Animal Models of Human Prostate Cancer: The Consensus Report of the New York Meeting of the Mouse Models of Human Cancers Consortium Prostate Pathology Committee

Michael Ittmann1,2, Jiaoti Huang3, Enrico Radaelli19, Philip Martin6, Sabina Signoretti9, Ruth Sullivan14,15,16, Brian W. Simons7, Jerrold M. Ward8, Brian D. Robinson17, Gerald C. Chu13, Massimo Loda11,12,13, George Thomas18, Alexander Borowsky4,5, and Robert D. Cardiff4,5

Abstract

Animal models, particularly mouse models, play a central role in the study of the etiology, prevention, and treatment of human prostate cancer. While tissue culture models are extremely useful in understanding the biology of prostate cancer, they cannot recapitulate the complex cellular interactions within the tumor microenvironment that play a key role in cancer initiation and progression. The National Cancer Institute (NCI) Mouse Models of Human Cancers Consortium convened a group of human and veterinary pathologists to review the current animal models of prostate cancer and make recommendations about the pathologic analysis of these models. More than 40 different models with 439 samples were reviewed, including genetically engineered mouse models, xenograft, rat, and canine models. Numerous relevant models have been developed over the past 15 years, and each approach has strengths and weaknesses. Analysis of multiple genetically engineered models has shown that reactive stroma formation is present in all the models developing invasive carcinomas. In addition, numerous models with multiple genetic alterations display aggressive phenotypes characterized by sarcomatoid carcinomas and metastases, which is presumably a histologic manifestation of epithelial–mesenchymal transition. The significant progress in development of improved models of prostate cancer has already accelerated our understanding of the complex biology of prostate cancer and promises to enhance development of new approaches to prevention, detection, and treatment of this common malignancy.

Introduction

Animal models, particularly mouse models, play a central role in the study of the etiology, prevention, and treatment of human prostate cancer. Although tissue culture models are extremely useful in understanding the biology of prostate cancer, they cannot recapitulate the complex cellular interactions within the tumor microenvironment that play a key role in cancer initiation and progression. Immune cells, fibroblasts, myofibroblasts, blood vessels, and nerves all interact with prostate cancer cells, and levels of circulating oxygen, nutrients, and endocrine factors also play a dynamic role in regulating tumor growth. Furthermore, there is a paucity of available prostate cancer cell lines as compared with many other common cancers due to the difficulty in establishing such cell lines. Analysis of human prostate cancer tissues has been a major focus of prostate cancer research for the past 2 decades, and the complex molecular alterations associated with various subtypes of human prostate cancer are beginning to be well delineated (1). Within the next several years, detailed molecular analysis of large numbers of prostate cancers by genomic sequencing and other high-throughput techniques will lay out the full complexity of human prostate cancer at the molecular level. However, ultimately such studies are correlative, and in vivo model systems to understand the underlying biology and to test novel prevention and treatment strategies are critically needed.
Mouse models of prostate cancer can be divided into 2 broad categories: genetically engineered mouse (GEM) models and xenograft models. Over the past 20 years, a significant number of GEM models of prostate cancer have been created and analyzed. In most cases, these models have used prostate tissue restricted expression of oncogenes or Cre recombinase (to inactivate tumor suppressors) via probasin or other prostate-specific promoters to create genetic lesions mimicking those identified in human prostate cancer (see Fig. 1). More recently, models using lox-stop-lox to engineer prostate-specific expression of genes from androgen-independent promoters gave also been used as cellular dedifferentiation during progression or therapies targeting androgen receptor (AR) can decrease probasin-driven oncogene expression. Indeed, analysis of GEM models has been critical in validating the biologic importance of the observed molecular alterations in human prostate cancer. Over time, these models have been combined to mimic the multiple genetic alterations in human prostate cancer and define the interactions between critical pathways in an in vivo context. GEM models have significant advantages in that they reflect tumor progression over time from the initiation of preinvasive lesions to invasive and in some cases metastatic lesions within the prostatic microenvironment including a fully intact immune system. The chief disadvantage of these models is that in some cases there may be important biologic differences in mouse versus human prostate and other tissues that may affect model phenotypes. For example, carcinogenesis is generally initiated at the time of sexual maturation in GEM models, so that aging-associated changes in the tumor microenvironment are not present as they are in human prostate cancer (2). Newer-generation models using chemically activated Cre recombinase can allow for activation of carcinogenesis at later times, although the spectrum of aging-associated changes in mouse stroma only partially overlaps with those seen in humans (3). At a practical level, GEM models also usually have higher costs and longer time frames to generate results compared with xenograft models, which acts as a barrier to their use.

In xenograft models, prostate cancer cells are implanted in host mice. Most commonly, human prostate cancer cell lines are implanted into immunocompromised [nude or severe combined immunodeficient (SCID)] mice either subcutaneously or injected orthotopically into the prostate. Orthotopic models have the advantage of growth within the prostate microenvironment, and in a number of models metastasis occurs at high rates. A second class of xenograft models, known as tissue recombination models, uses epithelial cells (benign or malignant) combined with mesenchymal cells, which are implanted either subcutaneously or under the renal capsule in immunocompromised mice. Such models represent a powerful method to study tumor–stromal interactions. In addition, in such models the epithelial and/or stromal compartment can be genetically manipulated to define the genetic lesions and paracrine signals capable of transforming stem cells from mouse or human prostate tissues (4–7). A third class of xenograft models is xenografts established from human

Figure 1. Methods for generation of GEM models of prostate cancer. A, a prostate specific promoter (such as the enhanced ARR2Pb probasin promoter) is used to drive prostate-specific expression of a gene of interest, typically an oncogene. The oncogene is expressed in the prostate epithelial cells at the onset of sexual maturity. B, mice are generated with loxP sites flanking critical exons in a gene of interest, typically a tumor suppressor gene. These mice are then crossed with mice expressing the Cre recombinase under control of the probasin or other prostate-specific promoter resulting in excision of key exons and inactivation of the targeted gene in prostatic epithelial cells bearing the targeted sequence in one or both alleles of the gene of interest. C, mice are generated with the gene of interest downstream of a strong constitutive promoter such as ROSA26. Transcription/translation is inhibited by a lox-stop-lox cassette upstream of the gene of interest. These mice can be crossed to probasin Cre mice, leading to excision of the stop sequence and expression of the gene of interest. This method has the advantage that expression of the gene of interest is no longer dependent on AR and other prostate-specific factors whose activity may be altered by tumor progression or treatment.
prostate cancer tissues and carried as xenografts. In most cases, cell lines have not been established because, as described earlier, it is often quite difficult to establish prostate cancer cell lines even from xenografts. Given the paucity of cell lines, these xenografts provide valuable models to study the impact of therapies in a larger number of prostate cancer models. Finally, mouse prostate xenografts can be established from mouse prostate cancer cell lines in syngeneic, immunocompetent host mice. These models are mainstays of immunotherapy studies. In addition, these mouse xenografts in immunocompetent mice are useful for studying the role of immune cells in the tumor microenvironment in tumor progression and therapeutic resistance (8). Unfortunately, the number of mouse prostate cancer cell lines is limited to date. The xenograft models described earlier have all proved extremely useful in evaluating the biology of human prostate cancer and are used routinely to evaluate prostate cancer therapies in a relatively quick and lower-cost way of evaluating new therapies in prostate cancer and mechanisms of therapeutic resistance. A major disadvantage of such models is that because they are almost all derived from advanced cancers their applicability to prevention studies is questionable. Similarly, because most xenografts and prostate cancer cell lines are derived from clinically aggressive lesions, they may not reflect the biology of precursor or less aggressive lesions. Finally, in almost all cases the tumor microenvironment is somewhat abnormal due to the location (subcutaneous or renal capsule) and/or due to abnormalities of the host immune system.

Other animal models are used much less often in studies of prostate cancer. Some rat strains have high rates of spontaneous carcinomas (9), and inducible carcinoma models using hormones and/or carcinogens in rats have been used by many groups (9). Transgenic rats can now be generated as well. An advantage of the rat over the mouse is the much larger size of the rat prostate. A disadvantage is that the number of analytic reagents for use in tumor analysis and transgenic and knockout lines for cross-breeding is far smaller than for mice.

Finally, spontaneous prostate cancer is relatively common in dogs and provides a potential useful natural model for evaluating novel prostate cancer therapies. Such spontaneous models can be extremely useful but are less easily genetically manipulated than xenografts or mice, which limits their use.

Scope and Objectives

The National Cancer Institute (NCI) Mouse Models of Human Cancers Consortium Prostate Cancer Steering Committee convened a panel of human and veterinary pathologists with expertise in prostate cancer to review the current state of the art in animal models of prostate cancer. A similar panel was convened more than 10 years ago resulting in the consensus report by Shappell and colleagues (10). That consensus report defined the underlying principles and techniques for the pathologic analysis of lesions in GEM models of prostate cancer and provided detailed definitions for various lesions of the prostate in GEM models. In the subsequent 10 years, numerous new models have been developed, and the scientific community has gained extensive experience in analyzing these models. The goal of the current report is to provide a consensus document updating the research community on current thinking in the pathologic analysis of GEM models and to provide a detailed description of a number of important GEM models. In addition, we have extended our analysis to include xenograft models and a limited number of models in other species.

Materials and Methods

A team of veterinary and human pathologists with expertise in prostate cancer was recruited by the senior investigators (R.D. Cardiff and M. Ittmann). Models to be reviewed were selected by the Mouse Models of Human Cancers Consortium Prostate Cancer Steering Committee, and blocks and/or slides were obtained from investigators. Slides were digitized using an Aperio ScanScope XT and uploaded to the Mouse Mutant Pathology Laboratory Spectrum public database (http://bit.ly/T9nHTN). The reader can view the accompanying digitized whole slide images (WSI) that are noted in the text by copying and pasting MC accession numbers (i.e., MC12-0269) into the search tool, or the WSI can be viewed using the live links in Supplementary Table S1. Detailed instructions for both approaches are appended to Supplementary Table S1. Preexisting models submitted previously to this site were also reviewed. Review teams were assembled and digitized WSI were reviewed and annotated online. The review team met at Columbia University (New York, NY) on April 17 to 18, 2012, and models were reviewed and discussed. More than 40 different models with 439 samples were reviewed, including GEM, xenograft, rat, and canine models. Following the review of the models, discussions were held about the optimal strategies for analysis of animal models of prostate cancer, lessons learned since the last consensus meeting, and emerging themes in the pathology of mouse models of prostate cancer.

There are several limitations to this study. First, it was not practical to review every GEM or xenograft model of prostate cancer, so this review is not encyclopedic in nature. Readers are referred to several excellent reviews for discussions of a number of models not represented in this report (11–14). Second, this report is focused on pathology rather than the biology of the cellular and molecular alterations that underlie the observed lesions. Third, the number of slides that could be reviewed for any model was limited, so that conclusions about rates of progression, frequency of metastasis, and other factors could not be assessed. The pathologists were able to determine if the interpretation of observed lesions in representative slides was concordant with the classification of the lesion by submitting investigator.

Results

Genetically engineered mouse models of prostate cancer

Methodologic considerations. The prior consensus report by Shappell and colleagues (10) describes in detail pathology methods for analysis of GEM models of prostate cancer. It is critical that a pathologist (medical doctor or veterinarian) be involved in the pathology evaluation of the mouse model. Several key points need to be reiterated. First, the mouse
prostate has a different anatomy and histology than the human prostate. It consists of the anterior prostate, ventral prostate, dorsal, and lateral lobes [the latter 2 often combined as dorsolateral prostate (MC02-0695)]. In contrast, the human prostate is a single gland with different histologic zones (peripheral, transition, and central zones; MC02-0713). The majority of human prostate cancers arise in the peripheral zone. In the mouse, the glandular epithelial cells are surrounded by a very thin fibromuscular stroma, which is in contrast with the abundant fibromuscular stroma in the human prostate. The seminal vesicles of mice are large and occasionally the site of the development of proliferative lesions and tumors. The periurethral and bulbourethral glands can also be the site of tumor development in GEM models. GEM models have differential phenotypes in different prostate lobes that may depend on the underlying biology of the genetic alteration, the exact constructs used for expression, the site(s) of integration of transgenes, and the mouse strain. It is the opinion of the panel that it is premature to conclude that lesions in any given lobe are more representative of human disease. Second, when initially analyzing models, it is preferable to submit the prostate and associated accessory sex glands and related organs en bloc for histologic examination as described by Shappell and colleagues (ref. 10; MC02-0695).

This allows examination of all prostate lobes as well as associated organs that may have neoplastic alterations, which can extend into the prostate. Lobe-specific harvesting can yield more tissue and is suitable for harvesting frozen tissue for molecular analysis, and thus complements the en bloc method in models that have undergone initial characterization. Third, it is essential to carry out time course studies (serial sacrifice by age) to evaluate the full biologic potential of the genetically engineered lesion. Although the exact time course is model dependent, it should be recognized that a year or more may be required for the most advanced lesions to develop. Examination of the genitourinary organs should be complemented by necropsy and, if possible, imaging studies to evaluate local extension and metastasis. Examination of age-matched controls of the same genetic background housed under the same conditions, preferably littermates, is essential.

Optimal pathologic evaluation of mouse models (and human tissues) is dependent on well-fixed, thin, well-stained sections. In almost all cases, neutral-buffered formalin is optimal for hematoxylin and eosin (H&E) sections and is compatible with most immunohistochemistry (IHC; with appropriate antigen retrieval). Obviously, immunohistochemical studies to confirm the expression of a transgene or loss of expression of a targeted tumor suppressor in abnormal tissues are critical for confirming that the genetic alteration is actually present in the lesions seen. Additional studies can be useful to evaluate stromal alterations and invasion including trichrome stain to assess fibrosis and IHC for α-smooth muscle actin to assess for the integrity of the smooth muscle investment of the glands. Markers of neuroendocrine differentiation of epithelial cells such as chromogranin or synaptophysin are useful in confirming the diagnosis of neuroendocrine carcinoma (MC02-0699). Neuroendocrine cancers also display the typical perinuclear staining with anticytokeratin antibodies seen in human neuroendocrine carcinomas. The role of evaluation of basal cell–specific markers is less clear. In human prostate cancer, loss of basal cells is a hallmark of prostate cancer, and stains to evaluate basal cytokeratins and/or p63 are used routinely to evaluate problematic lesions. However, in mice there is evidence that basal cell markers can be expressed by cancer cells so the presence of staining for such markers does not exclude prostate cancer. However, complete loss of basal cell markers in a focus that is histologically compatible with prostate cancer supports this diagnosis. IHC is now a routine part of clinical practice in human pathology, and inclusion of such studies enhances (but does not replace) histologic evaluation of H&E sections. In this regard, studies to evaluate proliferation by IHC (such as Ki67) or apoptosis [by IHC or terminal deoxynucleotidyl transferase–mediated dUTP nick end labeling (TUNEL)] can be useful adjuncts to histologic evaluation and can confirm the biologic impact of a given genetic lesion. However, changes in proliferation or apoptosis are not the equivalent to neoplasia, and increased proliferation and decreased apoptosis, while seen in neoplasia, are not adequate to define a lesion as neoplastic.

Analysis of progression in GEM models must be conducted with care. Local progression requires careful dissection and at times en bloc preparation of tumors and surrounding tissues (MC02-0695). Step and serial sections can often help define early lesions. The presence of metastatic lesions must be established by careful necropsy, which can be assisted by appropriate premortem imaging studies. Considerable caution must be taken to exclude spontaneous primary lesions at distant sites. Inbred mice, particularly older mice, can be prone to such tumors, which are often strain and site specific. For example, primary lung neoplasms are common in inbred mice and can occur in up to 41% of 24-month-old FVB mice (15) and can be multiple, mimicking metastasis (MC12-0377LA). Such lesions have characteristic morphology, but IHC for lung-specific markers such as surfactant protein can be used to confirm the lung origin of such tumors (Supplementary Fig. S1). TTF-1 is also a useful marker for primary pulmonary tumors. It should be noted that transgene expression does not necessarily prove a distant lesion is metastatic as it is possible that a putatively prostate-specific promoter may have transcriptional activity in nonprostate cells that is sufficient to transform such cells, particularly if the genetic lesion has potent transforming activity. Use of mice with a global germ-line genetic lesion (i.e., Pten+/– knockout mice) can also confound interpretation of lesions at distant sites, as the genetic change can lead to primary tumors at other sites. Similarly, use of non–prostate-specific promoters (i.e., keratins) obviously has similar limitations.

Mouse models also may develop distant spread of tumor via intravascular spread without invasion (TG02-1510LA) at the distant sites, primarily in the lung. An example is shown in Supplementary Fig. S2. Endothelial markers can be used to confirm the presence of the blood vessel around the tumor embolus if needed. Tumor emboli can be seen in other human cancers such as renal cell carcinoma but are rare in human prostate cancer, although widespread intravascular spread in the lymphatics of the lung is seen occasionally in advanced...
disease. The consensus panel felt that such lesions should be specifically identified as intravascular tumor emboli and not metastasis per se as the lesions do not invade the tissue at the distant site.

It should be noted that in a number of aggressive models extensive local invasion can occur. This is not considered metastasis as direct local invasion is pathologically and biologically distinct from spread to and colonization of distant sites by malignant cells. Direct local invasion of adjacent organs is analogous to pT4 lesions in human prostate cancer as defined by the American Joint Committee on Cancer.

**Classification of lesions of the prostate in genetically engineered mouse models.** Shappell and colleagues (10) have previously provided definitions and descriptions of the full spectrum of lesions seen in the prostate. In this report, we more narrowly focus on the main lesions seen in GEM models and summarize new insights into these lesions that have accrued in the 10 years since the original consensus conference. Many of the newer models involve crosses of the various GEM lines, and the pathology of such models may be the same or similar to one of the founder lines or may be unique. Lesions can be more progressive, more malignant, and more metastatic in the new complex line.

**Epithelial hyperplasia.** Epithelial hyperplasia is a non-neoplastic increase in epithelial cells within the prostate and can be manifested as simple hyperplasia, showing a single epithelial cell lining, or more complex, with architectural changes such as tufting, papillary, and even cribriform changes (MC12-0306BY-recut-H&E-CL). It may be focal or diffuse. Epithelial hyperplasia may show some minimal nuclear atypia, which is often associated with inflammation, but it should not be marked. Diagnosis of epithelial hyperplasia, particularly if focal, should be based on quantitative comparison of number and extent of hyperplastic foci compared with age-matched controls. Quantitative IHC analysis of proliferation markers and IHC/TUNEL for apoptosis can be useful for determination of a biologic cause of the observed hyperplasia.

**Mouse prostatic intraepithelial neoplasia.** Prostatic intraepithelial neoplasia (PIN) is characterized histologically by proliferation of atypical epithelial cells within preexisting glandular spaces but without frank invasion. In humans, it is widely accepted that high-grade PIN (HGPIN) is the precursor to invasive carcinoma, although only a fraction of such lesions ultimately progress to invasive carcinoma. Similar lesions, characterized by proliferation of atypical cells within preexisting glandular spaces, occur in GEM and are classified as mouse PIN (mPIN). They are differentiated from hyperplastic lesions by their morphology and natural history in a specific GEM model. Examination of multiple models developed over the past 10 years since the prior consensus conference has shown that such lesions often progress to frankly invasive carcinoma. Thus, there is increased confidence that such lesions do indeed reflect preinvasive neoplasia of the mouse prostate. mPIN has been seen in all prostate lobes in various models, although many publications do not clearly define which prostate lobes are actually involved with mPIN. A system developed by Park and colleagues to recognize and classify mPIN has been published (16), and this system has proved useful as a framework for evaluating such lesions. Examples of mPIN are shown in Fig. 2. In this system, mPIN is graded on a scale of 1 to 4 based on increasing degrees of architectural and cytologic abnormalities. mPIN1 has 1 to 2 layers of cells and mild nuclear atypia (Fig. 2A and B). Lesions of mPIN2 have increased nuclear atypia and contain 2 or more layers of cells often in papillary, tufting, or cribriform arrangements (MC12-0385DF). mPIN3 lesions have obvious nuclear atypia and fill or almost fill the duct lumens in papillary or cribriform patterns. mPIN4 lesions are similar to mPIN3 but have even more severe atypia, fill the ductal lumens, and may bulge into the surrounding stroma but without clear invasion (MC12-0310BY). The consensus panel found that mPIN1 and 2 are harder to reproducibly identify unless widespread, whereas mPIN3 and 4 are readily identified and are more consistently associated with the presence of or progression to invasive lesions (MC12-0396DF; TG04-0319-HE). Thus, it is reasonable to equate mPIN1 and 2 to low-grade PIN in humans, whereas mPIN3 and 4 are more like human HGPIN. It is generally agreed that the biologic potential of low-grade PIN in humans is not completely clear, and it can be difficult to reproducibly identify. As such, in human prostate pathology low-grade PIN is generally not reported. In addition, mPIN1 and 2 can be seen focally in mice, particularly older mice (see Fig. 2A). It is not advisable to report low-grade PIN as an endpoint without extensive blinded pathologic analysis of multiple animals and quantitative comparison with age-matched controls and suitable statistical analysis. However, the presence of mPIN1 and 2 lesions evaluated in this manner, particularly if widespread in younger mice, may be a valuable first indication of the potential for the development of subsequent higher-grade pathologies as a number of time course studies have shown that mPIN1 and 2 lesions precede the development of higher-grade lesions (16, 17). In contrast, high-grade PIN (mPIN3/4) is readily recognizable by trained pathologists and is often, but not always, associated with progression with age or biologic complementation. In humans, HGPIN is more reproducibly recognizable and is often (but not always) associated with cancer. Thus, the presence of mPIN3/4 is a useful pathologic endpoint and should always be reported, although it is critical to always examine age-matched control mice.

**Adenocarcinoma.** As described by Shappell and colleagues (10), invasive carcinoma can be recognized by an infiltrative and destructive growth pattern of atypical cells with at least focal glandular differentiation. In mice, this is usually associated with stromal desmoplasia, which is characterized by stroma with abundant fibrosis. The fibrotic stroma usually has increased numbers of spindle-shaped cells (fibroblasts and/or myofibroblasts) as well as chronic inflammatory cells. Most invasive carcinomas in GEM models are adenocarcinomas. Examples are shown in Fig. 3A and B.

Microinvasive adenocarcinoma in GEM mice can be difficult to reliably recognize owing to tangential cutting, irregular
out-foldings of epithelial structures, and preexisting stromal changes. This is also a problematic area in human pathology in many organ systems. Step or serial sections may be useful to identify frank invasion in mice where microinvasion is identified. Microinvasive carcinoma is not a commonly used diagnostic category in human prostate pathology, and such lesions would usually be called "HGPIN with adjacent small atypical glands suspicious for adenocarcinoma." The consensus panel agreed that microinvasive carcinoma can be diagnosed in unequivocal cases (Fig. 3C; MC12-0298ICR), but over-reliance on isolated foci of microinvasion as a definitive pathologic endpoint indicative of higher biologic potential is discouraged without extensive well-controlled studies with appropriate statistical analysis. If only a microinvasive lesion is seen as the most advanced prostate lesion in a new line of mice, one should be cautious in diagnosing this as invasive adenocarcinoma unless unequivocal adenocarcinoma forming larger grossly visible tumors or metastases are found.

Intraductal adenocarcinoma is a well-known entity in human prostate pathology although it is not common (18). Examples of lesions consistent with intraductal carcinoma were seen in some GEM models. These lesions are characterized by proliferating masses of atypical cells with marked expansion of preexisting structures without obvious infiltrative growth (Fig. 3D). The panel has advised using the term intracystic carcinoma for such lesions as its biologic potential is not currently clear, unlike intraductal adenocarcinoma in humans, which is associated with aggressive disease. Additional studies will be needed to determine the true biologic potential of intracystic carcinomas in mice.

A new development that was noted in several models was the presence of heterologous epithelial differentiation. This took the form of focal squamous or intestinal differentiation. Examples are shown in Supplementary Fig. S3. Both squamous metaplasia and mucinous gland metaplasia can be seen in the benign human prostate. However, such heterologous differentiation is not commonly seen in human prostate cancer; adenosquamous carcinoma of the prostate has been reported but is extremely rare. Presumably this heterologous differentiation represents divergent differentiation from a pluripotent transformed precursor. Unless extensive, it was felt that such
lesions should still be classified as adenocarcinoma in the same way that urothelial carcinomas are classified as such despite focal squamous or glandular differentiation. The biologic significance of such changes is unclear, particularly as they were typically focal.

Sarcomatoid carcinoma. As described later in this review, a number of GEM models, particularly models with aggressive behavior, show evidence of focal or extensive sarcomatoid differentiation within adenocarcinoma. This is characterized, as in human pathology, primarily by the presence of highly atypical spindle cells admixed with, or adjacent to, invasive adenocarcinoma. An example is shown in Fig. 3E. In some models, these lesions have been characterized by IHC and have been shown to coexpress mesenchymal and epithelial markers to variable degrees (ref. 19; EX09-0118-2). No instances of differentiation of the sarcomatoid elements into differentiated sarcomas, such as osteosarcoma, were observed in such lesions. While histologically identifiable sarcomatoid carcino-

Figure 3. Prostate malignancies in GEM models of prostate cancer. A, invasive adenocarcinoma, Hi-myc model. B, invasive adenocarcinoma, Pten null X Sox9 overexpression model. C, microinvasive adenocarcinoma, Pten null Smad4 null model are shown at 10 weeks. Focal microinvasion (arrows). D, intracystic carcinoma; APC model. Masses of poorly differentiated adenocarcinoma are shown within a cystic space (arrow indicates wall of cystic space). E, sarcomatoid carcinoma, Pten null p53 null model. Masses of atypical proliferating spindle cells are shown entrapping residual glands with PIN. F, neuroendocrine carcinoma, TRAMP model, metastatic to liver.

ma is rare in human prostate cancer, there is evidence that epithelial–mesenchymal transition (EMT) can promote prostate cancer progression (20) although this concept remains controversial.

It should be noted that GEM models often have lesions of variable histologic severity in the same lobe or gland, ranging from mPIN to adenocarcinoma and/or sarcomatoid carcinomas. Typically large numbers of prostate epithelial cells show activation of a transgene or loss of tumor suppressor genes at early times after activation of expression of the transgene or Cre recombinase. The individual cells presumably have variable rates of progression as they accumulate additional alterations, and this presumably accounts for the variable histology seen. Human prostate cancer also shows significant histologic heterogeneity, and neoplastic lesions can be geographically distinct within the prostate. There is evidence of molecular heterogeneity between these different tumor foci in human prostate cancer. High-grade
PIN is also often multifocal. Thus, there is evidence that GEM models and human prostate cancer both may be multiclonal although it seems likely that GEM models are more multiclonal than human prostate cancer. At increasing times after tumor initiation, in mice and in humans, the fastest growing tumor may come to dominate the histology seen in the prostate.

**Neuroendocrine carcinoma.** Focal neuroendocrine differentiation is common in human prostatic adenocarcinoma (21). Frank neuroendocrine cancers are rare as primary cancers but are more common in heavily treated men with prostate cancer dying of their disease (EX09-0054BI). Such neuroendocrine carcinomas are often admixed with more typical acinar adenocarcinoma and seem to arise from adenocarcinomas in most cases (21). Of note, they typically do not express AR, unlike most castrate-resistant prostate cancer, which continues to express AR that is active at castrate levels of circulating androgens. It is possible that more neuroendocrine cancers will be seen in the future as with the emergence of more effective therapies targeting AR and local AR ligand production. Neuroendocrine carcinomas are similar to human neuroendocrine carcinomas in appearance and are characterized by cells with high nuclear/cytoplasmic ratio, small amounts of cytoplasm, and granular chromatin. IHC for neuroendocrine markers such as chromogranin or synaptophysin can be used to confirm neuroendocrine differentiation (Supplementary Fig. S4), as is common practice in human pathology (MC02-0699). Neuroendocrine carcinomas in GEM mouse models are associated with rapid growth and metastasis and are highly lethal, as in human neuroendocrine prostate cancer. They are often seen in TRAMP mice and crosses of them to other GEM. They are the most widely metastatic and aggressive mouse prostate cancer (TG06-0293-6). An example is shown in Fig. 3F (TG06-0394).

**Pathology of specific genetically engineered mouse models**

**PTEN/AKT pathway.** The phosphoinositide 3-kinase (PI3K)/AKT pathway is a major signaling pathway that is activated in many human malignancies. Activation of PI3K/AKT pathway by a variety of mechanisms has been found in almost all of late-stage prostate cancers (1). PTEN is a major negative regulator of the PI3K/AKT pathway and early studies in germline *Pten*−/+− heterozygous knockout mice showed mPIN lesions (22). To model the involvement of this pathway in human prostate cancer, Wang and colleagues at the University of California, Los Angeles (UCLA) crossed *Pten* flox/flox mice to the AR2-Probasin-Cre transgenic line, PB-Cre, in which the Cre recombinase is under the control of an enhanced prostate-specific probasin promoter, and generated mice with prostate-specific homozygous deletion of *Pten* (23). Such mice showed mPIN and ultimately invasive adenocarcinoma (23). An important feature of the model is that it recapitulates the disease progression seen in human prostate cancer, with tumor initiation in the form of mPIN, followed by progression to invasive adenocarcinoma and metastasis, making this a widely used model for studying the PTEN/AKT pathway in prostate cancer. As described later in this review, sarcomatoid carcinoma develops in a number of PTEN deletion models with additional genetic alterations (MC12-0194NIH). Examples of pathology from models based on PI3K/PTEN/AKT pathway activation are shown in Fig. 4. Additional images are shown in Supplementary Figs. S5 and S6 with links and descriptions in Supplementary Table S2. The PTEN/AKT models have very similar cytology characterized by nuclei that are relatively large and round to oval with a clear chromatin and abundant light pink cytoplasm. It should be noted that in PTEN deletion models mPIN3/4, adenocarcinoma and sarcomatoid carcinoma can all be present in a single tumor. In addition, stromal fibrosis (desmoplasia) is almost always present in invasive disease.

With the increased realization that prostate cancer involves multiple signaling pathways, many studies have focused on engineering additional genetic lesions into the *Pten* null prostate cancer model to study their potential cooperation with *Pten* loss in prostate carcinogenesis. A number of such models were examined by the panel members. In several of the models, introduction of another genetic lesion led to more aggressive tumors.

Yu Chen (Memorial Sloan Kettering Cancer Center, New York, NY) has studied a model that overexpressed ERG in *Pten* null prostate cancer and found that in comparison with *Pten* loss alone, ERG significantly accelerated tumor development. Examination of tumors from this model revealed adenocarcinoma at 12 to 15 weeks of age and anaplastic invasive adenocarcinomas with sarcomatoid carcinomas by 25 to 30 weeks of age (MC12-0220S; Supplementary Fig. S5). Studies by Carver and colleagues (24) had previously shown that ERG expression in a germline *Pten*−/+− mice resulted in development of adenocarcinoma, whereas mice with heterozygous loss of *Pten* alone only developed mPIN.

Amanda Swain (Institute of Cancer Research, London, United Kingdom) submitted a model with loss of *Pten* and activation of β-catenin. Two 12-week-old mice were reviewed (MC12-0299IRC; MC12-0297IRC). The first was a mouse with loss of 1 *Pten* allele and overexpression of a stabilized form of β-catenin, which showed high-grade mPIN with focal squamous differentiation, whereas a mouse with loss of both *Pten* alleles and overexpression of a stabilized form of β-catenin showed multicentric invasive adenocarcinoma with focal squamous differentiation. The same investigator also submitted 2 slides from mice with loss of *Pten* and overexpression of SOX9 in the prostate. A 52-week-old mouse with loss of one *Pten* allele and overexpression of Sox9 showed microinvasive carcinoma in a background of high-grade mPIN, whereas a 12-week-old mouse with loss of both *Pten* alleles and overexpression of Sox9 showed multicentric, highly invasive adenocarcinoma (Fig. 3B; see also Supplementary Figs. S5 and S6). These findings are concordant with prior studies from the Swain laboratory implicating Sox 9 in enhanced prostate cancer progression (25).

Clegg and colleagues generated mice in which the prostate was *Pten* null and overexpressed human MYC (ref. 26; MC12-01895; MC12-01885). The prostate reviewed from these bigenic mice showed adenocarcinoma with focal intestinal
metaplasia in a background of high-grade mPIN, involving all lobes of the prostate. The bigenic mice are very similar to Pten null mice (i.e., involvement of all prostate lobes and neoplastic epithelial cells with abundant eosinophilic cytoplasm) but are remarkably different from MYC transgenic mice, which showed multiple invasive adenocarcinomas predominantly involving the lateral lobe of the prostate and composed of epithelial cells with abundant clear vacuolated cytoplasm, large vesicular nuclei with prominent nucleoli and marked apoptotic activity.

Mulholland and colleagues have developed mice with expression of mutant K-ras (G12D/WT) expression and Pten homozygous deletion in the prostate and showed that, whereas RAS activation alone was not sufficient to cause malignant transformation in the prostate, combination of Pten deletion and RAS activation led to accelerated progression of prostate cancer in comparison with Pten deletion alone (27). The slides reviewed by the panel showed adenocarcinoma with focal squamous differentiation at 10 weeks (MC12-0376LA-recut-H&E-CL). Between 35 and 50 weeks, the tumors showed features of sarcomatoid carcinoma (MC12-0106LA-HE-KB). Metastasis was observed with 100% penetrance in lungs (see Supplementary Fig. S1; MC12-0106LA-2-HE-KB). Of note, these investigators provide detailed molecular analysis of the sarcomatoid carcinomas, which are consistent with mesenchymal differentiation due to EMT. Thus, activation of the mitogen-activated protein kinase (MAPK) pathway in the Pten homozygous knockout model accelerates tumor progression and is associated with EMT.

Wang and colleagues have reported similar observations about the role of the MAPK pathway in tumor progression using a different strategy (28). Slides from mice with Nkx3.1CreERT2 knock-in allele simultaneously inactivating 1 allele of Nkx3.1 while driving tamoxifen-dependent Cre that induces conditional deletion of Pten were examined. The anterior prostate from a 24-month-old Nkx3.1 CreERT2/++Ptenfl/fl intact mouse showed diffuse high-grade PIN with adenocarcinoma, whereas the anterior prostate from a 17-month-old Nkx3.1 CreERT2/+Ptenfl/fl castrated mouse showed adenocarcinoma with focal...
squamous differentiation. The addition of a mutant K-ras allele activation (Nkx3.1CreERT2/+/Ptenlox/+KrasSL/SL mouse) with tamoxifen treatment initiated at 2 months of age showed adenocarcinoma with focal intestinal metaplasia in a background of high-grade mPIN at 18 weeks (MC12-0263CA-recut-H&E-CL). Examination of slides of tumors from mice with a mutant BRAF (V600E) with the same knockout background (Nkx3.1CreERT2/+/Ptenlox/+Braflox/lox), with tamoxifen treatment initiated at 4 months, showed sarcomatoid carcinoma at 35 weeks (MC12-0265CA-recut-H&E-CL). Metastasis was reported in 30% of these mice. Thus, MAPK activation was again associated with aggressive sarcomatoid prostate cancer in a PTEN knockout background.

Although p53 is the most commonly mutated tumor suppressor gene in human tumors, deletion of p53 alone does not produce a tumor phenotype in the prostate (29). Chen and colleagues have shown combined homozygous loss of Pten and p53 resulted in significantly more penetrant and rapidly developing prostate cancer than Pten deletion alone (29). Examination of a 7-month-old mouse with homozygous loss of both Pten and p53 revealed sarcomatoid carcinoma (Supplementary Fig. S5). Martin and colleagues have also generated mice with simultaneous homozygous deletion of Pten and p53 (30). There was diffuse development of high-grade mPIN in all lobes by 8 to 10 weeks of age, with invasive adenocarcinoma as early as 12 weeks (Fig. 3B). Adenocarcinoma predominated before approximately 16 weeks with subsequent development of sarcomatoid carcinoma (Fig. 3E). Sarcomatoid carcinoma is the predominant pattern in end-stage mice (18–22 weeks) with small foci of residual adenocarcinoma and entrapped high-grade mPIN glands. There is also occasional squamous differentiation. Rare metastasis to sublumbar lymph nodes and occasional tumor emboli in pulmonary capillaries were observed. Clonal cell lines were derived from these mice to investigate the differentiation and metastatic potential of different tumor-initiating cell populations when injected orthotopically into nude mice. 41 Potent clonal cell lines were derived from these mice to investigate the differentiation and metastatic potential of different tumor-initiating cell populations when injected orthotopically into nude mice. A bipotential clonal line capable of luminal and basal differentiation gave rise to adenosquamous carcinoma with a high rate of lung metastasis. Another clonal line with a luminal phenotype in vitro (CK8+/Vimentin−) gave rise to orthotopic sarcomatoid carcinoma (CK8+/Vimentin+) with no distant metastasis. Slides submitted by Akash Patnaik (Beth Israel Deaconess Medical Center, Boston, MA) showed phenotypes similar to other Pten/p53 null models described earlier (See Supplementary Fig. S6).

Ding and colleagues have generated a mouse model with homozygous deletion of Smad4 and Pten in the mouse prostate (31). This led to the emergence of invasive, metastatic, and lethal prostate cancers with 100% penetrance with metastases to regional lymph nodes and lungs (31). Examination of slides from this model revealed highly glandular adenocarcinomas at 13 weeks (Fig. 4C) and metastases to lumbosacral lymph nodes (Fig. 4D; MC12-0387DF) and lung (Supplementary Fig. S6) at 13 and 23 weeks, respectively. The same group has made an inducible telomerase reverse transcriptase (mTert) allele that was activated in mice with homozygous loss of Pten and p53 tumor suppressors in a telomerase null background carried for 3 to 4 generations before mTert induction (32). Telomerase reactivation in these mice resulted in very aggressive cancers. We reviewed 5 digital slides showing primary tumor masses and lesions involving vertebral bone and bone marrow identified as a metastasis. The prostate showed adenocarcinoma and highly pleomorphic sarcomatoid carcinomas (Fig. 4E). A large mass of sarcomatoid carcinoma adhered to the spine and invaded spinal muscle and spinal cord via spinal nerves and bone marrow (Fig. 4F; MC12-0381-IDF). We did not see metastatic lesions in bone marrow compatible with hematogenous or lymphatic spread, but rather the tumor cells in spinal bone and bone marrow seemed to have originated via direct invasion and spread from the primary prostate mass.

More recently, Chen and colleagues (29) have generated a mouse with homozygous deletion of the Lsf1 (Pokemon) transcription factor along with homozygous deletion of the Pten gene. Histologic examination of a tumor from a 7-month-old mouse revealed sarcomatoid carcinoma similar to the mouse with Pten and p53 homozygous deletion described earlier, consistent with enhanced progression due to Lsf1 deletion (MC12-0289BIL-recut-H&E-EH).

Using the Pten null mouse model, Hong Wu's laboratory has recently shown reciprocal feedback regulation of PI3K and AR signaling (33). AR deletion in prostate epithelial cells of Pten null mice promoted robust adenocarcinoma development in the dorsolateral lobes. The slide submitted to the panel showed invasive adenocarcinoma of the prostate with focal intestinal metaplasia. Lai and colleagues submitted 1 slide from germline Pten heterozygous knockout mice with deletion of stromal AR (34). The phenotype seemed similar to the control Pten−/− mouse with multifocal high-grade mPIN at 28 weeks of age.

Pten deletion has been used to study the cellular origin of prostate cancer in mice. Choi and colleagues studied the cell of origin of prostate cancer by deleting Pten in basal and luminal cells, respectively (17). A 41- to 42-week-old K14-CreERT2/Ptenlox/flx/bigenic mouse (Pten deletion in basal cells) showed multifocal intermediate- to high-grade PIN which was more severe in the dorsolateral prostate and ventral prostate. A 30- to 36-week-old K8-CreERT2/Ptenlox/flx/bigenic mouse (Pten deletion in luminal cells) showed adenocarcinoma in a background of diffuse high-grade mPIN, which was also more severe in the dorsolateral prostate and ventral prostate. Michael Shen (Columbia University) asked the same question using different promoters to drive Cre expression in basal and luminal cells, respectively. CK5CreERT2/flx/bigenic mice (Pten−/− mice (Pten deletion in basal cells) showed microinvasive carcinoma in a background of high-grade PIN detected at 6 months, whereas Nkx3.1CreERT2/flx/bigenic mice (Pten−/− mice (Pten deletion in luminal cells) showed adenocarcinoma in a background of diffuse high-grade mPIN, which was also more severe in the dorsolateral prostate and ventral prostate. Early studies of this pathway used a transgenic approach with prostate-specific expression of constitutively active myristoylated-Akt (myr-AKT) that showed mPIN lesions (35). The panel reviewed a Tet-inducible myr-AKT under the control of a
probein promoter. The prostate showed diffuse hyperplasia and low-grade mPIN after induction but no invasive carcinoma (Supplementary Fig. S5). To date, activated Akt is less potent at inducing neoplasia in the mouse prostate than homozygous Pten deletion.

**MYC pathway.** Increased copy number of the Myc oncogene and overexpression of MYC protein are common in human prostate cancer (1). Ellwood-Yen and colleagues have shown that overexpression of MYC in the prostate results in mPIN that progressed to invasive adenocarcinoma, thereby delineating the functional role of MYC in prostate cancer initiation and progression (Hi-Myc model; ref. 36). The reproducible kinetics and high penetrance of mPIN following adenocarcinoma combined with shared molecular signatures with human prostate cancer has made this a powerful mouse model to study the oncogenic program of MYC. An example of adenocarcinoma in a MYC transgenic model is shown in Fig. 3A. It is of interest to note that Iwata and colleagues (37) have shown by IHC that the characteristic cytologic features seen in mPIN are seen as soon as the MYC transgene is expressed, implying that they are a direct result of MYC activity and do not require additional genetic lesions. As in Pten null adenocarcinoma, there is a prominent stromal response with fibrosis and infiltration of chronic inflammatory cells (MC12-0192S-H&E-recut-KB).

Kim and colleagues have developed a model that included prostate-specific, Cre-inducible, focal overexpression of MYC, and floxed Pten allele and p53 alleles allowing Cre-mediated heterozygous or hemizygous loss to these 2 tumor suppressor genes (38). The slides submitted showed extensive prostate cancer in all lobes (MC12-0026V-H&E-CL). This is in contrast with the Hi-Myc model, which generally developed tumors in the dorsolateral and ventral lobes. The COMP model retained histomorphologic features associated with MYC overexpression such as prominent nucleoli and large vesicular nuclei.

**ERG pathway.** The TMPRSS2/ERG fusion gene is present in half of all human prostate cancers and results in expression of a slightly truncated ERG oncogene under the control of the androgen responsive TMPRSS2 promoter (39, 40). Yu Chen’s laboratory (Memorial Sloan-Kettering Cancer Center) has developed ERG-overexpressing C57BL/6 transgenic mice by knocking ERG-ires-nsLacZ into the first intron of the endogenous TMPRSS2 locus. The slides submitted for panel review showed earlier and more numerous foci of low-grade mPIN in the transgenic ERG mice when compared with controls. At around 20 weeks of age, low-grade mPIN lesions were noted in some of the transgenic mice, but not controls, and at around 70 weeks of age nearly all transgenic mice showed low-grade mPIN lesions, whereas controls showed only rare low-grade mPIN lesions. Although neither high-grade mPIN nor adenocarcinomas were noted in the transgenic mice overexpressing ERG only, ERG overexpression in Pten null mice induced earlier and more significant lesions, including sarcomatoid carcinomas, as noted earlier (see “PTEN/Akt pathway”, earlier).

Casey and colleagues have generated a transgenic ERG mouse using a recombinant bacterial artificial chromosome that incorporated a 25Kb human TMPRSS2 promoter plus exons 1 and 2 adjacent to the human ERG genomic region downstream of intron 7/exon 8 (41). These mice were mated to Pb-Cre; Ptenflox/; mice to produce TMPRSS2-ERG; Pten+/− mice. There were no significant lesions in the prostates of TMPRSS2-ERG mice at 1 year of age. At 28 weeks, there was an increased number and severity of mPIN lesions in the prostates of TMPRSS2-ERG/Pb-cre; Pten+/− mice as compared with Pbcre/Pten+/− alone mice. However, this difference disappeared by 1 year of age as there was no significant difference in number or severity of mPIN lesions at 52 weeks.

Klezovitch and colleagues previously reported on their transgenic ERG mouse model, which overexpresses an N-terminally truncated ERG protein using a modified probein promoter (42). In these mice, focal mPIN lesions (generally low grade) are detected in mice beginning around 5 to 6 months. The number and severity of lesions tend to increase with age, and at around 18 months adenocarcinomas are noted. At 2 years of age, high-grade pleomorphic and spindle cell tumors were present, consistent with sarcomatoid carcinomas (MC12-0114FH-2-HE-KB). The reason for the more aggressive phenotype noted by these authors is unclear but may be related to the very high levels of ERG transgene expression in this model (V. Vasioukin et al., Fred Hutchinson Cancer Research Center, Seattle, WA; unpublished data).

**Retinoblastoma pathway.** Hill and colleagues (43) developed a model of retinoblastoma (Rb) inactivation via ARR2-Pb driven SV40 T121 expression and Pten/p53 deletion in prostate under the control of Pb-Cre1 promoter (Pten and p53 floxed). Slides submitted show that by 17 weeks there is diffuse high-grade mPIN in all lobes with early adenocarcinoma. By 20 weeks, adenocarcinoma was common (MC12-0323NCI) and there were poorly differentiated foci with evidence of early sarcomatoid carcinoma. In older mice (>21 weeks) sarcomatoid carcinoma was seen. In the anterior prostate there was often intracytic carcinoma that filled the entire lumen of the gland with no invasion into the underlying stroma. Lung metastases were occasionally observed.

Yu and colleagues submitted multiple slides from different crosses on their LADY (LPb-Tag-12t) backbone with mice with activating lesions of oncogenic pathways including, MYC, β-catenin (44), and IκB. The LADY model expresses SV40 large-T antigen (without small t), which inactivates both Rb and p53, and these mice reproducibly develop mPIN. All models showed progression from mPIN to invasive adenocarcinoma, with extensive involvement of all murine prostate lobes. Some models exhibited a biphasic adenocarcinoma-neuroendocrine carcinoma pattern. Another interesting cross with LADY has been reported by Klezovitch and colleagues (45). In this model, LADY mice were crossed with transgenic mice overexpressing hepsin, a serine protease that is expressed at increased levels in human prostate cancer, using a probasin promoter. This model developed multifocal metastasis to multiple organs and notably to bone. The metastatic lesions all express neuroendocrine markers. The panel reviewed a metastatic lesion in bone, which showed infiltration by tumor cells (Fig. 5A). The tumor cells had more abundant cytoplasm than is typically seen in neuroendocrine carcinomas but...
expressed markers characteristic of neuroendocrine differentiation.

**Fibroblast growth factor pathway.** There is abundant correlative evidence from the analysis of human prostate cancer that fibroblast growth factors (FGF) and FGF receptors play an important role in prostate cancer (46). A number of mouse models expressing either FGF ligands or activated receptors have been developed (47–51). The most aggressive model is the ligand-inducible FGFR1 transgenic mouse model, JOCK1 (juxtaposition of CID and kinase1). In this model, a transgene consisting of an FGFR1 kinase domain that contains a drug-binding domain is localized to prostate epithelium via the ARR2PB promoter. In the presence of lipid-permeable dimerizer, AP20187 (a chemical inducer of dimerization, CID), the intracellular signaling domains of FGFR1 oligomerize and activate unrestrained FGFR1 signaling by juxtaposing FGFR1 kinase domains that contain a drug-binding domain. When mice are treated with dimerizing drug they develop hyperplasia, mPIN, adenocarcinoma, and sarcomatoid carcinomas (19). Marked desmosplastic stroma response was noted (MC12-0080BY-H&E-CL).

**Androgen receptor pathway (MC12-0261ST-H&E-KB).** The AR plays a central role in the biology of prostate cancer in humans. Zhu and colleagues submitted mice with an inserted human AR transgene with a LoxP-stop-loxP (LSL) cassette into the mouse ROSA26 locus (52). Conditional transgene activation is driven by Cre mediated recombination of the LSL cassette. The Osr1 promoter was used to drive expression of Cre recombinase at day 11.5 in the urogenital sinus epithelium resulting in AR expression in prostate epithelium. There is slow development of focal low-grade mPIN lesions, and rare high-grade mPIN was observed with increased frequency in the dorsal and anterior prostate. Large intracystic adenocarcinomas were observed in the anterior prostate (MC12-0261ST-H&E-KB). Despite their large size, there was only minimal invasion into the underlying stroma; however, focal lymphovascular invasion was observed in 1 mouse. Within these large adenocarcinomas, there were occasional foci of squamous differentiation (Fig. 5B).

**WNT/β-catenin/APC pathway.** One published model of adenomatosis polyposis coli (APC) gene deletion-mediated prostate neoplasia was submitted to the panel for review. In this model, Probasin-Cre–mediated deletion of APC was engineered in an undebrimed mixed strain of mice that contained proportions of C57BL/6J and 129Sv/J strains (53). Extensive mPIN was identified by the panel in submitted samples, and adenocarcinoma was observed in supplementary samples from aged animals. Adenocarcinomas were predominantly intracystic with areas of microinvasion (MC02-0241). Distant metastases were neither observed nor reported by the investigators. The investigators reported in their article that one feature of the model is that androgen is required for tumor formation but not maintenance.

Slides submitted by the Shahi and colleagues (Baylor College of Medicine, Houston, TX) of mice with ubiquitous expression (MHC1 promoter) of an inducible LRP5 construct, which induces constitutive β-catenin signaling when treated with dimerizer drug (see JOCK1 above), were examined (54). Slides from mice showed adenocarcinoma (Fig. 5C) and sarcomatoid carcinoma associated with reactive stroma in some mice.

**RAS/RAF/MAPK pathway.** Mutations of RAS have been observed in prostate cancer but seem to be uncommon, at least
in men in the United States. Similarly, RAF mutations are uncommon, activation of the MAPK pathway is common in prostate cancer, and introduction of mutant RAS or RAF oncogenes can activate this pathway. As described earlier, such lesions can enhance progression and the development of sarcomatoid carcinomas in mice with PTEN loss. Pearson and colleagues submitted slides from their model of Cre-driven Scribble deficiency in combination with G12D K-ras (55). The Scribble complex is involved in establishing and maintaining epithelial polarity. Biallelic loss of Scribble was noted to result in mPIN in the slides reviewed. The group reviewed slides from the cross with K-rasG12D mutation. This combination resulted in mPIN and extensive desmoplasia and foci highly suspicious for microinvasion.

**TGF-β pathway.** TGF-β has a well-established role in promoting carcinogenesis, including prostate carcinogenesis. Bhowmick and colleagues (Cedars-Sinai Medical Center, Los Angeles, CA) have generated mice with partial Tgfbr2 knockout in stromal fibroblasts by crossing Tgfbr2flox/floxE2 and Col1α2-Cre-ER mice in a C57BL/6 background. Systemic administration of tamoxifen activates Cre-mediated recombination, yielding mice deficient in stromal Tgfbr2-mediated cell signaling. Slides from these mice showed high-grade mPIN associated with stromal chronic inflammation (Fig. 5D; MC12-0047CSM-H&E-CL). Thus, loss of TGF-β signaling in stroma induces paracrine signaling that is sufficient to drive the initial stages of prostate carcinogenesis. Deletion of Smad4, a central mediator of TGF-β signaling, alone does not cause morphologic changes in the prostate (31); however, in conjunction with Pten deletion as reported earlier it causes an epithelial carcinoma with high metastatic potential.

**SV40 T-antigen.** TRAMP mice have been widely used in a variety of studies of the biology of prostate cancer with over 400 publications to date. This model expresses a complete SV40-T antigen (small and large-T). TRAMP mice have a high incidence of neuroendocrine tumors arising in the prostate that are highly metastatic to lung, liver, and other tissues (TG06-0394-3). TRAMP mice crossed to other GEM also have such lesions. TRAMP mice that have tumors of the seminal vesicles also develop polypoid tumors of epithelial cells admixed with stroma (phylloides tumors). They are often multiple and quite large. Invasion is not common, but some lesions have small foci of possible invasion. Addition of additional genetic lesions can inhibit development of the neuroendocrine carcinoma and metastasis. An example is the model reported by Qi and colleagues that was submitted to the group. In this model, knockout of the ubiquitin ligase Siah2 suppressed formation of neuroendocrine tumors (56). Similar suppression has been seen with other crosses of knockout mice with TRAMP (57).

**Xenograft models**

**Pure xenograft models.** Wang and colleagues have developed xenograft models of prostate cancer by engrafting fresh, histologically intact human primary prostate cancer tissues into SCID mice (58). Specifically, the investigators evaluated the graft take rate and the histopathologic features of cancer tissues implanted in the subcutaneous, subrenal capsule, and orthotropic sites. Although the efficiency of viable graft recovery was high at the subrenal capsule and prostatic orthotopic sites (~95% and 70%, respectively), the take rate of subcutaneously grafted tissues was only around 50%. Of note, the histopathologic features of the prostate cancer grafts overlapped with those of the preimplantation cancer tissues. Moreover, the differentiation marker prostate-specific antigen (PSA) was expressed at similar levels in the tumor specimens before grafting and in the tumor grafts grown in testosterone-supplemented SCID hosts (MC12-0118BC-AR_PSA-EH). Overall, these data suggest that patient-derived prostate cancer xenograft models could be valuable not only for investigating prostate cancer biology but also for preclinical evaluation of therapeutics in vivo. More recently, Lin and colleagues (59) were able to successfully propagate prostate cancer subrenal capsule grafts and generate several well-characterized transplantable xenograft lines. Most of these xenografts lines, when engrafted under the renal capsules of SCID mice, consist of tumor cells that express AR and PSA, show local invasion into adjacent host kidney parenchyma, and display androgen-dependent in vivo growth (Fig. 6A). A subset of the grafts has the capability of spreading to distal organs, mostly as intravascular tumor emboli (Fig. 6A). The investigators also successfully generated xenograft lines that are initially sensitive to castration in vivo but subsequently become castration resistant. The castration-resistant tumors consist of small round cells that express neuroendocrine markers, are negative for AR and PSA, and display androgen-independent growth in vivo. Finally, a xenograft line derived from a metastatic lesion of a small cell carcinoma of the prostate (i.e., LTL-352) is also available. The subrenal capsule tumors show histopathologic features of poorly differentiated neuroendocrine carcinoma (Fig. 6B) and are characterized by invasive growth into the host kidney parenchyma and metastases to distant organs. Additional details can be obtained at livingtumorlab.com.

The Vessella laboratory (5, 6, 60) has generated 28 novel prostate cancer xenograft lines designated as the LuCaP series, which were generated by implanting small fragments of human prostate cancer tissues subcutaneously in male BALB/c nu/nu mice. Their overall yield in achieving established xenografts is approximately 18%. About 15% of the xenografts have been derived from primary prostate cancer and the remainder from metastatic lesions obtained either at surgery or through the rapid autopsy program. Most of the LuCaP xenografts produce PSA that is detectable in the mouse sera, often up to several hundred nanograms per milliliter. Characterization of many of these xenografts including phenotypic, molecular, and response-to-therapy data have been reported in numerous publications. For example, Kumar and colleagues (61) have described whole-exome sequencing of a number of these xenografts revealing both recurrent and novel mutations, and the origin of a number of these xenografts is described in that report. Histologic examination revealed moderately to poorly differentiated adenocarcinomas, undifferentiated carcinoma, and, in some tumors, histology compatible with neuroendocrine carcinoma (see Supplementary Fig. S7; MC12-0085UW-HE-KB).
Prostate tissue recombinants engrafted under the kidney capsule of immunodeficient murine hosts are valuable tools to study the role of mesenchymal–epithelial interactions in both normal prostate development and prostate tumorigenesis (62). Several years ago, Hayward and colleagues established a nontumorigenic immortalized human prostate epithelial cell line (BPH-1) by expressing the SV40 large T antigen in primary epithelial cell cultures. Tissue recombinants generated using BPH-1 cells and normal human prostatic fibroblasts show the presence of small epithelial nests and cords exhibiting squamous differentiation. Tissue recombinants generated using BPH-1 cells and fetal rat urogenital sinus mesenchyme (rUGM) display the presence of large nests of keratinizing squamous epithelium that, however, do not invade the host kidney parenchyma (Fig. 7A). Of note, BPH-1 cells are able to form invasive tumors when recombined with human prostatic carcinoma-associated fibroblasts (CAF). These tumors do not histologically resemble prostatic adenocarcinoma but display morphologic characteristics of squamous cell carcinoma (Fig. 7B; ref. 63; MC12-0169V-H&E-recut-KB). To develop models that more closely mimic key futures of prostate cancer in patients, Jiang and colleagues from the same group recently established a spontaneously immortalized normal human prostate epithelial cell line (NHPrE1). Recombinants of NHPrE1 with rUGM show the presence of benign glandular structures that express prostate differentiation markers including AR, PSA, and NKX3.1 (Fig. 7C; ref. 64). The investigators are now planning to expose NHPrE1 cells to multiple genetic and environmental insults to develop a model of multistep prostate tumorigenesis.
Tuxhorn and colleagues from the Rowley laboratory have developed a tissue recombination model known as the differential reactive stoma (DRS) model using prostate stromal cells lines derived from normal human prostate (ref. 65; MC12-0313BY-recut-HE-EH). The LNCaP cell line is normally poorly tumorigenic when injected subcutaneously in nude mice but when mixed with stromal cell lines tumorigenesis is significantly enhanced. Of note, only certain lines robustly enhance tumorigenesis. Matrigel is also added in the "3-way" differential reactive stroma model. This model has been used to dissect stromal factors supporting prostate cancer tumorigenesis (66, 67). Stromal lines have also been developed from mouse prostates and can be derived from GEM mice with knockout of specific genes. Both the epithelial and stromal compartments can be manipulated to decrease or increase expression of specific genes allowing dissection of tumor stroma interactions. Histologically, tumors consist of nests of malignant epithelial cells admixed with stromal cells and blood vessels (Fig. 7D). Eosinophilic masses of Matrigel may also be present.

Nonmurine models

**Rat prostate cancer models.** Transgenic rat models of prostate cancer have been developed. We examined a transgenic rat model with the SV40 T antigen under probasin promoter control, allowing prostate-specific gene expression (68). Male rats are said to develop prostate cancer at 100% incidence before they are 15 weeks old. The slides examined show focal and diffuse hyperplasia and PIN and adenocarcinoma (Fig. 8A; MC12-0302DV). An area of neuroendocrine carcinoma was also seen (Fig. 8B).

**Canine prostate cancer (MC12-0313BY-recut-HE-EH).** Pet dogs have been identified as an animal model that can contribute significantly to our understanding of cancer and to the treatment of spontaneous neoplasms. Dogs develop spontaneous HGPIN and carcinomas of the prostate gland that are often both aggressive and metastatic, although generally androgen insensitive (69). Lai and colleagues have reviewed the histology of prostate cancer in the dog and identified multiple variants of adenocarcinoma, undifferentiated carcinoma, and sarcomatoid carcinoma (70). Examples submitted showed poorly differentiated adenocarcinoma (Fig. 8C) or undifferentiated carcinoma. Reactive stroma and inflammation were prominent. Clearly, there are important differences between the anatomy and histology of the canine and human prostate glands and in the neoplasms arising in them. However, human and canine prostate glands share a common embryologic origin and have many homologous anatomic and microanatomic structures, and even though prostate tumors in dogs tend to be hormone insensitive and many of the tumors arise in castrated dogs, they may perhaps provide a model of human advanced castrate-resistant tumors. Regardless of differences between tumors of dogs and humans, the dog may be a useful complementary model to studies of human prostate cancer. The domestic dog shares a common environment with humans and is subject to sporadic neoplasia of presumptive multifactorial etiology that develops slowly over time.
influenced by an intact immune system, as is the case in humans. In addition, dog breeds present a useful genetic resource in the study of diseases such as cancer. The NCI has identified pet animals as a potential population to incorporate into studies of new therapeutics (71). The use of the dog in studies of cancer therapeutics is further enhanced by the existence of resources for molecular studies in dogs: for example, the canine genome sequence, completed in 2005, represents an invaluable resource for studies of neoplasia in dogs. The dog's important contribution to translational research directed at helping humans can potentially benefit pet dogs themselves.

Discussion

Over the past 15 years, numerous animal models of prostate cancer, particularly GEM models, have been developed. The comprehensive analysis of the GEM models has identified 2 issues related to the pathology of these models that bear further discussion. The first is the observation that invasive cancers in the GEM models are associated with reactive stroma characterized by the presence of spindle cells (fibroblasts or myofibroblasts) and chronic inflammatory cells. Of note, some stromal changes are also noted in association with high-grade mPIN in the GEM models. Most human prostate cancer is not associated with histologically evident reactive stroma and tends to infiltrate the preexisting fibromuscular stroma with little histologically obvious reaction, although changes in gene and protein expression occur in this normal-appearing stroma (72). Ayala and colleagues have identified a subset of human prostate cancer with histologically evident reactive stroma (73, 74). The degree of reactive stroma formation can be graded, and cancers with the most extensive reactive stroma (reactive stroma grade 3) have significantly worse outcomes (73, 74). Of note, reactive stroma formation was seen in stroma adjacent to some foci of HGPIN, similar to the observation in GEM models. Thus, the stroma of GEM models of prostate cancer most closely resembles the stroma of this aggressive subgroup of human prostate cancers. Clearly, further work is needed to evaluate the biologic similarities and differences between the tumor microenvironment in human prostate cancer and GEM models. Of note, the tissue recombination xenograft models described earlier will be extremely useful in dissecting the relevant biology, at least for CAFs and myofibroblasts.

Another common pathologic phenotype noted was the high incidence of sarcomatoid carcinomas in the most aggressive GEM models. Of note, this phenotype was identified by the authors of published reports of some models, whereas in other models it was not specifically commented on. Sarcomatoid carcinomas were associated with aggressive local growth and invasion and increased metastasis in many GEM models. We noted this phenotype on multiple Pten null models with additional genetic lesions. It was also identified in older mice in GEM models with unrestrained FGFR1 signaling (JOCK1) and high-level ERG overexpression. However, it should be noted that the presence of sarcomatoid carcinoma was not always associated with increased metastasis. In a Pten null p53 null GEM model with sarcomatoid carcinoma, in 100% of mice with end-stage disease, lymphovascular invasion was ubiquitous; however, local lymph node metastasis was rare and distant metastases were not observed (21). In addition, an orthotopic transplant...
model that was developed using these prostate cancer cells showed that a clonal cell line that gave rise to sarcomatoid carcinoma did not develop distant metastasis. It is unclear whether all GEM models will necessarily display sarcomatoid differentiation when they display aggressive behavior, and this will need to be carefully evaluated moving forward. The histologic evidence of sarcomatoid differentiation has been shown to be due to EMT in several models. Histologically evident sarcomatoid differentiation is not uncommon in a number of human cancers such as lung and renal cell carcinoma and is associated with aggressive clinical behavior but is uncommon in human prostate cancer. Careful studies are needed to determine the extent to which this EMT reflects molecular events in aggressive human prostate cancer.

In general, specific genetic lesions were associated with characteristic histologic patterns that are readily recognizable. Models associated with the PTEN/AKT pathway develop mPIN and high-grade, invasive lesions with characteristic cytologic features that differ from similar lesions in the other models. The PTEN/AKT signature phenotype can be identified by the large nuclei with a delicate chromatin pattern and relatively small nucleoli (MC12-0699BIL). The light pinkish cytoplasm tends to be relatively abundant with well-defined cell borders (MC02-0699). In contrast, the TRAMP neuroendocrine tumors have small compact nuclei with dense hyperchromatic nuclei and very scanty cytoplasm without defined cytoplasmic membranes. The signature MYC pathway cells have round to oval nuclei with a coarse chromatin rim with prominent nucleoli (TG04-0319-HE). The lesions associated with the Ras oncogene display a wide variety of phenotypes rarely seen with the other neoplastic phenotypes. For example, many Ras-associated tumors have goblet cells indicative of intestinalization (MC02-0696). Squamous metaplasia was found in Ras-associated neoplasms (MC12-0254CA; MC12-0248CA). In addition, Ras expression was associated with sarcomatoid carcinomas. Other phenotypes were observed, but the limited number of slides available with other models precluded definitive conclusions.

The use of animal models has significantly enhanced our understanding of the pathobiology of prostate cancer. Accurate pathologic analysis of these models is critical for their optimal use. Moving forward it will be important to make use of these models to develop improved methods of preventing, detecting, and treating human prostate cancer. The optimal model or models for such studies is not yet clear, and it is anticipated that correlation of results in animal models and human patients will be the ultimate test of the use of these models.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors’ Contributions

Conception and design: M. Ittmann, S. Signoretti, M. Loda, A. Borowsky, R.D. Cardiff

Development of methodology: M. Loda, A. Borowsky, R.D. Cardiff

Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): M. Ittmann, J. Huang, G.C. Chu, M. Loda, A. Borowsky, R.D. Cardiff


Writing, review, and/or revision of the manuscript: M. Ittmann, J. Huang, E. Radaelli, P. Martin, S. Signoretti, R. Sullivan, B.W. Simons, J.M. Ward, B.D. Robinson, G.C. Chu, M. Loda, G. Thomas, A. Borowsky, R.D. Cardiff

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): M. Ittmann, E. Radaelli, J.M. Ward, B.D. Robinson, G. Thomas, A. Borowsky, R.D. Cardiff

Study supervision: M. Ittmann

Acknowledgments

The authors thank the Katie Bell, Judy Walls (Histology), Ed Hubbard, and Arishneel Ram (WSI) at University of California, Davis, for their technical assistance.

Grant Support

This study was supported by NCI: U01 Mouse Models of Human Cancers Consortium (U01CA141497 and U01 CA141582; P01CA125123 (M. Ittmann); UCLA. Pacific Northwest and D/HCC SPOREs in Prostate Cancer, P01 CA89901 (M. Loda) and ROI CA131945 (M. Loda); UWCCC Core Grant P30 CA04520 (R. Sullivan) P30 CA069533 ISSS through the OHU-Knight Cancer Institute (G. Thomas); Department of Defense Prostate Cancer Research Program (W81XWH-11-1-0272); J. Huang); and Prostate Cancer Foundation.

Received November 12, 2012; revised December 18, 2012; accepted January 22, 2013; published OnlineFirst April 22, 2013.

References


Downloaded from cancerres.aacrjournals.org on April 15, 2017. © 2013 American Association for Cancer Research.


transformation of the mouse prostate. J Biol Chem 2011;286:
33478–88.
149–59.
Animal Models of Human Prostate Cancer: The Consensus Report of the New York Meeting of the Mouse Models of Human Cancers Consortium Prostate Pathology Committee

Michael Ittmann, Jiaoti Huang, Enrico Radaelli, et al.


Updated version
Access the most recent version of this article at:
doi:10.1158/0008-5472.CAN-12-4213

Supplementary Material
Access the most recent supplemental material at:
http://cancerres.aacrjournals.org/content/suppl/2013/05/08/0008-5472.CAN-12-4213.DC1

Cited articles
This article cites 73 articles, 28 of which you can access for free at:
http://cancerres.aacrjournals.org/content/73/9/2718.full.html#ref-list-1

Citing articles
This article has been cited by 15 HighWire-hosted articles. Access the articles at:
/content/73/9/2718.full.html#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.