Animal Models for Prostatic Cancer

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The frequency of prostate cancer has been increasing (1). Afflicting 10% of men older than the age of 65, it repre-sents the most frequently diagnosed cancer in American men, with an even higher incidence in the African-American population.

Many investigators have tried to identify prognostic markers that distinguish indolent *versus* aggressive forms of prostate cancer, and to understand the genetic factors that evoke prostate cancer initiation and progression (2). Animal models have been developed to study the potential relationship of molecular mechanisms and clinical progression (3–5). The earlier models included xenograph and hormone induction models (3,6,7). Recently,transgenic and knockout models have become available (3–5,8). The most widely used models involve the SV40Tag gene behind various types of prostate-targeting promoters (9–17). These models involve a rapidly progressive,poorly differentiated, and metastatic neoplasm. The early lesions display varying degrees of epithelial atypia (9–12). The later lesions in some models frequently involve the entire epithelium. These lesions have been characterized and a tentative grading system has been developed under the heading of prostatic intraepithelial neoplasia (PIN) (18)

More recently, other mouse models of human prostate cancers have been developed using knockouts or trans-genes other than the SV40-Tag (3). These models develop a more indolent proliferative disease that rarely progresses to invasive carcinoma (19). They do, however,develop a variety of foci with atypical cells that are quite different from those observed in the SV40-Tag-based models.

We have studied the intraepithelial lesions occurring in nine of these models and have observed a continuum of structural and cytological changes that suggest increased severity and, thus, neoplastic progression. We have created a system to grade these lesions to assist others to evaluate their genetically engineered mice (GEM) models of prostate cancer. We describe and illus-trate here, using examples from a single model (Nkx3.1-/-,PTEN+/+), our proposed grading system, and the evidence to support progressive change as potentially useful guidelines for other investigators.

Atypical Hyperplasia

Many mice, including some elderly wild-type male controls, have increased numbers of prostatic epithelial cells with scattered cells that have enlarged, hyperchromatic nuclei. However, they generally do not have the abundant cytoplasm and other cytoplasmic changes described below. Further, the scattered atypical cells do not stand out from the general population as discrete foci. These changes are referred to here as hyperplasia, with atypia or atypical hyperplasia.

Prostate Intraepithelial Neoplasia (PIN)

Focal atypical lesions of the prostatic epithelium have been described in several models. As indicated, we have either developed or have access to at least nine mouse models of human prostate neoplasia and have studied others in slide sets developed for meeting workshops. These models include knockouts or transgenic mice from ras, Mxi, PTEN, p53 mutant, FGF8, and PyV-mT. Since detailed descriptions of some of these models are not yet published, we have chosen to illustrate the proposed criteria for GEM PIN using a single model system that we have thoroughly studied, the Nkx3.1-/-, PTEN+/+mutant mice. These mice progress to a relatively more severe phenotype that has been described and illustrated in some detail.

Whole Mounts

Stained or unstained whole mount preparations can be used to visualize and enumerate the atypical lesions in the mouse prostate. Whole mounts of mice with PIN demonstrate small masses scattered in the different lobes. These masses varied in size. Microscopic examination verified that these masses were PIN lesions. *Description of PIN* Foci of atypical cells were found in the prostatic lobes.However, in the models studied here, they were concen-trated in the ducts of the coagulating gland and dorso-lateral glands. The foci varied in the number of cell layers, the degree and pattern of atypia, and the relation to the fibromuscular stroma. The younger mice generally had fewer and less severe atypia. The low-grade lesions in younger mice did not necessarily occur in the context of more severe lesions. In contrast, prostate ducts with more severe atypia inevitably had less severe lesions. These observations implied a morphological continuum between the less and the more severe lesions. For the purpose of future studies, the lesions were classified into discrete classes fitting the criteria described below.

PIN I

Relatively small foci with one or two layers of atypical cells. The fibromuscular stroma is intact and the duct profile is undisturbed. The cells are generally more columnar, larger, and taller than adjacent normal cells. They have abundant pale cytoplasm with hyperchromatic but minimally pleomorphic nuclei.

PIN II

Larger foci with two or more layers of atypical cells that do not fill the lumen. The fibromuscular sheath is intact and the duct profile is undisturbed. The epithelial cells may have papillary, cribriform, or tufting patterns. The atypical cells are tall columnar with abundant pale pink cytoplasm with increasing but not severe nuclear pleomorphism and hyperchromasia. Increasing proportions of nuclei are larger and have vesicular chromatin patterns.

PIN III

The foci of atypical cells fill, or almost fill, the lumen of the ducts. The diameter of the glands may be enlarged but the fibromuscular sheath is present and the gland outline is smooth. The epithelial cells may have papillary, cribriform, or tufting patterns that are frequently associated with small intraepithelial blood vessels. PIN III lesions may extend along the duct to involve adjacent ducts. The atypical cells are frequently poorly oriented with abundant relatively pale cytoplasm with increasingly severe nuclear pleomorphism and hyperchromasia. The nuclear to cytoplasmic ratio is inverted. Mitotic figures are present. Variable host responses are present with some inflammation and foamy macrophages.

PIN IV

The foci of atypical cells fill the lumen of the ducts. The profiles of the ducts are distorted and irregular with bulging profiles. The fibromuscular sheath is irregular or ab-sent in most areas. However, the epithelium continues to be surrounded by a layer of laminin. The epithelial cells may have solid, cribriform, or tufting patterns that are associated with small intraepithelial blood vessels. Central necrosis may also be present. PIN IV level lesions extend along the duct to involve adjacent ducts. The atypical cells are poorly oriented with abundant pale cytoplasm and with increasingly severe nuclear pleomorphism and hyperchromasia. The nuclear to cytoplasmic ratio is inverted. Mitotic figures are present. Host inflammatory responses are marked with lymphocytes and macrophages.

Prostatic adenocarcinoma

The TRAMP model has a number of distinct advantages over existing models: 1) the

transgene is specific for the epithelial cells of the prostate; 2) the tumor tissue histologically resembles the human disease (with severe hyperplasia and cribriform structures appearing as early as 10 weeks); 3) the tumors arise with a short latency period and with 100% frequency; 4) the mice exhibit progressive stages of prostate cancer ranging from mild to severe prostatic hyperplasia with cribriform structures and focal adenocarcinoma and seminal vesicle invasion (SVI); 5) metastatic spread of the disease to the lymph nodes, lung and bone has been observed; 6) the mouse model can be used to follow the progression/multi-stage development of disease all within a 10 - 20 week time period; 7) the mouse models exhibit less morbidity and mortality due to complications with other organs as seen in other transgenic models; and 8) the TRAMP model was generated in an inbred strain to facilitate immunological studies. It is anticipated that the establishment of the TRAMP model will facilitate many new avenues of research towards better prevention, diagnosis and treatment of prostate cancer.