or be non-necrotizing. With less histologically defined granulomas, granulomatous prostatitis is recognized by the presence of a prominent infiltrate of histiocytes/macrophages, typically with abundant eosinophilic cytoplasm (“epithelioid histiocytes”). These cells are admixed with other inflammatory cells, including within the epithelial compartment, such that focal areas may resemble chronic active prostatitis as defined above. Granulomatous prostatitis may be due to infectious or noninfectious etiologies.

**Discussion.** So-called “nonspecific” granulomatous prostatitis is the most common form of “noninfectious” granulomatous prostatitis in humans, being present in up to 1% of transrectal biopsies (6). It is likely due to inflammatory reaction to prostatic secretions, potentially complicating intraprostatic duct obstruction and acinar rupture. Histologically, it is characterized by a mixed inflammatory infiltrate, without well-formed granulomas or giant cells, but with sheet-like growth of histiocytes (macrophages; Fig. 3H). Admixed eosinophils are an important diagnostic feature (6). The most common form of infectious granulomatous prostatitis in humans is an iatrogenic condition, caused by the instillation of *Mycobacterium Bacillus Calmette-Guérin* for the treatment of urinary bladder carcinoma in situ or superficially invasive bladder cancer. Necrotizing or non-necrotizing granulomatous inflammation can be seen in the human prostate as a part of systemic infections with fungal organisms or tuberculosis (6). Fungal or other infectious etiologies could in theory lead to granulomatous inflammation in the prostate of GEM models, especially if the genetic manipulation resulted in immunocompromise.
Abscess

**Definition. Abscess** [8]. An abscess is a focal area of acute inflammation and tissue destruction (necrosis). It differs from active prostatitis by the presence of neutrophils as the vastly predominant inflammatory cell type, the markedly greater extent/density of the inflammatory infiltrate, and the presence of associated tissue destruction. It is essentially always caused by infection, particularly bacterial and fungal.

**Criteria/Explanation.** Abscess is recognized by sheet-like infiltration of neutrophils, with a cavity formed in areas of frank tissue destruction. It may be surrounded by a wall of granulation tissue, with prominent small capillary formation, and may resolve by leaving a cavity surrounded by fibrosis.

**Discussion.** With the availability of antibiotic treatment, abscess is rare in the human prostate (6). It can be seen as a complication of acute prostatitis, potentially associated with urinary obstruction and infection with coliforms, or due to hematogenous seeding from another source, usually associated with staphylococcal infection. It is more likely to occur in the setting of immunocompromise. If identified in the prostate of GEM, local or systemic infection should be suspected. Frequent occurrence could also accompany immunosuppression due to the specific genetic manipulation involved.

Modifiers of Inflammation

**Coagulative Necrosis**

**Definition. Coagulative Necrosis** [9]. Coagulative necrosis is a type of cell death due to hypoxia or toxins identified by loss of nuclear detail with retention of cell outlines.

**Criteria/Explanation.** In the Bar Harbor Classification, necrosis is essentially synonymous with coagulative necrosis. Coagulative necrosis is a form of tissue necrosis in which the basic outline of the cells and tissues is maintained. Coagulative necrosis may occur in a manner potentially liberating cellular contents, and typically involving multiple cells and potentially large areas of contiguous cells within a tissue, and associated with an inflammatory response at the periphery. It is quite characteristic of hypoxic cell death (infarction). Necrosis (coagulative necrosis) may be manifested by soft, friable tissue grossly. It is confirmed microscopically by confluent areas with loss of nuclear staining, with cell and tissue outlines still evident as eosinophilic in H&E-stained sections.

**Discussion.** Necrosis in the Bar Harbor Classification is distinguished from cell death due to inflammatory injury in prostatitis and possibly associated with infection, which is properly classified as a form of prostatitis or abscess as described above. In any organ, coagulative necrosis is often seen as a consequence of hypoxic cell death, as in infarction. It is seen in the human prostate, for example, when BPH nodules undergo infarction, where coagulative necrosis may be surrounded by atypical reactive epithelium and squamous metaplasia (6). It has been seen either focally or more extensively in the prostate and seminal vesicles in occasional older transgenic animals with markedly enlarged prostates and subsequently engorged seminal vesicles,13 which is likely due to an ischemic process.

Apoptosis

**Definition. Apoptosis** [10]. Apoptosis or programmed cell death is a form of cell death triggered by various signals, which initiate a cascade of intracellular events identified morphologically by a fragmentation of the nuclear chromatin and karyolysis of the cytoplasm.

**Criteria/Explanation.** Generally apoptotic cells can be recognized by rounded red cytoplasm and pyknotic, fragmented nuclear chromatin. In addition to its usual microscopic appearance, it can be identified and even quantitated using ancillary techniques, such as terminal deoxynucleotidyl transferase-mediated nick end labeling assays and immunohistochemistry for enzymes activated as part of the cell death program, described elsewhere.

**Discussion.** Atrophy of the prostate after castration is in part the result of apoptosis of the epithelium. In several GEM models of prostatic neoplasia, increased epithelial proliferation has also been noted to be accompanied by increased apoptosis.

Fibrosis

**Definition. Fibrosis** [11]. Fibrosis is a deposition of extracellular collagen by activated fibroblasts.

**Criteria/Explanation.** The histological features of fibrosis may evolve with time. Early on, activated fibroblasts are surrounded by abundant eosinophilic staining collagenous material, possibly with edematous stroma, and adjacent chronic inflammation and granulomatous tissue, if occurring as a sequelae of inflammatory injury. With time, these areas of scarring evolve to hypocellular regions with dense eosinophilic collagen.

**Discussion.** In human and veterinary pathology, fibrosis is a well-recognized potential consequence of inflammation (i.e., scarring). In addition to a sequelae of inflammatory injury, transgenic manipulations involving growth factors or their receptors can lead more directly to stromal cell or fibroblast activation, with increased deposition of extracellular matrix. Such stromal alterations have been observed in the prostates of GEM, and should be considered when the “fibrosis” or stromal hyalinization is not accompanied or preceded by prominent inflammation.

Non-Neoplastic Proliferations of the Prostate: Hyperplasia

**Epithelial Hyperplasia (Fig. 4)**

**Definition. Epithelial Hyperplasia** [12]. Epithelial hyperplasia is a non-neoplastic increase in epithelial (glandular) tissue compared with age-matched wild-type control mice.

**Criteria/Explanation.** Epithelial hyperplasia is recognized as either an increase in glandular spaces or as an increase in epithelial cells within normal-appearing gland profiles, the latter primarily reflected by stratification of epithelial cells. Pronounced forms can achieve tufting, micropapillary, and even cribriform architecture. In the Bar Harbor Classification, epithelial hyperplasia is additionally classified as focal or diffuse. The modifier term focal refers to the involvement of one or a few gland spaces. Diffuse refers to a more extensive and uniform process. In practice, this can be distinguished as involvement of ≥50% of gland profiles in adequately sampled prostate lobes. Epithelial hyperplasia can be accompanied by cytologic (nuclear) atypia, which should be noted. Criteria for cytologic atypia include nuclear enlargement, pleomorphism, chromatin abnormalities, and increased prominence of nucleoli, as described. Epithelial hyperplasia is distinguished from mouse PIN (mPIN) by criteria of focality and progression for the latter, as detailed below. If accompanied by stromal proliferation, the lesion should be classified as mixed epithelial and stromal proliferation, as described below. Hyperplasia is the appropriate designation for epithelial proliferations in the mouse prostate that are not reactive to inflammation and that do not satisfy the definition of mPIN. Appropriate modifiers regarding extent and the presence of atypia should be included.

**Discussion.** A variety of characteristic epithelial hyperplasias occur in the human prostate, most notably adenomatous or glandular hyperplasia in the TZ as part of the glandular and stromal hyperplasia typical in BPH (Fig. 4, A–C; Ref. 6). Basal cell hyperplasia can be...
seen in association with BPH (Fig. 4D), as well as in a morphologically distinct form in the PZ, which, when accompanied by prominent nuclei, can mimic PIN (6, 65). Another described morphological entity is clear cell cribriform hyperplasia (Fig. 4E), which is also most commonly observed in the TZ with BPH (6). Importantly, the creation of new glandular spaces is not a diagnostic criterion specific for adenocarcinoma per se in the human prostate, nor should this be regarded as an absolutely specific feature for adenocarcinoma in prostate of GEM. Glandular proliferation is not accompanied by appreciable cytologic atypia in human BPH. Hypercellular BPH nodules can be observed, and atypical adenomatous hyperplasia, or adenosis, is characterized by a proliferation of admixed larger and small gland profiles, with similar nuclear and cytoplasmic features in each, and retention of at least a fragmented basal cell layer (6). Other lesions with increased glandular or epithelial tissue include sclerosing adenosis, also typically seen in the TZ, which can have nuclear atypia and mimic higher-grade Pcas (6). Hence, many benign lesions in the human prostate demonstrate increased glandular spaces in addition to or instead of increased epithelial cell stratification within pre-existing gland or duct spaces.

Epithelial proliferation classified as hyperplasia is not routinely recognized per se in the human prostate PZ. Lesions referred to as postatrophic hyperplasia are described above with atrophy. Epithelial proliferation in normal PZ gland spaces is usually accompanied by at least mild nuclear enlargement and atypia, and classified as low-grade PIN (9, 65). However, the anatomical restrictions of hyperplasia as a typical TZ histological alteration and PIN as a generally PZ-restricted entity in human prostate cannot be translated to specific lobes or regions within lobes of the mouse prostate. Hence, hyperplasia is the appropriate designation for epithelial proliferations in the mouse prostate not satisfying the definition of mPIN.

Hyperplasia defined as an increase in glandular tissue compared with age-matched wild-type control mice could certainly be a “developmental” consequence of transgene expression during prostate development. Attention should be given to the extent (diffuse versus focal) or possible uniform involvement of such epithelial proliferations (even if atypia is present) in effort to distinguish a generalized phenomenon versus a possible manifestation of the development of an epithelial neoplasm in GEM. Hyperplasia may also be seen as an increase in epithelial cells within otherwise normal-appearing gland spaces. Prostate epithelial hyperplasia has been observed in GEM (Fig. 4, F and G). The presence or absence of atypia should be noted.
As epithelial proliferation with nuclear atypia is the histological hallmark of PIN, atypia should be diagnosed with caution and preferably in a blinded fashion so that confidence can be had in the presence of this potentially subjective feature. As described below, other criteria are necessary for a classification of PIN in the mouse prostate, such as focality and progression.

Epithelial proliferation can result in an increase in basal cells or secretory cells or both, but as proliferative capacity is likely limited to basal cells, an increase in luminal cells likely represents accompanying cellular differentiation. When possible, the cell types involved (i.e., increased compared with wild-type controls) should be specified. Adjunctive immunohistochemical stains can be helpful, as described in the protocol section. Regarding classification of focal versus diffuse, although some gland lumens will be cut more longitudinally and others more transversely in any given section, and many of these are different profiles of the same connecting ducts or glands, the semiquantitative estimation of the percentage of involvement or rigid counting of gland spaces involved may be a useful objective parameter. Also, as normal proliferation and apoptosis in the rodent prostate may occur differentially along proximal and distal portions of ducts and glands, attention should be given if possible to differential involvement of proximal and distal portions of the duct/gland profiles within well-oriented sections (see “Protocol” section regarding sectioning). This is desirable, as genetic manipulations could accentuate, modulate, or negate these normal cell turnover mechanisms. As a supplement to blinded histopathologic assessment, more objective parameters of epithelial proliferation and turnover can be used, such as immunostaining for proliferation markers and tissue stains for apoptosis (see “Protocol” section). Such parameters can be assessed in an objective quantitative fashion (either by blinded counting or image analysis approaches), as long as sampling is random and equal and/or attention is given to the proximal versus distal portion of examined prostate lobes.

Stromal and Combined Epithelial and Stromal Hyperplasia (Fig. 5)

Definitions Stromal Hyperplasia [13]. Stromal hyperplasia is a non-neoplastic increase in the cellularity of the stromal component of the prostate compared with age-matched controls.

Combined Epithelial and Stromal Hyperplasia [14]. Combined epithelial and stromal hyperplasia is the simultaneous non-neoplastic increase of both epithelium (as described above) and stroma.

Criteria/Explanations. Stromal hyperplasia is recognized by an increase in the density of stromal cells compared with age-matched control mice. Cells are oval to spindle with general similarity to normal prostatic stroma, and their increase may be accompanied by an increase in the extracellular matrix, which should be noted. Stromal hyperplasia may be focal or diffuse. Focal refers to one or multiple areas comprising less than half of the sampled tissue. Diffuse stromal hypercellularity involves >50% of the sampled stromal compartment. Stromal hyperplasia may be accompanied by cytologic atypia, including nuclear enlargement, hyperchromasia, pleomorphism, and prominent nucleoli, which should be noted. Particularly if accompanied by nuclear atypia, increased stromal cells must be distinguished from undifferentiated or sarcomatoid carcinoma. The resemblance to normal prostatic stroma (which may be more evident at earlier time points), spatial relationship to normal or proliferative noncancerous epithelial elements, and immunohistochemistry (e.g., negative for pan-keratin and possibly positive for smooth muscle actin) may be helpful. Stromal hyperplasia should also be distinguished from stromal neoplasms based on criteria described below. Combined epithelial and stromal hyperplasia should be distinguished from adenoma and papilloma based on criteria described below.

Discussion. Stromal hypercellularity in the human prostate is an extremely common, almost characteristic, accompaniment to the glandular hyperplasia that occurs in the TZ in BPH (6). Stromal hypercellularity is noted to accompany other typically TZ lesions, such as basal cell hyperplasia and sclerosing adenosis (6), whereas it is not a usual accompanying feature (i.e., conspicuous by light microscopy) to the malignant glandular elements in typical PZ Pca in the human (8, 9).

Prominent stromal hypercellularity has been observed along with epithelial cell proliferation in multiple GEM models (Fig. 5, A–C). Whether a direct consequence of transgene expression or a possible paracrine effect from transformed epithelial cells is not defined. When uniform or diffuse, it is certainly unlikely to be a desmoplastic response to what is hopefully a focal invasive event in carcinoma (see below). Often the stromal hypercellularity in GEM prostate has been noted to be progressive and to have a general resemblance, especially in the early stages, to normal prostate stroma. In some models (Fig. 5, A and C), there is also noted a more condensed hypercellular stroma in closer proximity to the proliferating atypical epithelial compartment. Atypia and mitotic activity in this stroma can be conspicuous. In addition to possible classification as combined epithelial and stromal hyperplasia, in some models, the epithelial lesion accompanying the stromal alterations is properly regarded as mPIN (as defined below), and there has been development of subsequent invasive carcinoma. Such lesions can be designated as “mPIN with hypercellular stroma” or “mPIN with stromal hyperplasia.” Atypia in the stromal component should be addressed as well. In contrast to these hyperplastic processes, focally prominent proliferations of epithelium and associated stroma raise consideration of discrete neoplasms, potentially classifiable as adenoma or papilloma.

Neoplastic Proliferations of the Prostate

Benign Epithelial Neoplasms: Adenoma, Papillary Adenoma, or Papilloma (Fig. 5)

Definitions. Neoplasm [15]. A neoplasm is an autonomous new growth. The designations of adenoma and papilloma are intended for true neoplastic epithelial proliferations that lack the hallmarks of malignancy, such as destructive invasion and metastatic potential. Adenoma [16]. An adenoma is a benign neoplasm of gland-forming epithelium.

Papillary Adenoma [17]. A papillary adenoma or papilloma is a benign neoplasm of gland-forming epithelium with a well-defined fibrovascular stroma, wherein the vascular core and surrounding stroma is covered by neoplastic epithelium in a manner imparting a papillary or branching pattern.

Criteria/Explanation. Although in theory such lesions are clonal neoplasms, certainly this is not routinely demonstrated for the vast majority of lesions classified as such. Instead, these benign tumors are recognized by a combination of pathological features, including discrete growth of epithelial elements (with variable stromal component) that can be expansile or nodular or protrude into a lumen, but without destructive invasion. The stromal component may include a prominent vasculature, either throughout or at the base of the lesion. In papilloma, there is a well-defined fibrovascular stroma or “stalk” that is covered by an epithelial lining in a manner that assumes a characteristic papillary growth pattern (Fig. 5, F–H). Papillary adenoma and papilloma are, hence, synonymous. Cytologic atypia can be present in adenoma or papilloma, and if so, it should be described. Furthermore, if such atypia progresses (i.e., is increasingly prominent in lesions from older GEM), this should be noted and described. Such lesions may potentially parallel the development of high-grade dysplasia in some human adenomas in organs other than prostate. Similarly, if focally architecturally distinct epithelial regions are identified, such as...
Bard Harbor Classification of Mouse Prostate Pathology

Fig. 5. Combined epithelial and stromal proliferations in genetically engineered mice (GEM): prostatic intrapapillary neoplasia (PIN) versus hyperplasia versus benign neoplasms. A, combined epithelial and stromal proliferation in a GEM prostate. Low-power photomicrograph of dorosolateral prostate (DLP) of a LPB-Tag 12T5 mouse at 19 weeks, shows marked lobular expansion by a fairly symmetric and uniform proliferation of atypical epithelial cells (arrowheads) with hypercellular stroma (arrows). In stromal hyperplasia, the stromal elements may be fairly normal in appearance but show increased cellularity or they may show cytologic atypia, which should be described. Occasionally, hyperplastic stromal elements are more condensed and consist of crowded spindle cells with scant cytoplasm more immediately adjacent to atypical epithelium. In the LPB-Tag 12T5 mouse and related fast-growing LPB-Tag lines this epithelial lesion begins focally and quickly progresses in extent to an essentially diffuse lesion, and occasionally progresses to invasion. Thus, it has been regarded as PIN with a morphology distinct from PIN occurring within pre-existing gland spaces (see text). In SV40 or large T-antigen GEM models with such exuberant and diffuse atypical glandular and stromal hyperplasia, the distinction between true invasion versus herniation of glandular and stromal proliferations into periprostatic loose connective tissue or fat can sometimes be difficult (see text for details). B, photomicrograph showing a lobular expansion of glands in a 16-week-old TRAMP mouse prostate by atypical prostatic epithelium and a mildly hypercellular stroma (arrowheads). This is a somewhat uniform and symmetric epithelial lesion in which the small, peripheral acini appear to connect to the larger, more central lumen (+), with similar cytologic atypia. The stromal hyperplasia shown here is not in response to invasion and can be seen diffusely surrounding all three of the illustrated gland profiles. The distinction between lesions like those shown in A and B as PIN versus adenocarcinoma can be difficult. Histological features that help to distinguish adenocarcinoma, such as architecturally distinct foci and desmoplasia, are described in the text. The consensus of the Pathology Committee was that lesions like those shown in A and B represent in situ lesions. Compare these in situ lesions to the well-differentiated adenocarcinoma shown in Fig. 8E. C, hypercellularity of stroma (arrowheads) admixed with proliferating atypical glands (arrow) in 24-week-old TRAMP mouse prostate. These markedly hypercellular stromal elements consist of spindle cells with scant cytoplasm (arrowheads). Foci with these characteristics are also common in AP and DLP of fast-growing LPB-Tag lines (see text), and are different in appearance from the more smooth muscle-appearing hypercellular stroma also noted. These foci are usually seen in immediate apposition to atypical epithelium. The reactive versus neoplastic nature of this type of stromal proliferation or possible epithelial-mesenchymal transformation have not been thoroughly addressed. Cytologic atypia and mitotic activity can be noted. Possible prostatic stromal origin for poorly differentiated spindle cell lesions in metastatic foci should be considered in GEM models with such characteristics. Ancillary techniques described in the “Protocols” section can be useful for distinguishing metastatic carcinoma versus sarcoma. D, markedly atypical epithelial and admixed stromal proliferation in DLP of 25-week LPB-Tag 12T7s mouse. The overall histological appearance of the lesion shown here is very similar to the background glandular and stromal proliferations seen in these mice, and may constitute a simple physical herniation or protrusion of glands and stroma into duct lumens (+). Whether these lesions thus represent a focal exaggeration of the atypical prostatic epithelial hyperplasia or mouse PIN and stromal hyperplasia versus distinct neoplasms is not established. These foci are common with increasing age in the fast growing LPB-Tag lines and can show associated stromal edema, with an appearance reminiscent of phyllodes tumors in human breast, as have been described in TRAMP mice. E, low-power photomicrogram of multiple gland or duct lumens with intraluminal epithelial and stromal proliferations (+) in prostate of a TRAMP mouse. Lesions with these characteristics have had the descriptor “phyllodes-like” added to them, because of their histological resemblance to this human tumor, most often found in the breast. Lesions with these histological features are very rarely encountered in the human prostate. In the mouse lesions, the surface of the intraluminal component is typically covered by epithelium, and the polyploid portion contains an admixture of small glands and stroma. The small gland profiles in the polyploid portion often appear to connect to the surface epithelium, and the stroma is variably hypercellular, hyalinized, or edematous. Histologically, such foci have many features compatible with the designation of papilloma. In SV40 or Tag-based models, they are always seen in a background of more general atypical epithelial and stromal proliferation. A consensus was not reached on the nature of these lesions based solely on their histological features. Whether they constitute hyperplasia, as a focal exaggeration of a more general process, or distinct clonal neoplasms arising against a hypercellular background remains to be established. If encountered, either of these classifications is appropriate; however, their histological features should be described along with the appearance of the rest of the prostate. The term “phyllodes-like” can be added as a purely histological descriptive adjective, but this term does not imply any biological relationship of these lesions to phyllodes tumors found in human tissues. F–H, low-, intermediate-, and high-power photomicrographs of a discrete papillary lesion compatible with papillary hyperplasia or a papillary adenoma (papilloma) in a GEM prostate. F, this lesion protrudes into and partially fills the lumen (arrowheads) of the lateral prostate from a 73-week-old ARR.P5-FGF8b mouse. The lesion shows an expansile rather than destructive growth pattern. G, a well-vascularized stroma (arrow) is associated with the epithelial proliferation. Papillary structures (arrowheads) are present although the papillae are less evident in foci where the epithelium is more crowded. H, focal cytologic atypia with nuclear enlargement and macronucleoli (arrowheads) are also evident in these regions. In addition to discrete papillary lesions like the one illustrated here, lesions consistent with mouse PIN are also seen in these mice.
showing greater crowding, or accentuated atypia or apparent invasion into the stromal compartment of the lesion itself, this should be noted and described. Such lesions potentially parallel the development of early invasive carcinoma in some adenoma-carcinoma sequences in organs other than prostate.

Discussion. There are essentially no counterparts in human prostate pathology, in terms of benign neoplasms of variably differentiated secretory epithelial cells, compared with the common occurrence of adenomas in such tissues as colon. There are rare basal cell lesions described as basal cell adenomas (8, 9), but in general, this category in GEM was created based on already observed or possible lesions more comparable with those in other tissue sites or in veterinary pathology.

When occurring in the background of extensive or even diffuse epithelial and stromal hyperplasia, focal intraluminal polyploid protrusions may represent foci of essentially intraluminal herniations, with growth into an area of “less resistance” than surrounding stroma or adjacent tissue (Fig. 5, D and E). Some such foci in a few SV40 or Tag-based models develop a very characteristic edematous stroma as well, and lesions either representing combined epithelial and stromal hyperplasia or true neoplasms have been described by the term “phyllodes-like” on the basis of their histological resemblance to phyllodes tumors in the human. These tumors are particularly characteristic (although not necessarily common) in the human breast. Histologically similar lesions have rarely been described in the human prostate (8, 9). The hyperplastic versus neoplastic nature of the observed lesions in the prostate of GEM, which are seen especially with advancing age in certain models, is not established, and no consensus was reached on this matter. We do not recommend the term phyllodes tumor as a precise designation for these foci because of a lack of demonstrated homology to these uncommon neoplasms in the human (e.g., in terms of potential clinical behavior or natural history and the lack of more widespread background epithelial and stromal abnormalities in the human). However, the descriptive adjective “phyllodes-like” may be useful in describing the characteristic histology of such combined epithelial-stromal lesions.

A lesion homologous to papilloma is not recognized in the human prostate per se, but lesions satisfying typical histological criteria for papilloma in human and veterinary pathology have been observed in the prostate and seminal vesicles of GEM. When seen in a background of more widespread hyperplasia or PIN, such lesions again need to be distinguished from hyperplasia and are recognized by their focally distinct growth pattern. Destructive invasion is lacking. Given the possible background of widespread hyperplasia occurring throughout the GEM prostate, molecular data to support that such foci are truly distinct clonal neoplasms and not part of a more widespread process would be welcomed. For now, such lesions can be classified as adenomas or papillomas, or as combined epithelial and stromal proliferations, but they should be described in detail (including the presence or absence of atypia), as should the histology of the rest of the prostate in which they are arising.

Neoplastic Proliferation of Premalignant Potential: mPIN (Figs. 6 and 7)

Definition. mPIN [18]. mPIN is the neoplastic proliferation of epithelial cells within preexisting or normal basement membrane confined gland spaces. In the Bar Harbor classification, mPIN is further classified as mPIN: (a) with documented progression to invasive carcinoma [18]; or (b) without documented progression to invasive carcinoma [19].

Criteria/Explanation. mPIN is recognized histologically as proliferation of atypical epithelial cells within pre-existing glands. These basement membrane-bound glands may be architecturally similar to the wild-type prostate or increased uniformly as a consequence of transgene expression, but are still contrasted with those of invasive carcinoma (discussed below). Proliferation is most commonly recognized by stratification of epithelial cells. The epithelial cells demonstrate nuclear atypia. With progressive neoplastic epithelial growth, the focus can acquire a tufting, micropapillary, or cribiform growth pattern. In rare instances, the pattern may be flat, in which proliferation is not conspicuous, but the nuclear atypia and its progression are sufficient for the diagnosis of mPIN. Nuclear atypia can be in the form of nuclear enlargement, nuclear membrane irregularity, hyperchromasia, chromatin clumping, prominent nucleoli, or a combination of these features. As the category of epithelial hyperplasia can have atypia as described above, two other features that must be present for a designation of mPIN are focality and progression. The lesion should begin focally, as a manifestation of neoplasia, rather than being present uniformly throughout the prostate, as a perhaps more direct consequence of transgene expression or other genetic manipulation. Progression refers to either increased extent of involvement or increased nuclear atypia or both, with both being particularly supportive. Rigid criteria for the degree of these changes that constitute “progression” were not established (see discussion below for guidelines). However, they should be well documented, described, and hopefully illustrated over an appropriate time frame (depending on the rapidity of neoplasia development) in any given new model. Examples are shown in Figs. 6 and 7. Although initial evaluation of the spectrum of possible lesions in a new model can be unblinded, subsequent evaluation of lesions in mice of different ages using objective schemes to describe architectural and cytologic abnormalities that may allow for documentation of temporal progression should be blinded.

Within the Bar Harbor classification, the natural history of an mPIN lesion is an important component of its classification. Progression to invasion is recognized based on the criteria for microinvasive or more extensively invasive carcinoma as detailed below. It was the consensus opinion of the Pathology Panel that mPIN should not at present be graded histologically, because insufficient data exists to support general morphological criteria for a high-grade designation that might predict progression to or association with invasive carcinoma in any given model. This issue is considered in more detail in the discussion below.

Discussion. mPIN presumably shares some of the molecular alterations characteristic of carcinoma; however, the neoplastic cells have not invaded through the basement membrane into surrounding stroma. Regarded as a potential precursor for invasive carcinoma, by itself, PIN lacks metastatic potential. Because of the biological significance of PIN and the frequency with which GEM models develop lesions potentially classifiable as mPIN, there first follows a consideration of the biology, pathology, and clinical aspects of human PIN.

Human PIN. Human PIN is the neoplastic proliferation of epithelial cells within preexisting gland spaces, which occurs predominantly (almost exclusively) in the PZ (Fig. 6A; Refs. 8, 9, 66, 67). The proliferating, stratified cells are neoplastic and less-differentiated counterparts of secretory or luminal cells and do not immunostain with antibodies to high molecular weight CK. In fact, with increasing grades of PIN the basal cell layer is progressively lost or fragmented (19).

Grading Human PIN. Human PIN is now classified as low or high grade, replacing a previous three-tiered system whereby PIN I is low-grade PIN, and PIN II and PIN III are high-grade PIN (HGPIN; Refs. 8, 9, 65, 66, 68, 69). With increasing severity (grade), there is greater nuclear enlargement and increasingly prominent nucleoli (8, 9, 66). Macronucleoli (>2–3 μm) are typical and diagnostic of HGPIN (Fig. 6B). HGPIN architecturally can show multiple growth patterns, including tufting and micropapillary (most common; Fig. 6B), flat or cribiform (70).
Biological and Clinical Significance of HGPIN. In the human, HGPIN is considered the likely precursor lesion for the majority of invasive Pcas in the PZ (Fig. 6A; Refs. 12, 18). Multiple lines of evidence support the relationship between HGPIN and PZ-located Pca (8, 12, 19, 67). These include the earlier age occurrence of HGPIN compared with invasive Pca in autopsy studies. There is an increased incidence of HGPIN (but not low-grade PIN) in autopsy or RP prostates with cancer compared with those without. There is frequently close spatial association of HGPIN and invasive acinar-forming Pca (Fig. 6A). Numerous studies examining various molecular markers, including expression of oncogenes, growth factors, and their receptors, have demonstrated alterations in HGPIN that are intermediate between benign prostate and Pca or that are similar in HGPIN and Pca (18, 19). Similar to the frequent multifocality of
Fig. 7. Prostatic intraepithelial neoplasia (PIN) in non-SV40/Tag mouse models. A and B, mPIN in anterior prostate of 11-month-old PTEN +/− mouse. A, intermediate magnification showing two adjacent involved gland profiles (arrowheads) with tufting and cribriform growth and possibly mildly reactive surrounding stroma (*). Epithelial nuclear atypia includes enlarged nuclei with vesicular chromatin and prominent nucleoli (arrows), similar to that typical in human high-grade PIN. The degree of atypia and progression in extent are compatible with mouse PIN (mPIN). Spontaneous progression to invasive carcinoma is not a characteristic outcome in this genomic knockout model, with only rare possible invasion noted in older (>1 year) mice. B, higher magnification showing two adjacent involved gland profiles (arrowheads) with tufting and cribriform growth and possibly mildly reactive surrounding stroma (*). Epithelial nuclear atypia includes enlarged nuclei with vesicular chromatin and prominent nucleoli (arrows), similar to that typical in human high-grade PIN. The degree of atypia and progression in extent are compatible with mouse PIN (mPIN). Spontaneous progression to invasive carcinoma is not a characteristic outcome in this genomic knockout model, with only rare possible invasion noted in older (>1 year) mice. 

C and D, mPIN in dorsolateral prostate sections of PTEN +/− × p27 +/− mice. C, intermediate magnification showing focal prominent cribriform proliferation (arrowheads) within a gland lumen in an 8-month-old mouse. D, higher magnification showing cytologic atypia in cribriform mPIN in 6-month-old PTEN +/− × p27 +/− mouse, with enlarged nuclei and scattered prominent macronucleoli (arrows). Note the essentially normal surrounding thin fibromuscular stroma. Progression to frank invasion has not been observed in these mice, although it was reported in up to 25% of PTEN +/− × p27 +/− mice by other investigators (30). E, intermediate power photomicrograph of cribriform epithelial proliferation in multiple glands (arrowheads) in anterior prostate of 16-week-old metallothionein (MT)-transforming growth factor α mouse. Atypia and progression in this mouse are compatible with mPIN. 

F, high-power photomicrograph showing mild degrees of focal epithelial stratification (arrowhead) and nuclear atypia, characterized by mild nuclear enlargement and occasional prominent nucleoli (arrows). Ventral prostate section from 16-week-old MT-DNIR mouse. Atypia and progression are compatible with mPIN. 

Characterization of models with potentially subtle phenotypes including relatively mild epithelial proliferation and atypia is supported by the inclusion of adequate age-matched controls and blinded histopathologic analysis. Additional possible supportive objective analyses (e.g., for documenting progression) include quantitative assessment of indices of proliferation and apoptosis. See text for details.

G, mPIN, showing complex cribriform and microacinar epithelial proliferation, with nuclear atypia, extensively involving a gland lumen (arrowheads). A relatively uninvolved gland profile is shown at bottom right (arrow). High-power photomicrograph of lateral prostate from 24-month-old Pb-ras × mxl × +/− mouse. H, mPIN, showing cribriform epithelial proliferation, with nuclear atypia, including enlarged nuclei and focally prominent nucleoli. Section of lateral prostate from 82-week-old ARR-Pb-FGF8b mouse. I, mPIN, showing cribriform epithelial proliferation involving multiple pre-existing gland lumens (arrowheads) of the anterior prostate of an 8-month-old Nkx +/− × PTEN +/− mouse (low power). J, high magnification of same section as in I, showing complex cribriform growth pattern, including supporting delicate microvessels (arrowheads), and nuclear atypia, with enlarged nuclei and prominent nucleoli (arrow). Immunostaining for endoglin (CD105) demonstrated increased vessels in these cribriform lesions, which filled some duct lumens. This raises consideration about newly formed associated vessels and stroma and the existence of “back to back glands” within duct lumens, although the overall duct and lobular architecture was not altered (35). This lesion is described in the classification scheme of Park et al. (78), serving to document lesion progression sufficient for classification as mPIN as described in the text. Whether such lesions will eventually be associated with progression to unequivocal destructive invasion into the surrounding fibromuscular stroma in non-SV40 models remains to be more fully characterized, as described in the text.
invasive Pca in the human prostate, HGPIN is frequently multifocal, and genetic evidence supports that HGPIN and Pca within a prostate are commonly multiclonal (19, 71).

**Significance of Cribriform Lesions with Intact Basal Cell Layers in Human Pca.** Although in the human, HGPIN as a potential precursor lesion for invasive Pca may demonstrate a cribriform growth pattern within pre-existing ducts and glands (70), several recent studies have shown that “cribriform HGPIN” has a worse prognosis than cribriform invasive carcinoma in RPs or imparts an independent increased risk for progression when identified in RPs (72, 73). Such data have raised speculation that these lesions, which are associated with higher grade and larger volume Pcas, actually represent the spread of invasive carcinoma within ducts, so-called “intraductal carcinoma,” a postinvasive rather than preinvasive lesion (72–74), which is supported by recent molecular evidence (75).

The possible biological implications of these lesions in the human prostate should not prematurely be extended to mouse models based solely on architectural and histological similarities of “noninvasive” cribriform proliferations. Atypical cribriform lesions within ducts have been observed in numerous GEM models of Pca. In the mouse, however, these can be seen without associated invasion anywhere in the prostate (e.g., in contrast to the large volume of typically ≥ Gleason score 7 invasive carcinoma in human RPs) or with only small foci of invasion. Hence, these lesions likely represent PIN in the mouse (i.e., as a true potential precursor lesion), as they have typically been interpreted.

**Invasion in Association with HGPIN.** The earliest invasive forms of Pca associated with HGPIN in the human prostate (conceptually analogous to microinvasion in the mouse prostate classification) are not clearly defined. Not well recognized in the human is the penetration through the HGPIN gland basement membrane of individual tumor cells or small groups of cells with possible cytologic alterations or stromal response. Possible early invasive lesions composed of microscopic foci of larger HGPIN-like glands with closely arranged “sprouting” small acini have been described in RPs (76), but the application of such concepts to diagnosing early invasive carcinoma on biopsy is not established (77). Issues relevant to microinvasion in GEM models are described below in the section of microinvasive carcinoma.

**mPIN. General Considerations Regarding Possible Grading of mPIN.** In some previously published descriptions of GEM models, grading PIN as low grade or high grade has been accomplished based predominantly on progressive nuclear atypia. However, it should be borne in mind that human HGPIN represents a broader concept than just its morphological features; that is, by virtue of its being seen in association with invasive carcinoma and by having documented molecular alterations similar to Pca, it is considered to have true potential for progression to invasion (12, 18, 19). Obviously, documentation of progression to invasion and characterization of progressive molecular alterations in PIN and invasive Pca have not been accomplished in all of the mouse models. Because of the association of HGPIN with invasive Pca in the human, consideration was given to classifying as high grade only those mPIN lesions that occur in models that have documented progression to invasive carcinoma (and in which the invasion appears to be related to the PIN lesions). However, it was the opinion of the Pathology Panel that this definition may discourage development and characterization of models that could be useful for deciphering early changes leading to PIN in Pca development and for testing possible chemoprevention agents. Furthermore, because insufficient data exists on morphological features of PIN that correlate with progression (regardless of the specific mouse model), it was the consensus opinion of the Pathology Panel that mPIN should not at present be graded based on histological criteria as low grade or high grade. Any attempts to do such within the context of a formal classification scheme applicable to all of the GEM models would be premature.

should be additionally noted that based on the developed criteria for mPIN, in which focality and documentation of progression (of extent and atypia, not necessarily to invasive carcinoma) are required, many published models stating that they have PIN or PIN-like lesions would not actually satisfy the diagnostic criteria for mPIN. Investigators should place more emphasis on satisfying such criteria for a proper designation of mPIN in a GEM model. The number of foci or percentage of gland lumens involved, and descriptions of progressive architectural abnormalities and nuclear atypia with increasing age should allow for adequate classification of a GEM model as having mPIN. A histopathologic classification scheme for PIN lesions in one non-SV40 model, incorporating lesions also seen in other non-SV40 models, was described recently by a group of investigators including a member of the MHHCC Prostate Pathology Committee (78). PIN was divided into four categories based on progressive architectural and cytologic abnormalities (78). Although in this published study, it was not clear if all of the histological assessments were blinded, the application of this scheme allowed for the documentation of lesion progression in Nkx −/− × PTEN +/− mice (35, 78). Lesions similar to these PIN categories have been noted in several other non-SV40 based models listed in Table 1 (78). Detailed time course analyses (with sufficient numbers of animals at a range of time points) have not been reported in other models using this classification scheme to determine whether similar progression can be documented. However, it is likely that such a scheme can be applied in a blinded manner to objectively document progression of extent and severity of epithelial proliferations to allow for recognition of models satisfying mPIN criteria in the Bar Harbor Classification scheme (78). The reproducibility and interobserver agreement of this or any other mPIN classification scheme have not been documented thoroughly. This scheme (78) and lesions illustrated herein provide the investigator some idea of the phenotypes of mPIN lesions that may develop in non-SV40 based models. For now, although other descriptions may be adequate for documentation of progression, utilization of this particular scheme for documentation of progression for recognizing mPIN is the sole recommended application of this described scheme, and it is not recommended as a histological grading scheme for dividing mPIN into low or high grades. Histological classification schemes capable of documenting progression of mPIN lesions within an individual model, such as that described by Park et al. (78), may also be useful for blinded histological assessment of possible therapeutic benefit of chemoprevention strategies designed to inhibit or reduce progression.

The Bar Harbor Classification scheme does allow for grading/classification of mPIN, but stipulates that natural history be considered when classifying mPIN (i.e., whether mPIN is or is not associated with progression to invasive carcinoma). Although there are obvious histological differences in PIN lesions in different GEM models, it was the consensus opinion of the Pathology Panel upon uniform review of >20 models that these histological differences do not yet translate to differences in potential for development of invasive carcinoma that are an important component of the concept of a high-grade PIN lesion. For example, PIN has progressed to invasive carcinoma in multiple SV40 early region or large T antigen-based models. If based on these observations, the profound nuclear atypia in the PIN lesions of these mice were to serve as the histological

20 M. A. Moses, Jr., R. J. Matusik, R. L. Roberts, S. B. Shappell, unpublished observations.
hallmarks of HGPIN in GEM, few if any non-SV40-based models would have lesions classified as HGPIN. The morphological appearance of the PIN lesions in SV40 and non-SV40 models is quite distinct (see below). At the time of the Bar Harbor Pathology Workshop, none of the non-SV40 based models available for review (Table 1) demonstrated unequivocal invasion with any significant frequency. However, it appears that some recently created non-SV40-based models show progression to invasive and metastatic carcinoma (79–81). Future consensus conferences in which such models are reviewed may allow for the development of a grading scheme to be applied to non-SV40-based models, which may allow for predicting which new models may show progression to invasive carcinoma. Any such future histological mPIN grading scheme would likely be different from such a scheme for SV40-based models.

**Classification of mPIN in the Bar Harbor Classification.** Although mPIN should not be graded morphologically, this category is divided into those models that have documented progression to invasion or the capacity to invade and those that do not (Table 3). The natural history of a model is, thus, an important component of how an mPIN lesion should be classified. Criteria for invasion are detailed below. Because human HGPIN is associated with invasive carcinoma and when seen in isolation, may potentially progress to invasive carcinoma, the classification of mPIN with documented progression to invasive carcinoma is in some ways biologically analogous to human HGPIN. mPIN without documented invasion can be considered initially as a tentative category when a PIN lesion is observed in a new mouse model. If invasion is subsequently documented, the classification is appropriately modified to reflect this. The frequency with which such invasion should occur to warrant such designation was not established. However, it is intended that this should not be an exceedingly rare, isolated, or equivocal event. Progression should be reproducible. For example, development of unequivocal invasive carcinoma in ≥5–10% of animals in a particular age range of a model would be far more convincing than a single microscopic focus of possible invasion in a single mouse.

The “potential” for invasion or the “capacity to progress to invasion” are important concepts in precursor lesions. As such, it was additionally decided that demonstration of progression to invasive carcinoma of a PIN lesion in a transplant model would warrant classification as “PIN, with documented progression.” The criteria for recognizing subsequent invasion in a transplant model were not extensively discussed or clearly delineated, but in general, invasive carcinoma should be recognized on the basis of the sound criteria for invasion in the intact prostate as described below. Furthermore, standardized protocols for such acceptable transplantation models were not developed or approved, and may be a focus for future workshops. For now, complete details should be provided by investigators using this experimental approach, including the age of the source animal, the size, lobe, and histological nature of the transplanted material, and the time course for subsequent histological investigation of the transplanted tissue. In contrast, crossing of one GEM line with another (Table 1) with resulting progression of PIN lesions to invasion (which has been specifically reported in some instances; Refs. 30, 79) does not warrant classification of the PIN lesions in the parent lines as PIN with invasion. Such bogenic lines are considered distinct “models,” and should be characterized fully and classified separately.

**Descriptions and Biology of mPIN.** A spectrum of lesions has thus far been observed in GEM models that satisfy criteria for mPIN. Although sharing some general characteristics with PIN lesions in the human prostate, the morphology of PIN in some GEM models is distinct from that of the human prostate PZ in many aspects, including with regard to nuclear features. These differences are particularly pronounced for SV40 early region and Tag transgenic mouse lesions (Fig. 6). Tufting, micropapillary, and cribriform PIN lesions have been observed in SV40 or large T antigen-based GEM models. However, compared with human HGPIN, in SV40-based mPIN lesions, the atypical nuclei appear to be more elongated, are more hyperchromatic, and have a greater mitotic and apoptotic rate (Fig. 3, C–H; Ref. 16). In at least one SV40 model, the high proliferative and apoptotic rates have been confirmed by PCNA immunostains and tissue assays for apoptotic bodies. These indices increased with time in PIN lesions, paralleling other nuclear abnormalities in PIN, and were indeed much higher than those reported in human prostate (32). Some PIN lesions in SV40-based models have even demonstrated necrosis (e.g., CR2-SV40), which is not seen in human HGPIN, but such lesions were still compatible with in situ lesions and not intraductal carcinoma as described for human Pca (73). All of the reported SV40 early region and Tag antigen-based models have progressed from PIN lesions to invasive carcinoma (33, 37, 38, 82–84).

Tufting, micropapillary, and cribriform patterns of epithelial proliferation have also been noted in mPIN lesions in non-SV40-based models, with cribriforming and even duct distension particularly likely to be seen in older ages (35, 78). Compared with SV40-based models, nuclear atypia appears to be of a different quality in PIN lesions of these models, including growth factor and tumor suppressor gene manipulated GEM models (Table 1). Although nuclear enlargement occurs, there is less hyperchromasia and less nuclear membrane abnormalities. Nucleoli, occasionally multiple, are prominent (Fig. 7). The nuclear atypia in these models is thus more reminiscent of that seen in human HGPIN. Although progression to invasion has been reported by some investigators (30, 34), it was not consistently present in any of the models reviewed by the Pathology Panel. Since the time of the Bar Harbor meeting, at least three non-SV40-based models have been described that reportedly progress to unequivocal invasive carcinoma (79, 80, 81). All of these models have had participation by members of the MMHCC Prostate Pathology Committee in their pathology characterization. As mentioned above, future review may allow for development of histological mPIN grading schemes predictive of progression in non-SV40-based models.

**Modifiers of PIN Lesions in Individual Models.** For GEM lesions properly classified as mPIN, the pattern of growth should be described (e.g., flat, tufting, micropapillary, cribriform, and combinations). The extent of involvement should be described, with documented temporal progression as detailed above (e.g., as focal or diffuse or as the number of ducts/glands involved compared with the total assessed, or expressed as percentage). Nuclear atypia and its progression should be described. In all of the studies, but especially in models with perhaps more subtle alterations (e.g., Fig. 7, E and F), blinded pathological analysis compared with aged-matched controls should be performed. Supportive objective studies using tissue markers can be used, such as proliferation and apoptosis assessment, which are known to be altered in human PIN (19), and thus far in mPIN lesions that have been examined (30, 32, 54).

**Malignant Proliferations of the Epithelium**

**Microinvasive Carcinoma (Fig. 8)**

**Definition.** Microinvasive Carcinoma [20]. Microinvasive carcinoma is the earliest recognizable form of invasive carcinoma, with penetration of malignant cells through the basement membrane of PIN-involved glands into the surrounding stroma. It is distinguished from invasive carcinoma by the greater extent of the invasive focus of carcinoma in the latter.

**Criteria/Explanation.** Microinvasive carcinoma in the prostate of GEM is recognized by the extension of individual tumor cells or small nests or acini of cytologically atypical cells into the thin rim of stroma.
Fig. 8. Microinvasive carcinoma and invasive adenocarcinoma in prostates of genetically engineered mice. A, microinvasion occurring in association with prostatic intraepithelial neoplasia (PIN) lesion in LP of 7-month-old C3(1)-SV40 mouse. High power magnification showing extension of single cells and cords and small nests of cells (arrowheads) into thickened stroma underlying cribriform mouse PIN. Small nests of atypical cells with hyperchromatic nuclei, generally scant cytoplasm, and without evident glandular formation are invading into the stroma (arrowheads) surrounding a PIN-containing gland. C, progression to more extensive invasion in mouse prostate cancer model. Section of prostate from CR2-SV40 mouse showing almost circumferential invasion into the thickened stroma (arrowheads) surrounding a residual mouse PIN-containing gland (between *). Invasion is in the form of individual cells and cords and nest of cells, with apparent focal rosetting (arrows). A PIN-containing gland is also seen adjacent to this focus (middle), with more normal-appearing gland at bottom right corner. D, a microinvasive focus of well differentiated adenocarcinoma (demarcated by arrowheads). Section of prostate from 16-week LPB-Tag 12T-7/H11003 MT-DNIIR bigenic mouse. Such a lesion can stand out at low magnification as a more crowded small acinar focus compared with the more diffuse and symmetric lobular expansion by atypical epithelial proliferation (arrow) and hypercellular stroma. On higher magnification as shown, definitive alterations in nuclear and cytoplasmic features are evident, with larger more vesicular nuclei and more densely eosinophilic cytoplasm, compared with adjacent PIN. Similar cytologic alterations are well known with early invasive carcinomas (compared with associated in situ lesions) in a variety of carcinomas in the human, such as cervical and urothelial. This is in contrast to the faintly similar nuclear features of high-grade PIN and associated invasive acinar Gleason pattern 3 carcinoma in human prostate cancer. E, focus of well-differentiated adenocarcinoma in mouse prostate. Section of 24-week-old TRAMP mouse. On the left, there is extension of a focally distinct group of smaller and well-formed acini into surrounding stroma and connective tissue (arrowheads). Compared with widespread and essentially diffuse background of PIN containing glands or the diffuse symmetric lobular expansion with admixed large and connecting small gland profiles that is seen in some of the SV40 or large T antigen-based models with androgen-dependent promoters, the low power focality and architecturally distinct nature of the glands in question is a useful feature for distinction from possible complex in situ or atypical hyperplastic lesions. Although not characteristic of invasive adenocarcinoma in the human prostate, in optimal histological sections, a desmoplastic response in the surrounding fibromuscular stroma or surrounding looser connective tissue can also facilitate recognition of such foci in the mouse prostate. The invasive focus in this example is uniformly and completely composed of discernible gland formations, indicating the designation of well differentiated, as explained in the text. F, invasive adenocarcinoma in association with mouse PIN in LPB-Tag 12T-10 mouse prostate. Nests of tumor cells extending into the stroma show definitive gland formation (arrowheads), with clear lumens or light eosinophilic secretions, rather than features of neuroendocrine rosetting. G–I, invasive adenocarcinoma. Anterior prostate from 38-week-old C57Bl6/6TRAMP/+ × FVB/Jf1 TRAMP mouse, provided by National Institute of Environmental Health Sciences. G, intermediate power showing unequivocal invasive small acinar adenocarcinoma (arrowheads), extensively extending into stroma and periprostastic loose connective tissue, with two remaining PIN-involved glands seen at top and bottom right. H, higher magnification, showing discreet (arrowheads) and occasionally fused small glands, with nuclear enlargement and nucleoli. I, intermediate power showing very pronounced extension of malignant glands into surrounding periprostastic loose connective tissue (*), with possible desmoplastic response (arrowheads). Definitive well-formed glands are present. Because of occasional admixed more solid nests and fused glands, the lesion could be appropriately classified as a moderately differentiated adenocarcinoma in the Bar Harbor Classification scheme. This was considered by the Pathology Panel to be the best histological example of unequivocal invasive adenocarcinoma, with a uniform consensus designation as such. Such a focus was seen in only this particular mouse in material supplied for review.
underlying PIN-containing ducts or glands. Criteria for recognition of microinvasion in the GEM prostate include adequate spatial separation from larger PIN glands of small atypical acini, nests, or individual atypical cells; unequivocal penetration through the basement membrane of PIN glands based on special stains, immunohistochemistry, or electron microscopy; and a stromal response (e.g., desmoplasia).

**Discussion. Microinvasive Carcinoma in Human Pca.** A histologically recognizable form of microinvasive carcinoma as defined herein is not well characterized for human Pca. Clinically, when HGPIN and adjacent small acini of atypical cells are present on transrectal biopsy, it is important to distinguish a diagnosis of “HGPIN suspicious for invasion” or “HGPIN with adjacent small atypical glands” (which may warrant a repeat biopsy) versus “HGPIN with invasion” (which is sufficient for definitive therapy, such as radical prostatectomy; Refs. 65, 77, 85). However, even in the latter situation, this refers to small acini of malignant cells nearby larger HGPIN glands, but which are morphologically incompatible with just tangential sectioning of outgrowths of HGPIN glands. This is different from a recognizable penetration through the basement membrane of HGPIN containing glands of more closely adjacent individual tumor cells or small numbers of tumor cells as described for microinvasive carcinoma in GEM. Hence, despite the biologically important stage of initial invasion in Pca progression, the concept of microinvasive carcinoma has little clinical meaning in the human. Furthermore, because of sampling issues, a single small (i.e., <1 mm) focus of adenocarcinoma (with or without associated HGPIN) on biopsy cannot predict a small, clinically insignificant tumor that can be managed conservatively (86–88).

**Microinvasive Carcinoma in GEM: General Considerations.** Despite the above issues in human Pca, there are several reasons why microinvasive carcinoma is considered to be a definable and useful category in the prostate pathology of GEM. Biologically, the ability to identify early invasive carcinoma may allow for the characterization of the genetic alterations necessary for this crucial stage in the progression of Pca. Secondly, because many models are being generated that appear to be predominantly characterized by PIN, having histological criteria (or criteria based on ancillary techniques such as immunohistochemistry) to make a definitive diagnosis of microinvasion will allow for the identification of which models truly progress. Thirdly, unlike the difficulty in recognizing such a stage in a human Pca progression, it appears that such a stage of early progression can be reliably identified in the mouse. Early invasive carcinomas in certain GEM models do appear to take the form of individual cells or small numbers of cells invading through the basement membrane into surrounding stroma. The ability to sample evolving mouse lesions with time in contrast to tissue sampling issues in human patients is one advantage for defining these lesions. Recognition of microinvasion in the GEM prostate may be facilitated by the more uniform or smoother gland contours, with a generally flat or smooth epithelial-stromal interface. However, given the extremely thin rim of fibromuscular stroma in the mouse prostate, there cannot be a physically large amount of separation between PIN glands and invasive foci in a normal, non-desmoplastically thickened stroma. Hence, recognizing microinvasive foci arising from PIN is still a potentially challenging issue in mouse prostate pathology (16). The ability to define microinvasion in the mouse prostate is supported by several convincing reported examples, particularly in models based on the SV40 early region or large T antigen (Refs. 32, 33, 38; Fig. 8), but also potentially in growth factor and cell cycle regulator manipulated models (30). Blinded histopathologic analyses in GEM models support that this stage of microinvasive Pca, relative to HGPIN, increases with time (33, 38). Furthermore, histopathologic analyses in bigenic models to begin addressing key factors in tumor progression have shown that this is a definable endpoint, with documentable inhibition of progression to microinvasion from HGPIN lesions (89).

**Microinvasive Carcinoma in GEM: Definitive Classification of Microinvasive Carcinoma and Possible Use of Adjunctive Techniques.** Obviously, greater confidence in the classification of microinvasion is obtained for lesions in GEM models that clearly progress to unequivocally more extensive invasion (Fig. 8C). Possible criteria for more pronounced degrees of invasion include obvious destructive or extensive involvement of stroma, extension into periprostatic fat or loose connective tissue surrounding the contractile stroma, perineural invasion, lymphovascular invasion, and metastases (16).

For demonstrating unequivocal penetration of carcinoma into surrounding stroma in models with PIN and questionable invasion, adjunctive methodologies (special stains, immunostains, electron microscopy) may be warranted, especially in models not showing progression to more definitive and unequivocal invasive carcinoma over the time periods being investigated (78). However, these adjunctive techniques have not been rigorously applied and validated for this specific issue of invasive versus in situ lesions. Application of such techniques to tissue sections in models clearly progressing to microinvasive and more extensive invasive carcinoma may allow for the establishment of novel criteria to apply to models with more questionable early invasion from PIN.

**Microinvasive Carcinoma in GEM: Stromal Alterations with Early Invasion.** It is important to note that routinely detectable stromal alterations are not a typical feature in invasive human Pca. Although phenotypic alterations may be demonstrable using special techniques, prostate stroma does not show histologically defined inflammation, edema, or desmoplasia in response to usual invasive acinar carcinoma. Stromal hypercellularity is not seen in association with invasive Pca as a common or characteristic feature, and in fact, can sometimes argue against carcinoma in suspicious foci, being more typical in certain benign mimics of carcinoma in biopsy or transurethral resections of the prostate specimens (such as basal cell hyperplasia and sclerosing adenosis; Refs. 8, 9). This does not mean that stromal hypercellularity or desmoplasia cannot accompany invasive carcinoma in GEM models and that it will not be a useful feature in recognizing invasion in some models. However, it is crucial that such stromal alterations be truly focal and found in association with the possible invasive focus in question. It should not be observed more uniformly in association with proliferating atypical epithelium as a possible paracrine effect or as a possible more direct consequence of transgene expression in stromal cells. Attention should be paid to the focality of desmoplasia. Different pathologists may have different sensitivities or skills for recognizing such alterations, and it can be complicated by a more general background of stromal hypercellularity in some models (16, 29, 37, 83). No consensus opinion was reached regarding the validity of proposed examples of such desmoplasia in association with invasive carcinoma in the study sets for the Bar Harbor Pathology Workshop. However, this feature has been reported in association with invasion in some models (83), may be dependent in part on the genetic background of the mouse, and has been observed by some of the involved pathologists.27 Desmoplasia is certainly not a uniformly appreciable feature of early invasive carcinoma in general in GEM prostates, as it has not been observed or described in some published models having convincing early invasion (33, 38).

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27 M. M. Ittmann, unpublished observations.
Invasive Carcinoma (Figs. 8 and 9)

**Definition.** Invasive carcinoma [21]. Invasive carcinoma is a malignant epithelial neoplasm that exhibits destructive growth in prostate parenchyma.

**Criteria/Explanation.** An invasive malignant epithelial neoplasm has a growth pattern that in contrast to PIN is incompatible with architecturally normal glands, and in contrast to a benign tumor, exhibits destructive local invasion or is capable of metastasizing. Invasive carcinoma in the mouse prostate is distinguished from microinvasive carcinoma by an increased size or extent of the invasive focus (see criteria, below). Invasive carcinoma may show glandular differentiation classifiable as adenocarcinoma, show cytologic features of other types of carcinoma, such as NE or squamous cell carcinoma, or show no morphological features of specific differentiation, as in undifferentiated carcinoma.

The distinction of invasive carcinoma from microinvasive carcinoma (defined and described above) is essentially one of degree. Recommended criteria include one or more of the following, extension into a widened (potentially desmoplastic) stroma (e.g., Fig. 8C; compared with a more normal width stroma in microinvasive carcinoma; e.g., Fig. 8, A and B); extension into the loose connective tissue and periprostatic fat surrounding the contractile fibromuscular stroma (e.g., Fig. 8, G–I; Fig. 9; Ref. 38); and a focus size of >1 mm (33). Other possible morphological indicators of invasive carcinoma include perineural invasion, lymphovascular invasion, and metastases. Criteria for classification of invasive carcinomas into specific subtypes based on patterns of differentiation (Table 3) are described below.

**Discussion.** In contrast to PIN, because of the presence of lymphatics and vessels in prostatic stroma and surrounding tissue, invasive carcinomas in human and mouse have metastatic potential, as well as the increased capacity to cause morbidity due to local/regional effects. In addition to local invasive growth within prostatic parenchyma (including the contractile rim of fibromuscular stroma and the surrounding loose connective tissue in the GEM prostate), other properties of invasive carcinoma in human Pca that may be recognized in the GEM prostate include perineural invasion, lymphovascular invasion, and metastases. These properties are discussed in more detail, followed by definitions and characteristics of the subclassifications of invasive carcinomas in GEM prostates.

**Perineural Invasion in Pca.** Human Pca has a well-recognized characteristic tendency to invade perineural spaces. In human prostate pathology, properly defined perineural invasion is pathognomonic of carcinoma on biopsy (8, 9). It is observed in ~20% of transrectal biopsies with Pca, in which case it may correlate with ECE in subsequent RP (90, 91) and/or help in the decision to sacrifice the neurovascular bundle ipsilateral to the side with perineural invasion, potentially reducing the risk of positive surgical margins at foci of ECE in RPs (92). Perineural invasion is present in the vast majority (75–90%) of totally submitted RP specimens. The prognostic significance is the subject of ongoing investigation (93).

In the mouse prostate, nerve bundles are not histologically conspicuous within the thin rim of fibromuscular stroma surrounding individual acini, but rather are seen in the surrounding loose connective tissue. Prostatic perineural invasion by carcinoma has been observed in multiple GEM models (29, 33). However, these particular models develop obvious destructive prostatic stromal and periprostatic invasion by carcinoma, so that it is not yet known if perineural invasion will be observed in mouse models as the only definitive parameter of invasion in occasional cases.

**Lymphovascular Invasion in Pca.** Lymphovascular invasion can be identified in a significant minority of human RP specimens, including those without identifiable pelvic lymph node metastases (94, 95). The frequency of lymphovascular invasion is greater in stage pT3 (versus pT2) tumors, and in such patients, tumor lymphatic invasion may have independent prognostic significance for biochemical progression (elevated serum PSA) after RP (94, 95).

Lymphovascular invasion has been reported with carcinoma in the prostate of GEM (33, 37), presumably in lymphatic spaces in the loose connective tissue between acini rather than within the thin rim of fibromuscular stroma surrounding acini. It is also presumed that lymphovascular invasion in mouse prostate tumor models will uncommonly be identified in the absence of obvious (stromal) invasive carcinoma, but there is little specific information regarding this thus far. Furthermore, as in routine human surgical pathology, caution must be exercised regarding possible artificial histologically identified lymphovascular invasion. The presence of large amounts of potentially fragile or friable in situ atypical proliferations can lead to implantation of tumor within vessel spaces either during grossly cutting poorly fixed specimens or during histological sectioning. Attention should be paid to the usual morphological features of true lymphovascular invasion as described in human Pca (e.g., shape of focus, adhesion to vessel wall, and subtle cytologic changes; Ref. 94) as well as presence or absence of associated unequivocal invasion, its extent, and its spatial relationship to the focus of possible lymphovascular invasion.

**Metastases in Pca.** The presence of metastatic Pca is essentially diagnostic of invasive carcinoma, as neoplasia confined to in situ basement membrane-contained lesions should not have access to lymphatic or vascular spaces necessary for regional or systemic spread. However, as in human Pca, tumor metastasis would not be expected to be a very sensitive indicator of invasive disease in GEM. For example, all of the patients undergoing RP have documented invasive disease; however, in most current practice settings, lymph node metastases are present in only 2–5% of RP and bilateral pelvic lymph node dissection specimens (14, 17, 96).

All of the GEM models of Pca reported thus far as developing metastatic tumor have had unequivocal invasion at similar or earlier time points. However, metastases are occasionally noted in individual animals without documented invasive histologically similar primary tumors in the prostate. This could represent a sampling issue, as there is some indication that metastases from mouse Pcacs can occur in association with very small foci of invasive tumor (38). However, if this situation is encountered, several other explanations should be considered: (a) the metastasis could come from another completely unrelated site, which should always be excluded in transgenic mice made with nongenetic promoters or in genomic knockout models; (b) the primary could be in other male accessory glands, such as the periurethral or BUGs, which develop invasive tumors histologically similar to prostate tumors in some models made with reportedly prostate-selective promoters; and (c) a poorly differentiated spindle cell tumor in a distant site could actually represent a metastatic sarcoma, which should be a consideration in models that are developing stromal hyperplasia and possibly neoplasia in association with neoplastic epithelial proliferation. In this last case, ancillary studies, such as CK immunostains and electron microscopy, can be useful for distinguishing carcinoma from sarcoma. Sarcomas typically metastasize by hematogenous routes, very characteristically to liver and lung. However, several GEM models developing NE carcinomas have shown liver and lung metastases, and this pattern of visceral metastasis is also characteristic of Pca with NE differentiation in the human (97). Lymph node metastases are quite characteristic of carcinoma, in general, and pelvic lymph nodes are a typical site for metastasis of Pca in the human. Para-aortic/abdominal lymph node metastases have been noted in multiple GEM models of prostatic neoplasia.

It was the consensus opinion of the Pathology Panel that immuno-
Fig. 9. Invasive neuroendocrine (NE) carcinomas and carcinomas with morphological features suggestive of NE differentiation in prostate and metastases in genetically engineered mice models. A–C, invasive NE carcinoma in CR2-SV40 mouse. A, intermediate power showing invasive carcinoma (*) in association with prostatic intraepithelial neoplasia (PIN) in multiple gland profiles (arrowheads). B and C, low- and high-power photomicrographs of extensively invasive carcinoma. The invasive foci show a generally solid or sheet-like proliferation, but with evident rosettes throughout (arrowheads in C). On high magnification, cells with typical nuclear features of NE carcinoma focally have a moderate amount of eosinophilic cytoplasm, particularly evident in areas of rosette formation (arrowheads in C). Note that focal glandular differentiation can be seen in human NE carcinomas. The tumors in these mice are mucin-negative. In addition to the morphology illustrated here, foci in which tumor cells have less cytoplasm and more oval or spindle hyperchromatic nuclei can be seen, similar to human small cell carcinoma. The PIN, invasive, and metastatic lesions in this model show cytologic, immunophenotypic, and ultrastructural features indicative of NE differentiation. D–L, invasive and metastatic NE carcinoma in the LPB-Tag 12T-10 model. D, intermediate power showing extensive invasion by NE carcinoma, including extensive areas of “less differentiated” small cell carcinoma (*), in ventral prostate of a 40-week-old mouse. An entrapped PIN gland is seen (white arrowhead), as are two other PIN containing profiles at top and bottom left (black arrowheads). Some foci in the invasive tumor show “punched out” cribriform like areas with eosinophilia due to cell cytoplasm in areas of rosette formation. Areas in this field with more solid growth and extreme cellularity, with closely spaced oval or spindle cells show focal crush “artifact” or Azzopardi effect, which is also characteristic of human small cell carcinoma. E, intermediate magnification, showing extensive involvement of periprostatic tissue by NE tumor (*), encroaching on adjacent PIN-gland and its surrounding stroma (arrowhead). Extensive rosette formation is evident. F, strong focal chromogranin immunostaining (apical cytoplasmic granular, arrowheads) in the in situ component (PIN) of 12-month-old LPB-Tag 12T-10 mouse, which also had extensive invasive NE carcinoma. G, focal strong cytoplasmic granular chromogranin immunostaining (*) in liver metastasis. H, Metastatic NE carcinoma (*) in the liver of a 40-week-old 12T-10 mouse. Liver metastases were common with increasing age in this mouse, and often showed prominent rosetting. NE differentiation was demonstrated in such metastases by chromogranin immunostaining, as in G, and by electron microscopy. I, two pulmonary micrometastases (*) in 44-week LPB-Tag 12T-10 mouse. Lung metastases are typically smaller than liver lesions, are often in alveolar septa or peribronchial arterial spaces, and are more typically composed of oval or spindle cells with scant cytoplasm and without prominent rosette formation. J–L, invasive carcinoma with morphological features suggestive of NE differentiation in TRAMP mouse. J and K, intermediate- and high-power photomicrographs of prostate from 24-week-old TRAMP mouse showing extensive destructive invasion (*) in J) between residual PIN glands (arrowheads in J). The tumor shows relatively solid growth, but with evident cribriform or gland-like spaces, recognizable at lower magnifications, and confirmed at high magnification (arrowheads in K). This lesion, reported previously as moderately differentiated adenocarcinoma, shows most nuclei contain granular chromatin. Admixed spindle or oval cells with more hyperchromatic and/or pyknotic nuclei and abundant rosette or rosette-like spaces are noted. The consensus opinion of the Pathology Panel was that this morphology was highly suggestive of a carcinoma with NE differentiation, based on experience with human tumors as well as the morphological similarity to well-documented NE carcinomas in other genetically engineered mice (e.g., compare with B–E). Similar morphology has been noted in metastatic foci. L, less-differentiated focus in prostate from another 24-week-old TRAMP mouse. Tumor reported previously as poorly differentiated carcinoma was felt to show morphological features of NE differentiation, quite similar to human small cell carcinoma. Tumor is composed of closely spaced oval and spindle cells with scant cytoplasm and hyperchromatic nuclei. Metastatic foci, including pulmonary metastases, can show similar morphology. Similar cytologic features have been noted in the invasive and metastatic tumors in other mouse models (i.e., CR2-SV40 and LPB-Tag 12T-10) in which the tumor has been confirmed to show immunophenotypic and ultrastructural NE differentiation. The foci in TRAMP as shown in L and regarded as small cell carcinoma by the Pathology Panel have been subsequently shown to be synaptophysin immunopositive, whereas such staining was not noted in foci as shown in J and K.28
stains for a particular transgene (e.g., large T antigen in the case of e.g., a priori SV40 or Tag-based models) cannot be used as modular formation.

formed glands (Fig. 8, percentage of glandular formation. cinomas of the prostate of GEM should be additionally designated as for NE carcinoma below. In the Bar Harbor Classification, adenocarcinomas refer to tumors in which extensive gland formation is still evident, but there are admixed foci showing gland fusion or solid areas (Fig. 8, E and F). Poorly differentiated adenocarcinomas are those in which invasive foci are composed predominantly of nests or solid sheets, but in which glandular formation is focally present.

Discussion. It was the opinion of the Pathology Panel that specific percentages for the amount of an invasive tumor showing gland formation would not be specified for the purpose of the classification of differentiation of adenocarcinoma in GEM. If progressive dedifferentiation is observed in a given model, with less gland formation observed in invasive foci with age, the approximate percentage of gland formation in individual tumors can be stated as a function of time. It should be noted that the terms designating differentiation in the GEM classification of prostate adenocarcinoma do not translate to similar terms in human prostate pathology. Although differentiation in human Pca is more often stated by Gleason score or pattern, there is a general translation to these more descriptive terms, such that well-differentiated tumors correspond to typically TZ located Gleason pattern 1 and 2 (Gleason score 2–4) tumors (Table 4). Moderately differentiated tumors are composed of Gleason score 5 and 6 tumors, and, hence, predominantly include Gleason pattern 3 tumors, composed of well-formed small glands, as commonly seen in PZ tumors associated with HGPIN. A morphologically similar tumor (composed entirely or predominantly of discreet and well-formed glands) arising in association with mPIN in a GEM model would be classified as well differentiated. A summary of the classification of adenocarcinoma in GEM prostate is shown in Table 5. NE differentiation (e.g., based on ancillary methods such as immunohistochemistry or electron microscopy) in an otherwise more usual adenocarcinoma is addressed below. Poorly differentiated carcinomas, which do not show such NE differentiation, may be classified as poorly differentiated adenocarcinomas if focal gland formation is evident or if ultrastructural examination or other ancillary techniques show focal glandular or secretory differentiation. Otherwise, invasive epithelial tumors that do not show specific differentiation as outlined in Table 3 should be classified as undifferentiated carcinomas.

NE Carcinoma (Fig. 9)

Definition. NE Carcinoma [23]. NE carcinoma in GEM prostate is an invasive malignant epithelial neoplasm that shows light microscopic, immunohistochemical, and ultrastructural features indicative

| Table 4 Differentiation classification of human prostate adenocarcinoma |
|---------------------------------|-------------------------------|
| Differentiation                  | Gleason score | Common | Histologic/cytologic description |
| Well differentiated              | 2–4           | One    | Typically TZ: circumscribed, fairly uniformly sized, closely spaced glands; also BPH-like larger glands with ample clear cytoplasm (lipid), well-defined cell borders, basally situated relatively bland nuclei |
| Moderately differentiated        | 5–6           | Three  | Typically TZ: partially circumscribed, closely spaced but more variably sized glands with generally clear cytoplasm similar to benign prostate glands |
| Moderately poorly differentiated  | 7             | Four   | Common in PZ, can be seen in TZ; in PZ, commonly associated with HGPIN, small acinar forming, more infiltrative with greater intervening stroma and/or infiltration between benign glands; eosinophilic cytoplasm |
| Poorly differentiated            | 8–10          | Five   | Characterized by infiltrating cords of cells and single cells; large solid growths, including with comedo necrosis |

a TZ, transition zone; BPH, benign prostatic hyperplasia; PZ, peripheral zone; HGPIN, high-grade prostatic intraepithelial neoplasia.
of NE differentiation, corresponding primarily to the presence of
cytoplasmic neurosecretory-type granules.

Criteria/Explanation. The designation of a mouse tumor as an NE
carcinoma is based on the presence of characteristic histological and
cytologic properties, which can be confirmed by ancillary techniques.
NE carcinomas can take the form of solid and cribriform growth with
interspersed gland-like spaces from rosette formation. Although roset-
ettes can mimic gland formation, these tumors differ from adenocar-
cinoma in that NE carcinomas do not show well-defined true glandu-
lar formation or extensive secretory differentiation. Tumor cells may
have moderate eosinophilic cytoplasm, often appreciated in areas of
rosetting. The nuclei are oval or round and have finely granular
chromatin. Small cell carcinomas are a subtype of NE carcinoma,
which are histologically and cytologically similar or identical to the
well-known small cell carcinoma of the human lung. NE carcinoma
(not otherwise specified) and small cell carcinoma may coexist. Small
well-known small cell carcinoma of the human lung. NE carcinoma
chromatin. Small cell carcinomas are a subtype of NE carcinoma,
rosetting. The nuclei are often oval or round and have finely granular
appearance highly reminiscent of human lung small cell carcinoma,
in which one nucleus appears to indent into an adjacent nucleus, which
forms to the shape of the former.

NE carcinoma and small cell carcinoma should be immunopositive
for one or more markers of neurosecretory differentiation, such as
chromogranin (CG) and synaptophysin, at least focally. Ultrastructur-
ally, NE carcinoma cells should show dense core (NE type) secretory
granules, at least focally. The approach to the recognition and classi-
fication of NE carcinomas is outlined in Table 6.

Discussion. Invasive NE carcinoma has been observed in several
SV40 and large T antigen-based transgenic mouse models, based on
recognition of morphology typical of NE carcinomas in human pa-
thology and with subsequent confirmation by immunohistochemistry
and electron microscopy (Fig. 9, A–I; Refs. 33, 38, 84). At the time of
the Bar Harbor meeting, NE differentiation in the invasive tumors in
other SV40-based models was suggested by similar light microscopic
morphological features to these documented NE carcinomas in GEM
(Fig. 9, K and L). Classification of a mouse tumor as an NE carcinoma
is based on the presence of characteristic histological and cytologic
properties, which can be confirmed by ancillary techniques (Table 6),
and not the demonstration of focal immunostaining for traditional NE
markers in an otherwise histologically recognized adenocarcinoma.

It should be noted that NE differentiation can be seen focally in
many and maybe even most otherwise typical human prostate adenocar-
cinomas using immunohistochemistry (8, 9). The prognostic sig-
nificance of such NE differentiation, the increase in such differenti-
ation with tumor progression, and its relationship to the development
of hormone refractoriness remain a subject of controversy in human
prostate pathology (8, 9). In addition, there is a minority of human
PCs that show characteristic light microscopic morphological fea-
tures of NE carcinomas, including small cell carcinomas, as more
typically associated with other organ sites such as the lung (8, 9).
The designation of a mouse prostate tumor as an NE carcinoma is for
precise pathological characterization. Correct tumor classification will
allow the accumulation of information regarding phenotype and tumor
behavior, genetic alterations, and treatment response. Although there
are several preliminary observations relating this phenotype to andro-
gen insensitivity (33, 38, 55, 61), there is currently insufficient data to
allow any specific biological inferences regarding this morphology in
GEM models.

Because rosette formation mimicking gland formation can be evi-
dent in NE carcinomas (Fig. 9), “gland-like” spaces are not incom-
patible with a diagnosis of NE carcinoma. This pattern of NE carci-
noma, somewhat reminiscent of large cell NE carcinoma in the human
lung (98), may be admixed with or appear to transition to foci with the
appearance highly reminiscent of human lung small cell carcinoma,
either classic “oat cell” carcinoma or intermediate cell type (8, 9, 97,
98). In addition to hyperchromatic nuclei, which may show nuclear
“molding,” small cell carcinomas often show crush artifact, in which
smears hyperchromatic material is seen in hypercellular areas and
corresponds to DNA-rich material (“Azzopardi effect”). Small cell
carcinoma in the human prostate may be immunopositive for PSA and
negative for NE markers such as neuron-specific enolase or negative
for PSA and positive for neuron-specific enolase. Although data are
based on relatively small numbers, patients with these tumors appear
to be hormone insensitive, have an increased incidence of visceral
(i.e., liver and lung) rather than lymph node metastases, and may
respond to established small cell chemotherapy (97). Because tumors
in human and mice with this small cell carcinoma morphology can be
seen in association with NE carcinomas with rosetting and more
cytoplasm, in the Bar Harbor Classification, small cell carcinoma is
regarded as a less-differentiated form of NE carcinoma, rather than as
a separate entity (Table 3). Although little data exist yet in GEM
regarding degree of NE differentiation in tumors with these two

Table 6. Neuroendocrine (NE) carcinoma: considerations and classification in genetically engineered mice (GEM) prostate

<table>
<thead>
<tr>
<th>Criteria/Explanation</th>
<th>Discussion</th>
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<tr>
<td>NE differentiation may be demonstrated in usual acinar carcinoma.</td>
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<td>Small cell carcinomas are commonly seen in association with usual acinar carcinoma or arise in patients with a prior diagnosis of more usual acinar adenocarcinoma.</td>
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<tr>
<td>Small cell carcinomas have a variable immunophenotype regarding presence of usual NE markers. Small cell carcinoma in the human is diagnosed on the basis of its cytologic appearance.</td>
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<tr>
<td>Approach to tumors in GEM with histologic features suggestive of NE differentiation:</td>
<td></td>
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<tr>
<td>Tumors with light microscopic appearance similar or identical to those illustrated as NE carcinoma herein (that have been substantiated by immunohistochemistry and electron microscopy), should be regarded as potentially being NE carcinomas. NE differentiation should be confirmed by ancillary techniques. Immuno staining should employ at least two different markers, e.g., chromogranin and synaptophysin. Positive immunostaining for either is sufficient to designate such tumors as NE carcinoma (when morphology is as shown for NE carcinoma herein). Punctate perinuclear CK8 immunostaining is supportive but less specific. Positive immunostaining for NE markers can be substantiated by ultrastructural examination in order to demonstrate dense core neurosecretory granules. Negative immunostaining should be followed by electron microscopy. As NE type granules can be focal and small in number, examination should be rigorous. Consultation with members of the MMHCC Pathology panel is encouraged. If NE type secretory granules are identified, they are sufficient to designate such tumors as NE carcinoma (when morphology is as shown for NE carcinoma herein). In the absence of immunohistochemical or ultrastructural confirmation of NE differentiation, tumors with suggestive morphology can be designated as carcinoma with NE differentiation or carcinoma with NE features. Tumors with glandular differentiation (adenocarcinoma) or without histologic features of either glandular or NE differentiation (undifferentiated carcinoma) that have NE differentiation demonstrated by immunohistochemistry or electron microscopy can be designated as adenocarcinoma (or carcinoma) with NE differentiation. The specific ancillary techniques employed and their results should be specified. Tumors with cytologic features typical of small cell carcinoma in human lung and human prostate should be designated as small cell carcinoma, regardless of the immunophenotype. It is encouraged that immunohistochemistry for NE markers still be performed on these tumors in order to collect potentially useful data for future classification.</td>
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Summary of NE differentiation in human and GEM prostate carcinoma

The relationship between NE differentiation determined immunophenotypically and prognosis in human Pca is not established.

Glandular differentiation and NE differentiation in human Pca and potentially in GEM tumors are not mutually exclusive. In human Pca:

- NE differentiation may be demonstrated in usual acinar carcinoma.
- Small cell carcinomas are commonly seen in association with usual acinar carcinoma or arise in patients with a prior diagnosis of more usual acinar adenocarcinoma.
- Small cell carcinomas have a variable immunophenotype regarding presence of usual NE markers. Small cell carcinoma in the human is diagnosed on the basis of its cytologic appearance.

Approach to tumors in GEM with histologic features suggestive of NE differentiation:

- Tumors with light microscopic appearance similar or identical to those illustrated as NE carcinoma herein (that have been substantiated by immunohistochemistry and electron microscopy), should be regarded as potentially being NE carcinomas. NE differentiation should be confirmed by ancillary techniques. Immuno staining should employ at least two different markers, e.g., chromogranin and synaptophysin. Positive immunostaining for either is sufficient to designate such tumors as NE carcinoma (when morphology is as shown for NE carcinoma herein). Punctate perinuclear CK8 immunostaining is supportive but less specific. Positive immunostaining for NE markers can be substantiated by ultrastructural examination in order to demonstrate dense core neurosecretory granules. Negative immunostaining should be followed by electron microscopy. As NE type granules can be focal and small in number, examination should be rigorous. Consultation with members of the MMHCC Pathology panel is encouraged. If NE type secretory granules are identified, they are sufficient to designate such tumors as NE carcinoma (when morphology is as shown for NE carcinoma herein). In the absence of immunohistochemical or ultrastructural confirmation of NE differentiation, tumors with suggestive morphology can be designated as carcinoma with NE differentiation or carcinoma with NE features.
- Tumors with glandular differentiation (adenocarcinoma) or without histologic features of either glandular or NE differentiation (undifferentiated carcinoma) that have NE differentiation demonstrated by immunohistochemistry or electron microscopy can be designated as adenocarcinoma (or carcinoma) with NE differentiation. The specific ancillary techniques employed and their results should be specified.
- Tumors with cytologic features typical of small cell carcinoma in human lung and human prostate should be designated as small cell carcinoma, regardless of the immunophenotype. It is encouraged that immunohistochemistry for NE markers still be performed on these tumors in order to collect potentially useful data for future classification.

* Pca, prostate cancer; MMHCC, Mouse Models of Human Cancer Consortium.
Squamous Cell Carcinoma and Adenosquamous Carcinoma

**Definitions.** Squamous Cell Carcinoma [24]. Squamous cell carcinoma is an invasive malignant epithelial neoplasm that shows light microscopic features of squamous differentiation, typically in the form of keratinization and/or intercellular bridges.

Adenosquamous Carcinoma [25]. Adenosquamous carcinoma is an invasive malignant epithelial neoplasm that shows an admixture of foci with squamous differentiation and foci with glandular differentiation.

**Criteria.** These lesions should be diagnosed based on the presence of features recognized in such tumors in human and veterinary pathology in prostate and other sites (8, 9). Features of adenosquamous carcinoma are described above.

**Discussion.** Primary squamous cell carcinomas are extremely rare in the human prostate, and most typically arise from the prostatic urethra. They have not been reported in the prostate of GEM. The same is true for adenosquamous carcinoma.

Spindle Cell or Sarcomatoid Carcinoma

**Definition.** Spindle Cell Carcinoma [26]. Spindle cell or sarcomatoid carcinoma is a malignant spindle cell lesion in which there is unequivocal epithelial differentiation detected by immunohistochemistry or by ultrastructure (8, 9). It differs from adenocarcinoma by the absence of foci with well-formed glands.

**Criteria.** Such tumors should be diagnosed based on the presence of features recognized in such tumors in human and veterinary pathology. Tumors composed of foci showing glandular differentiation and foci of spindle cell carcinoma should be classified as adenoscarcinoma (moderately or poorly differentiated depending on extent of glandular differentiation), with a detailed description of the less-differentiated or sarcomatoid area, including documentation of its epithelial nature. The latter is best demonstrated by CK immunostaining and/or by ultrastructural examination as in standard pathology practice. These tumors differ from carcinomas in that the spindle cell component contains markers indicative of epithelial differentiation. They have been referred to recently as epithelial-mesenchymal-transition tumors in the mouse pathology literature.

**Discussion.** Spindle cell or sarcomatoid carcinoma has not been specifically reported in the prostate of GEM, although it needs to be considered in the differential diagnosis of poorly differentiated (undifferentiated) carcinomas, carcinomas, carcinosarcomas, and sarcomas, as described below.

Undifferentiated Carcinoma

**Definition.** Undifferentiated Carcinoma [27]. Undifferentiated carcinoma is an invasive malignant epithelial neoplasm (carcinoma) that shows no glandular, NE, squamous, or spindle cell differentiation by light microscopy.

**Criteria.** Undifferentiated carcinomas show a nested or sheet-like growth pattern, and their content of cytoplasm and cohesive growth help support the diagnosis of carcinoma over other neoplasms (e.g., sarcoma). Epithelial nature can be confirmed by CK immunostaining or ultrastructural examination. If features of specific differentiation are evident only with special techniques (e.g., electron microscopy), such tumors may be appropriately referred to as poorly differentiated carcinoma with reference to the direction of differentiation (e.g., poorly differentiated carcinoma with glandular differentiation or poorly differentiated adenoscarcinoma; poorly differentiated carcinoma with NE differentiation or poorly differentiated NE carcinoma).

Neoplastic Proliferation of the Stroma

**Benign Soft Tissue Neoplasms and Sarcomas (Fig. 10)**

**Definitions.** Benign Soft Tissue Tumor [28]. A benign soft tissue tumor is a neoplasm that arises from or differentiates toward prostatic stroma or specific differentiated mesenchymal tissues (such as smooth muscle) and lacks features indicative of malignancy.

Malignant Soft Tissue Tumor [29]. A malignant soft tissue tumor (sarcoma) is a neoplasm that arises from or differentiates toward prostatic stroma or specific differentiated mesenchymal tissues (such as smooth muscle) and possesses one or more features indicative of malignancy.

**Criteria/Explanation.** These designations are used for neoplastic growths satisfying usual human and veterinary pathology diagnostic criteria for benign and malignant mesenchymal neoplasms. Features indicative of malignancy that are absent from benign tumors and variably present in sarcomas include destructive invasive growth, extensive necrosis, prominent mitotic activity, and markedly increased cellularity, particularly if accompanied by nuclear atypia or prominent pleomorphism. Such soft tissue neoplasms have rarely been observed or reported in GEM. For now, if such lesions are encountered, they should be diagnosed and classified according to standard pathology practice. A few practical considerations are offered in the following discussion.

**Discussion.** Mesenchymal Neoplasms in the Human Prostate. In the human prostate, the most common sarcoma in the pediatric population is rhabdomyosarcoma, usually of the embryonal type (8, 9). In the adult human prostate, benign stromal nodules are extremely common, increasing with age (Fig. 10, A and B). They are located typically in the glandular poor periurethral region, and are usually accompanied by glandular and stromal hyperplasia in the TZ as part of BPH. Benign circumscribed lesions with histology and immunophenotype of smooth muscle, and hence classifiable as leiomyomas, occur uncommonly (Fig. 10, C and D; Refs. 8, 9). Rare proliferations of the specialized prostate stroma occur that demonstrate increased cellularity, atypia, and mitoses (8). Some stromal proliferations in the human prostate appear to have an associated epithelial component, imparting a biphasic appearance. The epithelial or glandular spaces...
can exhibit a “leaf-like” growth pattern, quite similar to the phyllodes tumor more typical in the human breast (8, 9). Such lesions in the human prostate are best regarded as low-grade and, less commonly, high-grade malignant neoplasms (rather than hyperplasias), as they have occasionally demonstrated local recurrence and even metastases (8, 9). The most common sarcoma in the adult human prostate is leiomyosarcoma (8, 9). Criteria for distinguishing potentially malignant from benign smooth muscle neoplasms include the presence of infiltration, nuclear atypia, significant pleomorphism, necrosis, and appreciable mitotic activity (8, 9).

Mesenchymal Lesions in the GEM Prostate. Stromal hypercellularity is a prominent feature of the prostate in some GEM models (Fig. 5, A-C). It is a prominent feature of the AP and DP/DLP of the faster growing LPB-Tag lines (Ref. 37; Fig. 5A). The stromal hypercellularity is diffuse in these lobes (accompanying the marked epithelial proliferation), generally increases with age, and the cells and surrounding extracellular collagenous stroma have morphology in keeping with prostate stroma. Similar, but potentially lesser, stromal hypercellularity has been observed in the prostate of TRAMP mice (Ref. 83; Fig. 5B).

Similar extensive or focal stromal hypercellularity has also been observed in the smooth muscle wall of the seminal vesicle in LPB-Tag and TRAMP mice (84). In the prostate, these changes are too diffuse and too morphologically similar to normal mouse prostate stroma to represent desmoplasia. Whether they occur as a paracrine response to neoplastic epithelial cells or as a more direct consequence of transgene expression in these cells remains to be further resolved. When not associated with a distinct circumscribed or destructive growth pattern suggestive of neoplasia, such lesions should be described as hyperplasia, either focal or diffuse, and with or without atypia, as described above.

We have noted in the background of more diffuse stromal hypercellularity in some LPB-Tag mice, somewhat discreet foci that stand out as more cellular, and which show increased pleomorphism and mitoses (85). The natural history of such lesions in this or other models

29 S. B. Shappell, R. Barrios, R. Herbert, et al., unpublished observations.
has not been examined. Neoplasms properly classified as sarcomas have rarely been noted in the prostate of GEM, so that their true incidence in different models or under different experimental manipulations remains to be clarified. In addition to poorly differentiated carcinomas with histological features suggestive of NE differentiation emerging in castrated animals from fast-growing LPB-Tag lines, foci of increased stromal hypercellularity with atypia similar to that described above and lesions compatible with frank sarcomas are occasionally noted (55). One lesion in a 12T7s mouse castrated at 25 weeks and maintained for an additional 25 weeks developed a sarcoma with histological features compatible with leiomyosarcoma. The tumor is composed of highly atypical and mitotically active spindle cells organized in well-formed intersecting fascicles typical of leiomyosarcoma (Fig. 10, E and F). As the mouse had other foci involved by poorly differentiated carcinoma with NE differentiation, consideration could be given to a designation of carcinosarcoma as described below. Some of the specific sarcoma subtypes are included in the Bar Harbor Classification for completeness and anticipation of the possible spectrum of neoplasms that may occur in the GEM prostate. However, to our knowledge, tumors such as rhabdomyosarcoma, chondrosarcoma, and osteosarcoma have not been observed. If encountered, they should be diagnosed based on standard practices in veterinary and human pathology.

**Distinction of Carcinoma and Sarcoma.** When a poorly differentiated neoplasm with oval or spindle cells is encountered in the prostate and/or as a metastatic lesion, designation as carcinoma should be supported if necessary by either convincing CK (typically a pan-CK or CK8, not HMWCK such as CK5 or CK14) immunostaining or by ultrastructural examination. Spindle cell neoplasms may also be small cell carcinomas as described above, which can have weak CK immunostaining. Inclusion of appropriate NE markers (CG or synaptophysin) can address this potential differentiation (along with electron microscopy). Vimentin immunostaining is also theoretically useful in this setting, as strong vimentin and absent CK staining support a sarcoma. However, few if any successful mouse vimentin immunostaining protocols have been described. If the differential diagnosis includes a specific sarcoma, antibodies for characteristic differentiation markers, such as smooth muscle actin for possible smooth muscle tumor, can be added. Sarcomatoid (or spindle cell) carcinoma defined above can have focal or weak vimentin immunostaining (in addition to CK immunostaining).

**Neoplastic Proliferations of Stroma and Epithelium**

**Carcinosarcoma**

**Definition.** Carcinosarcoma [30]. Carcinosarcoma is a truly biphasic malignant lesion in which areas of unequivocal carcinoma and unequivocal sarcoma are both present.

**Criteria/Explanation.** Any detected lesions in GEM should be diagnosed according to similar principles in human prostate pathology (8, 9). The epithelial component may be poorly differentiated adenocarcinoma, or show morphology of another carcinoma, such as squamous cell carcinoma. These tumors differ from the sarcomatoid carcinomas in that the spindle cell component does not demonstrate epithelial markers. The sarcoma may be a sarcoma, not otherwise specified, show features of leiomyosarcoma, or show heterologous differentiation, such as a rhabdomyosarcoma or osteosarcoma (8, 9).

**Discussion.** Carcinosarcomas are rare, highly aggressive tumors in the human prostate. Most have been observed with disease progression after hormonal and/or radiation treatment in patients with previously diagnosed more typical prostate adenocarcinoma (8, 9). Although radiation treatment could in theory contribute to the tumor “dedifferentiation,” these tumors can develop without the history of such pretreatment, precluding definitive association. Epithelial-mesenchymal transformation could underly the development of any such biphasic lesion observed in GEM prostates.

**Pathology of Disorders of the Periurethral Glands and BUGs (Table 3; Fig. 11)**

In general, definitions and diagnostic criteria are similar to those described above for the homologous lesions in the prostate. Pertinent classification guidelines and discussion based on observed lesions in existing GEM models are offered below.

**Developmental Disorders of the Periurethral Glands and BUGs.** Given the relationship between the embryonic development of the prostate and these other ductular/glandular derivatives of the urogenital sinus and their shared androgen dependence, genetic manipulations resulting in abnormal prostate development may be expected to also give rise to related abnormalities in these other male accessory glands. As such, agenesis and hypoplasia should be defined and these designations applied as described above for the prostate. For example, abnormal development of secretory differentiation compatible with hypoplasia in the BUGs was noted in Nkx3.1 knockout (Nkx3.1 −/−) mice, with reduced mucin-producing epithelium accompanying a corresponding increase in ductular epithelium (54).

**Hyperplastic and Neoplastic Proliferations of the Periurethral Glands and BUGs. Hyperplasia and Atypical Hyperplasia.** Epithelial and/or stromal hyperplasias should be described as for prostate, with attention to their focal or diffuse nature and presence or absence of atypia. Description of extent can include involvement of multiple discreet periurethral or BUG gland lobules as well as that within any given focus. Presence or absence of bilateral nature of involvement should be noted for the BUGs. Thus far, observed epithelial proliferations in these sites have been accompanied by cytologic atypia, similar to the prostate lesions in their corresponding mouse models, and stromal proliferation has not been appreciated. Possible precursor lesions in which atypical epithelium is noted in periurethral glands or BUGs without obvious stromal invasion can be designated as atypical hyperplasia (Fig. 11, A, B, and H). These lesions show nuclear enlargement and hyperchromasia with frequent mitotic figures and apoptotic bodies. These foci typically appear to conform to the normal glandular architecture, the smallest of which appear to be localized in the ducts of these accessory glands. More expansile forms may be seen with progression, with an apparent increase in slightly irregular new ductular or glandular profiles, but still without extension into surrounding stroma (41). More extreme forms with a nodular or micropapillary growth pattern have been reported as atypical nodular hyperplasia (41). These lesions (without frank invasion) should be classified as atypical hyperplasia, and terms such as nodular or micropapillary can be added as modifiers, along with other descriptions, such as increased nuclear atypia, to document apparent progression of the lesion in any given model.

**Carcinoma.** Invasive carcinomas in the periurethral glands and BUGs should in general be classified according to the same guidelines described above for the prostate. Carcinomas with focal and ill-defined, but definitive, glandular formation have been observed, including lesions associated with prominent stromal fibrosis and referred to as scirrhous carcinoma. Such a lesion would be classified as poorly differentiated adenocarcinoma in the current classification scheme, based on the criteria outlined above for prostate (Table 5). In addition, invasive tumors with histological and cytologic features typical of NE carcinoma have been observed in multiple models (Fig. 11, C–G). As described above for prostate, such tumors should be

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30 J. M. Ward, personal communication.
arrows acini are seen (arrowheads). As this promoter targets a neuroendocrine epithelial cell population in the prostate in an apparently androgen-insensitive manner, it is tempting to conform to the normal duct lining. There is nuclear enlargement, hyperchromasia, and chromatin clumping, and ample eosinophilic cytoplasm. Residual periurethral gland shown herein, however, these lesions have not been noted to progress to frank carcinoma.

Speculate whether a minor NE cell population in these other accessory glands is the target for this lesion. In contrast to at least the C3(1)-SV40 and occasional LPB-Tag tumors in the C3(1)-SV40 mouse. Low power (A) shows a large tumor focus involving periurethral region (urethral lumen, black * arrowhead). Distinguishing the actual site of origin could be difficult. Note more normal-appearing prostate gland lumens at right. The single small nodule of tumor in this region (arrow) would be most compatible with secondary involvement at this site (i.e., extension or spread from tumor of periurethral region or from other part of prostate) as no prostatic intraepithelial neoplasia is present in adjacent portions of prostate. Prostatic intraepithelial neoplasia is commonly noted with invasive tumor originating in the prostate; D, high-power magnification of the tumor in C, showing occasional gland or rosette formation (arrowhead). Most tumor cells show scant cytoplasm, with a high nuclear:cytoplasmic ratio. Many of the nuclei are hyperchromatic, with occasional nuclear molding, features typical of NE differentiation. The differential diagnosis includes poorly differentiated adenocarcinoma versus NE carcinoma. Tumors with morphology typical of NE carcinomas (as illustrated for the prostate in Fig. 9 and for other accessory glands below) should be designated as such. Immunohistochemistry or electron microscopy can be used to confirm the NE nature suggested by the characteristic histological appearance. Adenocarcinomas in human, and potentially in mice, may show features of NE differentiation upon ultrastructural or immunohistochemical analysis. In lesions with definitive and predominant glandular differentiation, this can be designated as carcinoma, or adenocarcinoma, with NE differentiation, rather than as NE carcinoma. Envolvement of bulbourethral gland by NE carcinoma in a 44-week LPB-Tag 12T-10 mouse. Focal tumor cell apoptosis is present in center (arrowheads). Urethral lumen is to top left (*). F, involvement of periurethral glands by NE carcinoma in a 17-week-old LPB-Tag 12T-7s mouse. Urethral lumen (+) and urethral mucosa are to top left. Nuclear features and rosetting (arrowhead) typical of NE differentiation identifiable by light microscopy alone are present. Note similar morphological appearance of tumors in E and F to NE carcinomas arising in the prostate as shown in Fig. 9. G, extensive involvement of periurethral region (center) and proximal portions of prostate (far right and far left) by poorly differentiated neoplasm compatible with NE carcinoma (including poorly differentiated or small cell carcinoma) in 24-week-old TRAMP mouse. Tumor bulges into urethral lumen (+). Focal residual normal periurethral glands are noted at left and bottom left relative to urethral lumen (arrowheads). Whether such tumors in this region are ever found without morphologically identical large tumor apparently arising in the prostate has not been described in this model. The androgen regulation of the periurethral and bulbourethral glands mandates careful examination of these tissues in transgenic mouse models made with androgen-regulated promoters. H, focal atypical hyperplasia apparently involving duct of periurethral gland (arrowheads) in a CR2-SV40 mouse. These tall atypical epithelial cells appear to conform to the normal duct lining. There is nuclear enlargement, hyperchromasia, and chromatin clumping, and ample eosinophilic cytoplasm. Residual periurethral gland acini are seen (arrow). As this promoter targets a neuroendocrine epithelial cell population in the prostate in an apparently androgen-insensitive manner, it is tempting to speculate whether a minor NE cell population in these other accessory glands is the target for this lesion. In contrast to at least the C3(1)-SV40 and occasional LPB-Tag tumors shown herein, however, these lesions have not been noted to progress to frank carcinoma.
tentatively classified as NE carcinoma, but efforts should be made to confirm the diagnosis by immunohistochemistry and/or electron microscopy, as outlined in Table 6.

**Discussion.** The relationship of the periurethral glands and BUGs to the prostate embryologically, their regulation by androgens, the documented and possible expression of transgenes in these tissues with prostate selective or prostate “specific” promoters, and the observed occurrence of neoplasms in these sites in GEM all contributed to the decision to include a classification of their neoplastic proliferations in the Bar Harbor Classification (Table 3). Neoplasms arising in the corresponding human sites are extremely rare. However, in situ and invasive tumors involving these tissues have been observed in multiple transgenic mouse models generated with androgen-dependent and even androgen-independent potentially prostate-specific promoters (Fig. 11). Whether tumors arise in these sites or secondarily involve them is not always clear. However, origin of tumors in the periurethral glands and BUGs has been described in some models, along with likely precursor lesions. Tumors arising in the prostate can extend to involve the periurethral region, either by growth along prostatic ducts to where they enter the urethra or by direct invasion into periurethral stroma. Conversely, tumors arising in the periurethral glands can extend into prostate lobes, potentially mimicking a primary prostate tumor. Finally, neoplasms may originate in both the prostate, and the periurethral glands and/or BUGs (either synchronously or metachronously), and either or both could give rise to metastases. As reported and unreported examples of periurethral gland and BUG carcinomas have morphological characteristics quite similar or identical to those arising in the prostate in given models, the latter also raises issues of determining the primary site for metastases.

Periurethral gland and BUG neoplasms have been best characterized in the C3(1)-SV40 mouse model (Ref. 41; Fig. 11, A–D). In fact, most metastases in this model may come from periurethral gland and BUG tumors. Invasive tumors, essentially uniformly with NE differentiation, have also been noted in the periurethral glands and BUGs of intact LPB-Tag 12T-10, and castrated and even intact fast-growing LPB-Tag lines (Refs. 38, 55; Fig. 11, E and F). Similar tumors were noted to involve the periurethral region of TRAMP mice from multiple institutions (Table 1) based on slides reviewed by the Pathology Panel (Fig. 11G). However, the potential origin or isolated occurrence of such tumors in the periurethral glands in this model versus their simultaneous occurrence with tumors of similar morphology in the prostate has not been described in detail. The combined experience in different models with androgen-dependent promoters would certainly support the possibility of neoplastic transformation due to transgene expression in these androgen-dependent other male accessory glands, and transgene expression in these sites has been documented with C3(1)-SV40 and LPB-Tag models (38, 41). Interestingly, atypical hyperplastic lesions have been noted in the ducts of periurethral glands in CR (2)-SV40 mice (Fig. 11H). As this androgen-independent promoter appears to selectively target NE cells in the prostate (33), this suggests possible targeting of a normal likely very minor NE component in these other male accessory gland sites. However, these lesions do not appear to progress in this model, and involvement of the periurethral region by more extensive NE carcinomas appears to be by direct extension from unequivocal prostate tumors.

Although tumors originating in the homologous sites in the human are rare, given the relationship of the development of these sites to that of the prostate and the potential similar androgen dependence or independence of tumors arising therein, if mouse models of Pca have relevance to human Pca, tumors in these other related sites may share at least some of that relevance (41, 100). Pathways regulating cell proliferation, apoptosis, invasion, and angiogenesis, and the role of hormones and stromal interactions may be closely related in tumors of the prostate and other male accessory glands in the mouse. Additionally, promoters that can selectively target transgenes to the prostate versus these other male accessory glands may be developed. Knowledge of how to grossly procure these sites for pathological analysis and how to classify neoplasms therein is essential for understanding the natural history of current models, characterizing future models, and analyzing selectivity of new promoters. Techniques for tissue sampling are described below in the Protocols section.

**Basic Protocols in Characterization of Prostate Lesions in GEM**

**Necropsy of Newly Established Models and Prostate Dissection Ages for Sampling.** To fully characterize a GEM model for prostatic disease, the suggested sampling should include time points that mark the sexual maturity and reproductive milestones of the mouse, in addition to the time points determined by the investigator, based on previous findings, published reports, natural history, and so forth. The recommended initial/minimal sampling periods include: (a) day 1, at time of birth; (b) week 3, at time of weaning; (c) week 6, at time of reaching sexual maturity; and (d) week 40, at time of end of reproductive capability. Described time courses of phenotype development for published models, such as those listed in Table 1 and others, may also be helpful.

**Examination and Dissection of GEM Prostates.** At necropsy, macroscopic examination of the prostate (and/or its individual lobes) should be carried out with relevant data recorded, such as: (a) size; (b) weight; (c) descriptive features (e.g., enlargement and atrophy); (d) tumor, if identified (with documentation of location, size, extent of local invasion or characteristics of other local effects, such as bladder obstruction, seminal vesicle distension, and so forth); (e) necrosis; and (f) presence of visible metastases in distant organs.

**Prostate Dissection and Tissue Submission.** The method of prostate dissection and tissue submission for histology depends in part on the intended use of the tissues, particularly whether ancillary studies such as gene expression analyses are being performed. Histological examination and tissue-based analyses such as immunohistochemistry and in situ hybridization are routinely performed on standard formalin-fixed, paraffin-embedded sections. Caution should be observed regarding the duration of exposure of tissues to 10% buffered formalin, which is adequate for most if not all analyses. If tissues are not to be processed right away, tissues or cassettes with tissues in them should be switched to 50 or 70% ethanol after 4–6 h. Prolonged exposure to formalin can reduce tissue antigenicity, compromising some subsequent immunohistochemical or in situ hybridization analyses.

Separate identification of individual prostate lobes (grossly or in microscopic sections) and characterization of pathology in specific lobes is important for thorough model characterization. For analyses only involving histopathologic assessment and possible subsequent immunohistochemical and/or in situ hybridization studies on paraffin sections, the prostate and associated organs can be submitted “en bloc” as described below and in more detail on cited websites. For protocols involving submission of snap-frozen tissues, the individual lobes of the prostate can be dissected with the aid of a dissecting microscope (31). Lobe dissection is accomplished after removal of the entire genitourinary bloc (prostate lobes, seminal vesicles, ampullary glands, proximal ductus deferens, bladder, and proximal urethra) after transection of the urethra. The individual prostate lobes can be weighed and representative portions of each can be snap frozen, and
the remaining tissue fixed and processed for standard paraffin sections. High quality RNA can be obtained from tissues procured in this manner. Representative portions of both right and left lobes or an entire lobe from one side can be snap frozen, and the remaining tissue from specifically designated lobes can then be submitted in its entirety in individual tissue cassettes. If such protocols are used, sections of seminal vesicles and the remaining genitourinary bloc should be submitted (in a similar fashion as described below and shown in Fig. 12), to allow for sampling of the ampullary and periurethral glands. The amputated segment of distal urethra is longitudinally oriented at bottom. D, same bloc after transverse section through urethra as indicated in B, generating the lower and middle portions of tissue shown. An additional transverse section through seminal vesicles and anterior prostate has been made (top). The free portions of the seminal vesicles can be embedded on end for sectioning. The two portions after the transverse cut through the urethra should be embedded with their cut surfaces downward (sectioning into the cut surfaces). E, microscopic section illustrating the resulting tissue section, allowing typically adequate visualization of DP, LP, and ventral prostate, as well as other tissues that may have pathology (e.g., ampullary glands, shown, and periurethral glands, not well visualized in section, but typically demonstrable in deeper sections). 1, urethra in cross-section; 2, paired ductus deferens in cross-section; 3, paired ampullary glands in cross-section; 4, ventral prostate; 5, lateral prostate; 6, dorsal prostate.

Fig. 12. Technique for histological examination of the prostate with en bloc submission. A, exposed intact prostate, bladder, and seminal vesicles [genitourinary (GU) bloc] after linear ventral abdominal incision. The white curvilinear seminal vesicles are readily apparent. B, schematic diagram of removed GU bloc (anterolateral view), after transection of the urethra (UR). VP, ventral prostate; LP, lateral prostate; DP, dorsal prostate; SV, seminal vesicles; CG, coagulating gland or anterior prostate; DD, ductus deferens; UB, urinary bladder. Horizontal black line indicates the level of transverse sectioning through the urethra to include both dorsolateral prostate and ventral prostate, at or near level of SV junction. C, removed GU bloc from animal in A, corresponding to that illustrated schematically in B. The amputated segment of distal urethra is longitudinally oriented at bottom. D, same bloc after transverse section through urethra as indicated in B, generating the lower and middle portions of tissue shown. An additional transverse section through seminal vesicles and anterior prostate has been made (top). The free portions of the seminal vesicles can be embedded on end for sectioning. The two portions after the transverse cut through the urethra should be embedded with their cut surfaces downward (sectioning into the cut surfaces). E, microscopic section illustrating the resulting tissue section, allowing typically adequate visualization of DP, LP, and ventral prostate, as well as other tissues that may have pathology (e.g., ampullary glands, shown, and periurethral glands, not well visualized in section, but typically demonstrable in deeper sections). 1, urethra in cross-section; 2, paired ductus deferens in cross-section; 3, paired ampullary glands in cross-section; 4, ventral prostate; 5, lateral prostate; 6, dorsal prostate.
Table 7 Immunochemical assays utilized in characterization of prostate lesions in genetically engineered mice (GEM)

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Application</th>
<th>Host</th>
<th>Specificity</th>
<th>Source</th>
<th>Catalog number</th>
<th>Dilution</th>
<th>Antigen retrieval</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ki67</td>
<td>Proliferating cells</td>
<td>Rabbit</td>
<td>polyclonal</td>
<td>Mouse, human</td>
<td>Novacastain</td>
<td>CLKi67 1:1,800</td>
<td>Standard microwave</td>
<td>(78)</td>
</tr>
<tr>
<td>AR</td>
<td>AR</td>
<td>Rabbit</td>
<td>polyclonal</td>
<td>Mouse, human</td>
<td>ABR</td>
<td>pal-111a 1:250</td>
<td>Standard microwave</td>
<td>34</td>
</tr>
<tr>
<td>Laminin</td>
<td>Basal lamina membrane</td>
<td>Rabbit</td>
<td>polyclonal</td>
<td>Mouse (human?)</td>
<td>Upstate Biotechnologies</td>
<td>06-686 1:1,000</td>
<td>Standard microwave</td>
<td>(78, 99)</td>
</tr>
<tr>
<td>Chromogranin A</td>
<td>Neuroendocrine cell</td>
<td>Rabbit</td>
<td>polyclonal</td>
<td>Mouse, bovine</td>
<td>Santa Cruz</td>
<td>N-20 (sc-816) 1:100</td>
<td>Microwave, 1 M Urea</td>
<td>(33, 38)</td>
</tr>
<tr>
<td>Synaptophysin</td>
<td>Neuroendocrine cell</td>
<td>Rabbit</td>
<td>polyclonal</td>
<td>Mouse, human</td>
<td>Diasorin</td>
<td>L9393 1:1,000</td>
<td>Standard microwave</td>
<td>(78)</td>
</tr>
<tr>
<td>Alpha-Actin</td>
<td>Smooth muscle</td>
<td>Mouse</td>
<td>monoclonal</td>
<td>Mouse (human?)</td>
<td>Sigma</td>
<td>20085 1:1,000</td>
<td>Standard microwave</td>
<td>(33, 38)</td>
</tr>
<tr>
<td>E-cadherin</td>
<td>Secretory epithelial cells</td>
<td>Mouse</td>
<td>monoclonal</td>
<td>Mouse, human</td>
<td>Transduction lab (BD)</td>
<td>C20820 1:800</td>
<td>Standard microwave</td>
<td>(78, 99)</td>
</tr>
<tr>
<td>CK14</td>
<td>Basal epithelial cells</td>
<td>Mouse</td>
<td>monoclonal</td>
<td>Mouse</td>
<td>Biogenex</td>
<td>146M 1:50</td>
<td>Standard microwave</td>
<td>34</td>
</tr>
<tr>
<td>CK5</td>
<td>Basal epithelial cells</td>
<td>Mouse</td>
<td>monoclonal</td>
<td>Mouse</td>
<td>Binding Site Covance</td>
<td>PH503 1:200</td>
<td>Standard microwave</td>
<td>(78)</td>
</tr>
<tr>
<td>p63</td>
<td>Basal epithelial cells</td>
<td>Rabbit</td>
<td>polyclonal</td>
<td>Human, mouse</td>
<td>Lab Vison Corporation</td>
<td>4A4 1:50</td>
<td>Standard microwave</td>
<td>(46)</td>
</tr>
<tr>
<td>Pancytokeratin</td>
<td>Epithelial cells (secretory and basal)</td>
<td>Mouse</td>
<td>monoclonal</td>
<td>Mouse</td>
<td>Dako</td>
<td>Z0622 1:700</td>
<td>Protease K</td>
<td>(35)</td>
</tr>
<tr>
<td>CK8</td>
<td>Luminal (secretory) epithelial cells</td>
<td>Mouse</td>
<td>monoclonal</td>
<td>Human, mouse</td>
<td>Research diagnostics</td>
<td>RDI-PRO65138 1:1</td>
<td>Standard microwave</td>
<td>34</td>
</tr>
<tr>
<td>PECAM-1 CD31</td>
<td>Vessels/MVD</td>
<td>Rat monoclonal</td>
<td>Mouse</td>
<td>PharMingen</td>
<td>550274 1:50</td>
<td>Protease K</td>
<td>(99)</td>
<td></td>
</tr>
<tr>
<td>Endoglin CD105</td>
<td>Vessels/MVD</td>
<td>Mouse</td>
<td>monoclonal</td>
<td>Mouse, human</td>
<td>Dako</td>
<td>M3527 1:10</td>
<td>Protease K</td>
<td>(35)</td>
</tr>
<tr>
<td>DLP</td>
<td>DLP secretory protein</td>
<td>Rabbit</td>
<td>polyclonal</td>
<td>Mouse</td>
<td>From investigator</td>
<td>N/A</td>
<td>1:5,000</td>
<td>Standard microwave</td>
</tr>
</tbody>
</table>

When only one section per prostate lobe is initially stained, several additional sections can be prepared on charged slides, either for possible subsequent staining, or ancillary immunohistochemical or other studies.

In sections obtained to characterize a new model, wherein the extent of any phenotypic alterations are uncertain, more extensive sectioning of paraffin blocks would typically be required. However, we do not recommend necessarily H&E staining of all serial sections, sectioning of paraffin blocks would typically be required. However, extent of any phenotypic alterations are uncertain, more extensive possible subsequent staining, or ancillary immunohistochemical or additional sections can be prepared on charged slides, either for when only one section per prostate lobe is initially stained, several additional sections can be obtained on charged slides. Despite the slightly greater cost compared with uncharged slides, this will better allow for ancillary immunostaining or in situ hybridization. Every third or every fifth slide can be stained initially. If lesions are present, the intervening slides from adjacent sections can be used for ancillary procedures to provide additional characterization of focal lesions. If lesions are not identified, additional intervening sections can always be stained with H&E. It is impossible to make rigid recommendations that will apply to all studies, and these are intended as general guidelines. Members of the MMHCC Prostate Pathology Committee can be consulted to facilitate such protocol design.

A method for tissue submission of the male accessory glands en bloc is demonstrated in Fig. 12. Procedures have been described previously (1, 101), and detailed protocols are available on line.

**Ancillary Studies. Immunohistochemistry.** Immunohistochemistry is a vital component in the characterization of GEM models and in the assessment of the effects of treatment interventions or genetic crosses. Many examples are described in the published literature, including for some of the models listed in Table 1. Immunohistochemical assays for antigens of special interest include the determination of expression of the particular transgene used in a given GEM model (e.g., Tag), alteration of expression of specific proteins accompanying tumor progression (e.g., PTEN, NKX3.1, and p53), and modification of proteins reflecting activation of certain signaling pathways during tumor progression (e.g., phospho-AKT). The results of many such assays are published, and interested investigators should consult specific publications for assay methodology and results. In addition, there is a growing number of commonly used immunostains that may have particularly broad utility in model characterization as well as in interventional trials. A list of these antigens and some of the specific antibodies and tissue fixation conditions that have been successfully used is shown in Table 7. The included references are intended to provide examples of successful protocols and are not exhaustive. These include markers of epithelial differentiation, including for basal and luminal cell subpopulations and NE differentiation. In addition, immunohistochemical staining for proliferation markers, such as PCNA and Ki-67, can be useful for demonstrating increased proliferation with progression as well as determining effects on proliferation of genetic crosses or therapeutic interventions. Similarly, multiple antibodies have been used to stain endothelial cells in the mouse to assess microvessel density (MVD) and to determine gland
altered patterns of angiogenesis with tumor progression, or the effects of genetic crosses or therapeutics on tumor angiogenesis.

In addition to polyclonal antibodies, murine monoclonal antibodies are routinely used to stain mouse sections. References cited herein as well as additional publications using specific models and the related websites provide specifics regarding antigen retrieval, secondary antibodies, blocking strategies, and detection techniques. Additional immunohistochemistry information can be found on the internet.33

**In Situ Hybridization (ISH).** ISH is a vital component in the characterization and utilization of GEM models of prostatic neoplasia. Applications include documentation of transgene expression in new transgenic models, as well as lost expression in genomic or selective knockout models. Although not quantitative, ISH is highly specific, and certainly adequate for detecting major alterations in mRNA expression. Despite less precise resolution than immunostaining in tissue sections, it is typically sufficient to demonstrate epithelial versus stromal localization. ISH may be useful for demonstrating altered expression of specific genes with tumor progression or with therapeutic intervention. It is anticipated that ISH will be useful in confirming results of cDNA microarrays when novel genes are indicated as altered in a given model, as well as indicating the site of such gene expression. The techniques are established and more widely available than laser capture microdissection coupled to quantitative gene expression analyses. ISH may be a suitable screening option that can then be supplemented by more quantitative techniques if needed.

It is recommended that investigators involve collaborators with technical expertise in ISH.

**Apoptosis Assays.** Apoptosis may be useful to assess and even quantify in the analysis of possible progression in new GEM models. Quantitating apoptosis may be useful in the investigation of possible beneficial effects with certain therapeutic interventions. Increased apoptosis has been noted with tumor progression in some models of mPIN and invasive carcinoma. The ApoTag system from Intergen has been successfully used by multiple investigators.36 When possible, and particularly in therapeutic trials, apoptotic cells should be quantitated in a blinded manner, either by cell counting or by computer-assisted image analysis.

**Electron Microscopy.** Several studies have successfully used ultrastructural analysis in characterization of tumor cell differentiation or other aspects of GEM model characterization (32, 33, 38, 84). In general, standard techniques are applied. Protocols are available on the internet.37

Web sites for additional information include, RENI trimming guide for prostate and seminal vesicles;38 University of California, Davis, resource site;39 and prostate molecular profiling at the National Cancer Institute.40 (Whereas this site is primarily for human prostate cancer, it has detailed information on tissue preparation, slide preparation, microdissection, and processing of tissue for molecular analysis, as well as protocols in development).

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Prostate Pathology of Genetically Engineered Mice: Definitions and Classification. The Consensus Report from the Bar Harbor Meeting of the Mouse Models of Human Cancer Consortium Prostate Pathology Committee

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