Genetically defined mouse models that mimic natural aspects of human prostate cancer development

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Abstract

This review is focused on mouse models for prostate cancer that have been designed on the basis of genetic alterations that are frequently found in human prostate cancer. It begins with an analysis of the similarities and differences in the gross and microscopic anatomy of the mouse and human prostate glands, and extends to the pathologies induced in the genetically manipulated mouse prostate in comparison with the sporadic development of the disease in humans. Major achievements have been made in modeling human prostate cancer in mice in recent years. There are models which display slow, temporal development of increasingly severe preneoplastic lesions, which are remarkably restricted to the prostate gland, a property similar to the aging-related progression of these lesions in humans. Other models rapidly progress to local invasive adenocarcinoma, and, in some of them metastasis is manifested subsequently with defined kinetics. Global assessment of molecular changes in the prostate of the genetically manipulated mice is increasingly underscoring the validity of the models through identification of ‘signature’ genes which are associated with the organ-confined primary or distant metastases of human prostate cancer. Taken together, various ‘natural’ models depicting stages of the disease, ranging from the early preneoplastic lesions to metastatic prostate cancer, now provide new tools both for exploring the molecular mechanism underlying prostate cancer and for development or testing of new targeted therapies.

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Introduction

Prostatic adenocarcinoma, the most common malignant visceral neoplasm of men, is a complex disease, and its natural history has been difficult to ascertain. It is extremely difficult to predict whether a particular carcinoma will remain indolent throughout a man’s lifetime or become clinically aggressive (Gittes 1991, Abbas & Scardino 1997). Cancer of the prostate displays extensive phenotypic heterogeneity, both morphologically and molecular genetically, and is typically also admixed with non-cancerous cells within the glands (Roy-Burman et al. 1997, DeMarzo et al. 2003). Such complexities are further intrigued by the presence of multiple malignant foci in a majority of cancerous glands (Miller & Cygan 1994). Despite these difficulties, there has been some progress in understanding the clinical course and the molecular parameters of this disease, albeit in a fragmentary manner. It is widely perceived that the most likely primary precursor of human prostate cancer are the histopathological lesions known as prostatic intraepithelial neoplasia (PIN). PIN lesions can be described as low grade (LG) or high grade (HG), and it is believed that HGPIN is a precursor of prostatic adenocarcinoma (McNeal et al. 1991, Haggman et al. 1997, Isaacs et al. 2002). While initially the growth of prostate tumor or cancer is dependent on androgens, after hormonal therapy most cancers become androgen-independent,
thereby making continued androgen-deprivation therapy ineffective (Pilat et al. 1998, Feldman & Feldman 2001). The molecular pathways that contribute to genesis of subclinical, microscopic PIN lesions, their progression to invasive cancer and androgen-independence remain largely unknown, although certain molecular candidates have been implicated in the overall process of disease progression. For example, aberrations in specific signaling molecules including extracellular growth factors, protein tyrosine kinase cell surface receptors, intracellular anti-apoptotic or transcription factors, nuclear receptors and their ligands, growth suppressors, cell cycle regulators and others have been indicated in some prostate carcinomas. Some of these issues have constituted various topics for a number of recent reviews (Abate-Shen & Shen 2000, Elo & Visakorpi 2001, Gao & Isaacs 2002, DeMarzo et al. 2003, Litvinov et al. 2003, Powell et al. 2003).

There is currently a strong interest in the application of the knowledge gained to date from the analyses of human prostate cancer in modeling this disease in mice in order to recapitulate its natural course of development and progression (Abate-Shen & Shen 2000, Park et al. 2002, Powell et al. 2003). This interest is, however, a logical derivative of the earlier successful efforts to derive transgenic mouse models with prostate-specific expression of viral oncogenes. The first transgenic mouse line, designed with the anticipation to induce prostate cancer, used a potentially prostate-restricted promoter, that is of the prostate steroid-binding protein C3(1) gene to drive expression of the SV40 viral large T-antigen (Maroulakou et al. 1994, Shibata et al. 1998). Since then, several other models have been developed using other types of promoters to attain prostate-specific expression of viral oncogenes. Two such models, which are well studied, are the so-called transgenic adenocarcinoma of the mouse prostate (TRAMP) (Greenberg et al. 1995, Gingrich et al. 1997) and LADY (Kasper et al. 1998, Masumori et al. 2001) mouse lines, which used two different versions of the rat probasin gene promoter to direct expression of the transgene(s) to the prostate epithelium. Besides the size variations of the promoter, another interesting difference between the two systems is that the TRAMP mice may express both large T and small t-antigens of the SV40 virus, while the LADY is designed to express only the large T-antigen. These models have been useful in the analyses of various parameters in the disease progression, resistance of anti-androgen therapy, and other aspects. However, there are some inherent limitations in these systems which are noteworthy. For example, the viral antigens and their pleiotrophic effects are not naturally associated with the human cancer and these models manifest extensive neuroendocrine differentiation (Garabedian et al. 1998, Masumori et al. 2001, Abate-Shen & Shen 2002, Powell et al. 2003), a phenotype morphologically and biologically distinct from the adenocarcinomas seen in most human prostate cancers. These issues, in turn, motivated investigators to focus on genetic alterations or aberrations in gene expression that are frequently encountered in human prostate cancer in the design of mouse models. There is remarkable success with these second generation models. The first series of genetic manipulations, which yielded prostatic preneoplastic disease with a stochastic pattern of increasing degree of abnormalities in the lesions, has now been complemented with newer models that further progress to prostatic adenocarcinoma followed by metastatic disease. This review is targeted at the second generation models with the underlying expectation that they could still be further refined in the future to better recapitulate the physiological and clinicopathological features of human prostate cancer. To this end, however, it will be critically important to recognize that, although both men and mice harbor functionally equivalent prostate glands, there are differences in the organization of this tissue in the two species. Therefore, an emphasis is placed, at the beginning, on the understanding of the similarities and differences in the gross and microscopic anatomy between human and mouse prostates and their pathologies.

**Mouse vs human prostate: the realities of comparative anatomy**

**Anatomy and histology of the prostate**

There are both significant similarities and differences in the gross and microscopic anatomy of the mouse and the human prostate, and these need to be considered when evaluating murine models of human prostatic disease. Both mouse and human have similar epithelial cell types present in their prostates (secretory, basal and neuroendocrine), which presumably carry out the same physiological functions, but the ratio varies between species. Both species have columnar secretory epithelial cells which secrete prostatic proteins and fluids from their apical surfaces into a lumen. Humans have a continuous layer of basal cells between the secretory cells and the basement membrane, whereas mice have fewer basal cells and a discontinuous layer around the glands (Marker et al. 2003). Neuroendocrine cells are scattered and rare in human prostate, and even more rare in the mouse (Garabedian et al. 1998). The prostates of both species are composed of glands and ducts, but there are significant differences in the stroma. The human prostate has a robust fibromuscular stroma. The mouse prostate has a very modest stromal component. Both species have prostates and accessory glands that develop from Wolffian ducts and urogenital sinuses and both are...
androgen-sensitive. There are many embryological, anatomical and histological similarities between mouse and human prostate tissues. However, the two species are sufficiently different with respect to this tissue that collaboration with a pathologist who is familiar with both mouse and human prostate tissue is a necessary resource when evaluating murine models of human prostate disease.

Anatomically, the human prostate gland is located between the base of the bladder and the rectum, and it completely surrounds the proximal urethra (Fig. 1A). It is a single alobular structure with central (CZ), peripheral (PZ) and transitional (TZ) zones. In contrast, the mouse prostate is not merged into one compact anatomical structure. It comprises four paired lobes situated circumferentially around the urethra, immediately caudal to the urinary bladder—namely, anterior (AP), dorsal (DP), lateral (LP), and ventral (VP) prostate (Fig. 1B). Often, the dorsal and the lateral lobes are thought of in combination and referred to as the dorsolateral (DLP) lobe as they share a ductal system. The mouse AP is considered analogous to the human CZ, which is rarely a site of neoplastic transformation in humans. The mouse DLP is considered most similar to the human PZ, which is the zone in which most carcinomas arise (Xue et al. 1997).

These analogies, however, are limited as they are based solely on descriptive data and need to be re-evaluated using molecular techniques before the relationship between specific mouse prostate lobes and the human prostate zones is definitively asserted (Abate-Shen & Shen 2000). The mouse VP does not have a human homologue, and the human TZ does not have a murine homologue (Table 1).

The DLP originates dorsally at the base of the seminal vesicles and wraps ventrally around the urethra. Grossly, it is fern-like in morphology with a clear, gelatinous appearance in the unfixed state. The acini are variably sized with an intermediate amount of epithelial infolding compared with the VP (which has the least amount of infolding) and AP (which has the greatest amount of infolding) (Fig. 2A). The DP has simple, columnar epithelium with basophilic granular cytoplasm and centrally placed nuclei. Nucleoli are absent or indistinct. Both the size of the nuclei and the height of the epithelium are slightly greater than those of the VP. The stroma in the DP is relatively dense compared with the other lobes. The secretions are eosinophilic and homogeneous. In the LP, the epithelial cells can be more cuboidal and less basophilic than those of the DP. The secretory cells of the LP have smaller nuclei that are basally located, as well as hyperchromatic nucleoli. The stroma is much thinner than that of the DP, and the secretions are granular and more eosinophilic.

The ventral lobe is also clear, gelatinous and leaf-like, but can be difficult to identify without a dissecting microscope. All of the male accessory glands become easier to visualize after fixation. The ducts of the ventral lobe enter the urethra ventrally. The most unique
Table 1 Description of anatomy and histology of human and mouse prostate

<table>
<thead>
<tr>
<th>Human anatomy and histology</th>
<th>Mouse anatomy and histology</th>
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<tbody>
<tr>
<td>Overall anatomy: Postero inferior to bladder, completely surrounding the urethra. Base of prostate abuts bladder neck, apex of prostate rests on the urogenital diaphragm. Funnel shaped, 20g, 4 × 3cm.</td>
<td>Overall anatomy: Caudal to the bladder, incompletely surrounding the urethra. Not a discrete, compact unit.</td>
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<tr>
<td>Subgross anatomy: Alobular with three zones.</td>
<td>Subgross anatomy: four paired lobes.</td>
</tr>
<tr>
<td>Overall microanatomy: Ducts and glands with simple columnar epithelium in a compact, encapsulated organ composed of both glandular and non glandular elements which are tightly fused together. Epithelial cell types: secretory, basal, neuroendocrine. Fibromuscular stroma is dense with abundant smooth muscle.</td>
<td>Overall microanatomy: Ducts and glands with simple columnar epithelium and folded mucosae in loose connective tissue. Epithelial cell types: secretory, basal, neuroendocrine. Fibromuscular stroma is looser and considerably less than human; minimal smooth muscle component.</td>
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<tr>
<td>Central zone: ~25% of glandular prostate; surrounds ejaculatory ducts and converges with the prostatic urethra at the verumontanum. Acini are larger than other zones. Glands are complex with prominent intraluminal ridges +/- tufting and cribriform pattern. Glands are surrounded by a thin, compact band of smooth muscle. Epithelial to stromal ratio is 2:1. Secretory cells are haphazardly arranged, have darker granular cytoplasm, and large nuclei. The epithelium appears crowded and/or pseudostratified.</td>
<td>Anterior prostate: Grossly clear and gelatinous; apposed to the lesser curvature of the seminal vesicles, bilaterally. Complex glands with the greatest degree of infolding and prominent intraluminal ridges +/- tufting or cribriform pattern. Simple columnar epithelium. Secretory cells have round, centrally located nuclei and inconspicuous nucleoli; cytoplasm is granular and eosinophilic. Secretion is abundant, homogeneous, and less eosinophilic than the secretions of the adjacent SV. The stroma has prominent connective tissue layer with a smooth muscle component.</td>
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<tr>
<td>Peripheral zone: ~70% of glandular prostate; represents the posterolateral aspects of the prostate; surrounds the TZ and CZ from the apex posteriorly to the base. Epithelial to stromal ratio is 1:1. Secretory cells have pale cytoplasm with basally–centrally located nuclei. The epithelium appears orderly and is composed of a single layer of columnar cells. The stroma is relatively loose.</td>
<td>Dorsal prostate: Grossly, clear and gelatinous; originates dorsally at the base of the SVs, bilaterally. Simple columnar epithelium with intermediate degree of infolding. The secretory cells have centrally located nuclei and indistinct nucleoli; cytoplasm is granular. The secretion is eosinophilic and homogeneous. The stroma is relatively dense. Lateral prostate: grossly, clear and gelatinous with a leaf like appearance; originates dorsally and incompletely wraps around the urethra ventrally, bilaterally. Simple columnar epithelium with negligible infolding. Secretory cells have small, basally located nuclei with nucleoli; the cytoplasm is less granular than the DP. The stroma is thin.</td>
</tr>
<tr>
<td>Transitional zone: ~5% of glandular prostate; two independent interiorly located, pear-shaped lobes surrounding the proximal urethra. Epithelial to stromal ratio is 1:1. Secretory cells have pale cytoplasm. The stroma is dense and has interlacing smooth muscle fibers.</td>
<td>No counterpart to transitional zone.</td>
</tr>
<tr>
<td>No counterpart to ventral prostate.</td>
<td></td>
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<tr>
<td>Proximal prostatic urethra: Bladder neck to verumontanum. Distal prostatic urethra: verumontanum to apex.</td>
<td>No counterpart to proximal prostatic urethra.</td>
</tr>
<tr>
<td>Capsule: An extension of the stroma, not a distinct capsule; separates prostate from periprostatic fat; defective anteriorly and anterolaterally.</td>
<td>No counterpart to distal prostatic urethra.</td>
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<td></td>
<td>No counterpart for capsule that surrounds entire prostate; however, individual lobes are surrounded by a thin, delicate capsule.</td>
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The histological feature of the VP is that its simple columnar epithelium lacks infolding. Like the LP, the stroma is thin and the secretory cells have basally located nuclei; however, they do not have distinct nucleoli.

The coagulating gland is also known as the AP (although the term ‘anterior’ can be confusing as it has a different meaning in bipeds and quadrupeds). The APs are cranial to the other prostatic lobes and are apposed to the lesser curvature of the seminal vesicles (Fig. 3A and B). They are tubular in shape and have a clear, gelatinous gross appearance. Histologically, the AP will be on the same slide as the seminal vesicles, as these tissues are adjacent and usually submitted as a unit, but will have comparatively less eosinophilic secretions and more papillary projections into the lumen. The epithelium of the AP will have the greatest degree of infoldings with possible cribriform pattern. The epithelial cells are columnar with round, centrally placed nuclei (Fig. 3B; see also Fig. 6B and C).

The human CZ surrounds the ejaculatory ducts, which enter the prostate from the posterosuperiorty located seminal vesicles. It converges with the prostatic urethra at the verumontanum, surrounding the TZ from the base of the bladder to the angle of the urethra. The ducts and acini are larger than the other zones, and, like the mouse AP it has the most complex epithelium with prominent intraluminal ridges and/or cribriform pattern. The epithelial to stromal ratio is highest in this zone at 2:1, while in TZ or PZ the ratio is 1:1 (Sternberg 1997). The glands are surrounded by a thin, compact band of smooth muscle. The secretory cells are remarkable for their haphazard arrangement giving the epithelium a crowded or pseudostratified appearance. These cells have granular cytoplasm that is slightly darker than the other zones and large nuclei. The human TZ consists of two independent, interiorly located, pear-shaped lobes which surround the proximal urethra. It is roughly 5% of the glandular prostate and the most common site for nodular hyperplasia. The secretory cells have pale cytoplasm, and the stroma is dense with interlacing smooth muscle fibers.

The PZ in the human is where 70% of adenocarcinomas arise. It represents approximately 70% of the glandular prostate and comprises the posterolateral aspects of the prostate as it surrounds the TZ and the

**Table 1 continued**

<table>
<thead>
<tr>
<th>Human anatomy and histology</th>
<th>Mouse anatomy and histology</th>
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<tr>
<td>Nerves and vessels: Paired branches originating posteriorly and arborizing into prostatic stroma.</td>
<td>Nerves and vessels: Found in the loose connective tissue that separates the glandular areas from their capsules.</td>
</tr>
<tr>
<td>Anterior Fibromuscular stroma: An apron of tissue composed of smooth muscle fibers.</td>
<td>No counterpart to anterior fibromuscular stroma.</td>
</tr>
<tr>
<td>Hormone-responsive</td>
<td>Hormone-responsive</td>
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SV, seminal vesicle.

**Figure 2** Microanatomical comparison of mouse and human prostates. (A) Mouse prostate (×20) with variable epithelial infolding in different lobes. (B) Human prostate (×10) contains dense fibromuscular stroma, which surrounds the glandular epithelium.
CZ. The orderly epithelium has a single layer of columnar cells with basally located nuclei. The secretory cells have pale cytoplasm and the fibromuscular stroma in this zone is loose.

The non-glandular elements of the human prostate differ significantly from those of the mouse. The human prostate has dense fibromuscular stroma with abundant smooth muscle, fibroblasts, and collagen, which surrounds the glandular elements (Fig. 2B). Anteriorly, the prostatic stroma merges with the fibromuscular tissue of the urogenital diaphragm. The ‘capsule’ of the human prostate is actually an extension of the prostatic fibromuscular stroma and not a distinct capsule, but it does separate the prostate from the periprostatic fat. The capsule is defective anteriorly and anterolaterally where it converges with the anterior fibromuscular stroma. The anterior fibromuscular stroma is an apron of tissue composed of smooth muscle fibers. The most remarkable difference between the human and the murine prostates, anatomically and histologically, is that the human prostate is composed of significantly more fibromuscular stroma, which unifies the zones into a discrete organ, whereas the mouse prostate lobes are separate entities. Not only does the mouse prostate have looser and much less fibromuscular stroma, the stroma has only a minimal smooth muscle component. The ducts and glands of the mouse prostate are primarily surrounded by a loose connective tissue. The mouse prostate does not have a distinct capsule. However, the individual lobes are surrounded by a delicate, thin fibromuscular sheath.

The differences in the overall anatomy and microanatomy between human and murine prostates have an impact on how one evaluates cancer models. Major concerns in carcinogenesis are the ability of a tumor to invade locally and metastasize distantly. Lack of a capsule-like entity in the mouse confounds the ability to draw conclusions about local invasiveness of a similar cancer in humans. Likewise, the difference in the anatomical location of the neurovascular bundles in the human and the mouse prostates presents another difficulty in assessing invasive potential of prostatic cancer in the mouse. In the human, the nerves and vessels originate from either the base or the apex posteriorly, sending penetrating branches into the fibromuscular stroma. As mentioned above, the mouse does not have a compact unit of fibromuscular stroma enveloping the glandular prostatic elements, and the neurovascular bundles course through the loose connective tissue that separates the glandular areas of the lobes from their thin capsules, rather than in the stroma itself. Perineural invasion is a very common route of extracapsular spread in humans, so this difference must be taken into consideration when evaluating a mouse model of prostatic cancer.

**Pathology of the prostate**

The mouse is not prone to developing spontaneous benign or malignant prostate pathology. If left unmanipulated, it would be rare to see any significant abnormalities in the prostate at necropsy. When mice are manipulated genetically, chemically, or surgically, certain alterations that occur spontaneously in humans can be reproduced. The human prostate, on the other hand, is prone to pathology, especially with advancing age. Nodular hyperplasia, which is a benign proliferation of the epithelium and stroma of the TZ, starts developing in the fourth decade of life and has a nearly 100% prevalence by the ninth decade (Damjanov & Linder...
Atrophy of the prostate is very common in older men. Prostate cancer is seen in up to 80% of 80-year-old or older men at autopsy.

Epithelial hyperplasia with or without atypia can be seen in genetically manipulated mice (Suwa et al. 2002). It appears as an increase in the number of epithelial cells or an increase in the size of glands, and it can be focal or diffuse. It is not a considered precancerous or progressive lesion. With increased atypia, the diagnosis changes from hyperplasia to dysplasia, also called PIN. When this lesion is seen in the mouse, it is referred to as mPIN (Fig. 4B and D). Human PIN occurs spontaneously in men and increases in prevalence with age. It occurs almost exclusively in the PZ, and it is graded by its degree of atypia as either LG PIN (LGPIN) or HG PIN (HGPIN). HGPIN is considered a putatively precancerous lesion in human pathology, and increasing grades of PIN are associated with progressive loss of the basal cell layer. Several genetically manipulated models have been induced to develop mPIN, but often they do not progress to invasive cancer despite a high degree of nuclear atypia.

The term HGPIN suggests progressiveness, and invasion should be demonstrated before using this term with respect to mPIN. Further, mPIN appears histologically distinct, depending on what type of gene was manipulated and what method was used to manipulate it. However, the basic criteria of proliferation of atypical epithelial cells within normal gland spaces with evidence of progression remain consistent.

Microinvasion through the basement membrane and into the adjacent stroma is a subsequent step in cancer progression. Since the mouse and human prostatic stromal elements are so dissimilar, caution must be taken when making interspecies correlations concerning microinvasion. In human diagnostic pathology, when the stromal invasion becomes more extensive and/or perineural invasion can be demonstrated, a diagnosis of invasive carcinoma is given (Fig. 4C). Here again, the method of genetic manipulation can affect the type of carcinoma produced, as will be discussed later. Examples of invasive adenocarcinoma produced in a genetically engineered mouse prostate are shown in Fig. 5.

**Figure 4** Histopathological lesions in the prostate. (A) Human PIN; (B and D) examples of mPIN. (C) Human adenocarcinoma.
Human prostatic adenocarcinoma is graded according to the Gleason system, which assesses the glandular architecture, size and appearance of the glands, and the pattern of invasion. Murine prostatic adenocarcinoma is graded as well-, moderately- and poorly-differentiated based on glandular architecture and cellular atypia (Gingrich et al. 1997). Neoplastic cells typically have enlarged nuclei with prominent or multiple nucleoli. Carcinoma lacks a basal cell layer, and immunostaining of high molecular weight cytokeratin is frequently done in both species to detect its presence or absence. In human carcinoma, intraprostatic perineural invasion is commonly seen, and murine models should be assessed for their ability or lack thereof for perineural invasion. Neuroendocrine cells can be present in significant numbers in human adenocarcinoma, but they are not neoplastic, whereas the neuroendocrine cell of the SV40 T-antigen models are neoplastic. Similar neuroendocrine tumors in humans are rare, constituting only around 5% of all prostatic neoplasms (Powell et al. 2003).

Tumor stage or the anatomical extent of disease should also be assessed. Pathological staging of human prostate cancer is assessed by determining whether there has been capsular perforation, seminal vesicle invasion, invasion into surrounding structures (e.g. bladder) and lymph node metastases. The usual sites of metastases of prostatic adenocarcinoma in the human are the regional lymph nodes, bones, and lung. In SV40 T-antigen mouse models, the most common sites of spread are lymph nodes and lung (Abate-Shen & Shen 2002). Similarly, another recent model, which is based on Pten conditional knockout, displays distant metastases to lymph nodes and lung, although there are some early clues for bone abnormalities in this model as well, which, however, remain to be verified (Wang et al. 2003).

**Figure 5** Examples of invasive adenocarcinoma in the Pten null prostate mouse model. (A) Prostate cancer cells invading the blood vessels, a higher magnification of which is shown in (B). In the same model, lung metastatic cells are shown to be positive for AR immunohistochemically (C) while negative for PTEN-staining (D) (from Wang et al. 2003).

**Incidental lesions and age-related changes in the prostate**

Incidental lesions are those that are considered ‘within normal limits’ and without consequence. In the murine
prostate, it is not unusual to see focal aggregates of well-differentiated lymphocytes (Maronpot et al. 1999). True prostatitis, in mice, 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 (R D Cardiff, unpublished observations). Likewise, patchy acute and chronic inflammation is present in the prostates of most adult men and is not considered a significant finding. ‘Concretions’ in the murine prostatic urethra are also commonly seen as incidental findings. These usually represent semisolid coagulum rather than calculi (Maronpot et al. 1999). Lastly, the accumulation of brown pigment lipofuscin or hemosiderin in murine prostatic tissue is seen with age, or remote hemorrhage respectively. These incidental findings are seen in wild-type as well as genetically manipulated mice.

Spontaneous prostatic atrophy in wild-type laboratory mice is extremely rare. Manipulation of sex hormone levels can result in physiological atrophy of the prostate epithelium. Grossly, atrophy may be suspected when prostatic lobes appear smaller at necropsy. Histologically, atrophy will be manifested by shrunken glands, and the normally columnar to cuboidal epithelium of the prostate becomes decreased in size (less secretory cytoplasm) and appears pseudostratified or flattened (Maronpot et al. 1999). Similarly, estrogen-supplemented or androgen-depleted mice may develop prostatic epithelial metaplasia, with replacement of the normal epithelium by transitional, squamous or mucinous epithelium. This is a reversible and non-neoplastic process. In humans, atrophy due to aging or secondary to remote inflammation is very common, and demonstrates the same general histological characteristics as prostatic atrophy in the manipulated mouse.

The prostate is hormone-responsive in both humans and mice. The epithelium matures at adolescence in response to testosterone. In humans, the prostate atrophies with advanced age in response to decreasing levels of testosterone. Manipulation of hormone levels, genetically, chemically or surgically, will alter the appearance of the prostate epithelium. In pre-pubescent mice, the testes will be undescended unilaterally or bilaterally, and the glandular epithelium resembles immature human prostatic glandular epithelium with crowded gland spaces and multilayered dark nuclei (Sternberg 1997).

**Dissection techniques for mouse prostate**

There are a number of protocols for dissecting the murine prostate. Effort has been made recently to standardize the sectioning of this tissue (Suwa et al. 2002), and this standard will be presented here. However, the protocol should be modified according to the particular study and necropsy findings, keeping in mind that the prostate is a hormone-sensitive organ that matures as sexual maturity is reached. Before beginning, it is necessary to familiarize oneself with the orientation of the male accessory sex organs in the mouse. As with human prostate, orientation can be difficult. The UC Davis Center for Comparative Medicine, Visible Mouse Project’s Comparative Anatomy of the Prostate (http://ccm.ucdavis.edu/mmhcc/prostate/comparativeindex.htm) is an excellent resource for prostate anatomy and histology, and it provides a detailed protocol for those interested in microdissecting each lobe of the prostate separately. In most cases, en bloc dissection and fixation with specific sectioning will suffice, as an experienced mouse pathologist will be able to identify the separate lobes based on histological differences and anatomical relationships. To perform a basic, en bloc dissection, the killed mouse is placed ventral-side up with extremities pinned to the dissecting board. The abdominal fur is soaked with 70% ethanol prior to making a ventral abdominal incision from the xiphoid process to the anus; this helps keep loose fur from sticking to visceral organs, which makes gross photography easier. Key orientation landmarks are penis, seminal vesicles (in a mature mouse), and bladder. The abundance of fat in the epididymal fat pad can be quite variable, but an effort should be made to trim as much fat as possible prior to fixation. Starting either cranially (from the seminal vesicles down) or caudally (from the penis up), the reproductive tract and urinary bladder are removed as a unit. Lesions should be measured, noted, and photographed at this time. In order to maintain anatomical relationships, the unit is wrapped in tissue paper and placed in a cassette for fixation. At trimming, the unit is placed dorsal-side up and, using the distal ends of the seminal vesicles as a landmark, a transverse section through the urethra is made. The two resulting tissue fragments will include both DLP and VP (Fig. 6A and B). Finally, sagittal cuts through both seminal vesicles are made. Submit them in a separate cassette with APs still attached (Fig. 6C).

**Single-gene overexpression models**

A number of transgenic mouse lines have been produced in which genes that are known to be overexpressed in human prostate cancer are targets. All of the models reviewed here, however, concern usage of a robust prostate-specific promoter to drive the expression of the transgene, and thus exclude those which were based on promoters that are not sufficiently prostate-specific. For example, the transgenic line constructed for c-myc overexpression under the control of the promoter of the rat
C(3)1 gene, expresses the transgene not only in the prostate but also other tissues, including testes and the uterus of the females (X Zhang et al. 2000). Although these mice develop epithelial cell abnormalities in the VP, their utility is limited due to the ultimate loss of reproductive capability, perhaps from abnormal \(c\)-myc transgene expression in the reproductive tissues. Another example is the important growth factor, namely insulin-like growth factor-I (IGF-I), that may also be a good tumor marker in prostate cancer (Woodson et al. 2003), and which was targeted for expression in the mouse using the bovine keratin 5 promoter (DiGiovanni et al. 2000a). These mice develop squamous papillomas, some of which progress to carcinomas of the skin. The increased IGF-I

**Figure 6** A guide for dissection of mouse prostate. (A) Dorsal view of prostate, accessory glands, bladder and penis, with appropriate transections. (B) Sagittal cuts of seminal vesicle (SV) and AP embedded whole in one cassette (×1). (C) Cranial face of urethra transection from (A) (×4).
levels also lead to pathological changes in the prostate and in other male accessory glands of these transgenic mice (DiGiovanni et al. 2000b). The pathology of the prostate includes mPIN lesions, small and focal well-differentiated adenocarcinomas, which are more like carcinoma in situ, and tumors with neuroendocrine differentiation.

Androgen receptor (AR)

The AR is expressed in normal prostate in the secretory epithelial cells at high levels and in a subset of stromal smooth muscle cells (Cussenot et al. 1994, Leav et al. 1996). All phases of prostate cancer ranging from preneoplastic lesions to androgen-dependent adenocarcinoma to androgen ligand-independent disease require the activity of this nuclear receptor (Feldman & Feldman 2001, Balk 2002, Litvinov et al. 2003). The failure of androgen-ablation therapy and development of ‘andro-gen-resistant’ prostate cancer is thought to be, at least partly, due to gain of function in AR signaling that operates in the absence of optimal levels of the ligands (Litvinov et al. 2003). Although the mechanisms for the continued AR signaling in the androgen-deprived environment largely remain to be elucidated, there are some clues for either overexpression of the receptor, gain-of-function mutations in AR gene, AR coactivator overexpression or ligand-independent activation of the AR in a substantial proportion of this cancer (Buchanan et al. 2001a, Feldman & Feldman 2001, Edwards et al. 2003). For example, in about 20–30% of primary prostate cancers following androgen deprivation and in a similar fraction of metastatic foci, there is amplification of the AR gene (Koivisto et al. 1997, Bubendorf et al. 1999, Linja et al. 2001). The incidence of somatic mutations in AR-coding sequence in such advanced cancer is even higher, up to 50% (Taplin et al. 1995, 1999, Marcelli et al. 2000); many of these mutant receptors appear to display altered, and generally enhanced, activation by androgens and some non-classic ligands (Gelman 1996, Buchanan et al. 2001, Jia et al. 2003). To this end, it is important to note that in the TRAMP mouse model, androgen deprivation also results in the detection of AR mutations in the cancer and some of the mutant receptor forms could indeed contribute to altered signaling (Han et al. 2001b). Since the stromal microenvironment is known to be critical in malignant transformation of the prostate epithelium (Cunha et al. 2002), AR activation within the nuclei of stromal smooth muscle cells is another important parameter in prostate cancer. For example, while expression of certain cytokines, such as transforming growth factor-β1 is inhibited by AR activation in the stroma, secretion of andromedins or various androgen-induced stromal peptide growth factors may have a regulatory influence on differentiation, proliferation or survival of the epithelial cells (Lu et al. 1999, Planz et al. 1999, Litvinov et al. 2003).

To assess the effects of AR overexpression in the normal prostate secretory epithelium, a transgenic mouse line was produced (Stanbrough et al. 2001), by using the rat probasin gene minimum promoter (Greenberg et al. 1995) to drive a murine AR transgene expression. These mice display histologically normal prostate up to 1 year of age, but with further aging (> 1 year) begin to develop focal areas of mPIN lesions in the VP and DLP. In addition to making the point that increased AR levels in the epithelium may be a strong factor in the generation of moderate to severe dysplastic lesions, the results from this model have several other interesting implications. Although a marked proliferation of secretory epithelial cells of the prostate is noted, hyperplasia is not prominent in these animals, apparently because of the simultaneous increase in the rate of apoptosis. Since the mPIN lesions are focal and increased with age, it is suggested that a balanced increase in proliferation and apoptosis may be a conducive factor for secondary genetic or epigenetic events that lead to dysplasia (Stanbrough et al. 2001). The model remains to be investigated for the nature of these secondary events and whether these changes are dependent on AR-induced positive cell growth regulation or on unknown effect of AR signaling on genome stability and integrity. Because of the important role of the stroma in prostate carcinogenesis, it would be also interesting to determine whether the progression and severity of the dysplastic lesions could be enhanced by AR manipulations in the stroma as well. Simultaneous increase in andromedins and their paracrine effects may be some of the additional factors which, if available, potentially could alter the temporal pattern of the lesions in this model, a proposition that may be initially tested by designing appropriate tissue recombinant analyses (Cunha et al. 2002).

Fibroblast growth factors (FGFs)

The FGF family of heparin-binding proteins are intercellular signaling molecules, of which at least 23 different members (FGF1–FGF23) have been identified to date. Most FGF proteins are secreted and their effects as extracellular components are mediated by a complex system of FGF receptor (FGFR) tyrosine kinases through either autocrine or paracrine mechanisms (Basilico & Moscatelli 1992, Johnson & Williams 1993, Wilkie et al. 1995, McKeehan et al. 1998). Dysregulation of several of these growth factors has been implicated in prostate development and tumorigenesis (Djakiew 2000, Thomson 2001). One member of the FGF family, FGF8 is of considerable interest in prostate cancer (Ghosh et al. 1996, Leung et al. 1996, Tanaka et al. 1998, Dorkin et al. 1999).
The conventional knock-out of the fgf8 gene in the mouse led to early embryonic lethality (Meyers et al. 1998) because FGF8 is a crucial signaling molecule for outgrowth and patterning, such as the elongating body axis, midbrain-hindbrain junction, limb and face (Crossley & Martin 1995, Mahmood et al. 1995, Crossley et al. 1996). Alternative splicing of the ‘first’ exon of the fgf8 gene in mouse gives rise to eight potential protein isoforms that vary in their amino termini (Tanaka et al. 1992, 1995, MacArthur et al. 1995a,b). In humans, however, only four protein isoforms (FGF8a, 8b, 8e and 8f) are predicted due to a blocked reading frame in the exon 1B of the human gene (Gemel et al. 1996, Tanaka et al. 1995). Of the four possible isoforms, FGF8b has been demonstrated to possess the most transforming and tumorigenic potential (Kouhara et al. 1994, MacArthur et al. 1995a,b, Ghosh et al. 1996, Daphna-Iken et al. 1998), and it appears to be the primary species in prostate epithelial cell lines or malignant epithelium (Ghosh et al. 1996, Dorkin et al. 1999). Its expression is practically undetected in the stromal component of prostate cancer (Tanaka et al. 1998, Dorkin et al. 1999, Valve et al. 2001). The overexpression of FGF8b in human prostate cancer LNCaP cells increases their growth rate, soft agar clonogenicity, and in vitro and in vivo invasion ability (Song et al. 2000). Furthermore, it is demonstrated that the growth of stromal cells can be strongly upregulated when cocultured with FGF8b-producing LNCaP cells (Song et al. 2000). Downregulation of FGF8 mRNA by antisense RNA expression reduces the growth rate, inhibits the soft agar clonogenic activity and diseases in vivo tumorigenicity of prostate tumor cells (Rudra-Ganguly et al. 1998). Increased expression of FGF8b in prostatic lesions beginning from PIN to adenocarcinoma to its persistence in androgen-independent disease has been detected (Dorkin et al. 1999, Valve et al. 2001).

Interesting information is emerging on the regulation of FGF8 gene expression. Addition of retinoids to prostate cancer cells appears to switch the expression from the highly transforming isoform b to the weakly transforming isoform a (Brondani & Hamy 2000). Analysis of the promoter region has led to identification of two distinct functional motifs cis-responsive to retinoids or their receptors (Brondani et al. 2002). One is a canonical retinoic acid (RA) response element, which is the cis target of an RARx–RXR liganded heterodimer, and the other representing a novel type of response element with two half-binding sites with spacer. The novel element is demonstrated to be the target of unliganded RARx homodimer phosphorylated on the Ser77 residue. Combined activities of these cis- and trans-acting factors may support a model of complex regulation leading to an isoform switch (Brondani et al. 2002). There is also evidence that expression of FGFRs in prostate cancer is, at least in part, regulated by the AR at the transcriptional level (Gnanapragasam et al. 2002). Besides these attractive links between hormones and growth factor, a report points to angiogenic activity of FGF8b (Mattila et al. 2001). A correlation of both tumor and stromal expression of vascular endothelial growth factor, a mediator of neo-angiogenesis in prostate cancer with clinical parameters as well as its correlation to FGF8 expression has been described (West et al. 2001).

Among the FGFRs, the ‘c’ splice form of FGFR2 or FGFR3, as well as FGFR4, are reported to be most efficiently activated by FGF8b (MacArthur et al. 1995c, Blunt et al. 1997). Although these receptor isoforms are considered to be largely expressed in mesenchymal cells, there is evidence of aberrant expression of FGFR isoforms in prostate cancer (McKeehan et al. 1998). It is also interesting that prostate appears to exhibit wide expression of the general classes of FGFRs. For example, a moderate level of expression of FGFR1 and FGFR2 is found in prostate epithelium and the microvasculature, while stromal smooth muscle cells exhibit a weak level of expression of FGFR3 (Hughes 1997). In another study (Ittman & Mansukhani 1997), in primary cultures of human prostate epithelial and stromal cells, FGFR3 was found to be the primary product in epithelial cells with smaller amount of FGFR2, while stromal cells express primarily FGFR3 and small amounts of FGFR1 and FGFR2. Together these observations indicate that FGF8–FGFR signaling plays an important role in prostate biology and cancer.

Accordingly, it was of interest to develop a transgenic mouse model to examine the effect of FGF8b overexpression in the prostate. Four independent lines of transgenic mice were generated with targeted production of FGF8b in the prostatic epithelium using an improved rat probasin promoter (Song et al. 2002). This promoter, ARR2PB, has been demonstrated to confer a high level of reporter transgene expression specifically in the prostatic luminal epithelium and is strongly regulated by androgens (J Zhang et al. 2000, Wu et al. 2001). The histopathology of the prostate tissues was followed in different age groups of the various lines, but most extensively in one line (line 3), starting from 1 month of age to 24 months. Prostatic hyperplasia appears in the LP and VP in some animals as early as 2–3 months, and in other lobes, DP and AP, between 6 and 16 months. Beginning at 5–7 months, mLGPIN is detected. During the first 14 months, 100% of animals exhibit multifocal epithelial hyperplasia; 35% also had areas of mLGPIN. This profile changes in subsequent months (15–24 months) to a higher incidence of mLGPIN (66%) along with mHGPIN lesions (51%). Some of the mHGPIN lesions appear similar to the...
histopathology of human prostatic carcinoma in situ. This progression is depicted in Fig. 7. Similarly to mHGPIN, stromal proliferation and appearance of papillary hyperplasia with atypia, display a delayed pattern. Together, these findings, which are consistent in four independent transgenic lines, are biologically highly significant since the expression of FGF8b, a powerful mitogen, is associated with progression of human prostate cancer, beginning with PIN lesions. It can be concluded from the study of these transgenic mice that FGF8b is an etiological factor in prostate tumorigenesis. Clearly, an increased expression of FGF8b is sufficient to drive proliferation in the prostatic epithelium that precedes the development of the histopathologically identifiable lesions. The stochastic pattern of disease progression also implies that initiated hyperplasia is conducive to the manifestation of other genetic lesions, which may represent the rate-limiting factors responsible for a temporal progression from hyperplastic changes to preneoplastic lesions up to carcinoma in situ. Furthermore, a delayed but fairly common development of stromal hypercellularity in the prostate of the transgenic mice mimics the results of the epithelial–stromal cocultures in vitro (Song et al. 2000), implicating an indirect effect of FGF8b signaling in the epithelial cells on the stromal cells. Thus, FGF8b is likely to act not only as an initiation factor but also, possibly, as a progression factor. In fact, there is evidence to suggest that FGF8b overexpression can confer strong tumorigenic as well as invasive properties to weakly tumorigenic prostate carcinoma cells (Song et al. 2000). The angiogenic properties of FGF8b (Mattila et al. 2001) are similarly noteworthy in this regard.

In contrast to FGF8, which is produced in the prostate epithelium, FGF7 (keratinocyte growth factor) is an andromedin made by the stroma while its cognate receptor FGFR2 isoform b is found on the epithelium (Leung et al. 1997, Matsubara et al. 1999). There is strong evidence for FGF7 to be a mediator of stromal–epithelial interactions in prostate development (Rubin et al. 1995, Sugimura et al. 1996). A report describes that a switch in the spatial pattern of FGF7 expression may occur in clinical prostate cancer such that cancer cells, rather than stromal cells, display stronger expression, which is further increased with the increase in the Gleason grade of the specimens (McGarvey & Stearns 1995). This observation is consistent with findings in the SV40 T-antigen models in which unscheduled FGF expression could be detected in the epithelial compartment during the progression of the prostate cancer (Foster et al. 1997, Huss et al. 2003). These changes in FGF7 or other FGF members may be linked to malignancy or gain of independence from stromal control as there appears to be concomitant changes in FGFRs as well. For example, during prostate cancer progression, FGFR2 may switch from isoform ‘b’ to ‘c’ in the rat Dunning tumor system (Yan et al. 1993, McKeehan et al. 1998) or to FGFR1 isoforms in the TRAMP model (Foster et al. 1997). To convert FGF7 from a paracrine to an autocrine factor, transgenic mice
were produced in which the minimal rat probasin promoter was used to target FGF7 transgene expression directly to prostatic epithelium (Foster et al. 2002). After 1 year of age, these mice mostly develop prostatic epithelial hyperplasia, which, however, does not progress to dysplasia or tumor. Thus, it appears that FGF7 misexpression in the prostate could be a contributing factor, at least, in the induction of proliferation of the epithelial cells, although the primary role of FGF7 may rest on its paracrine signaling in prostate biology and disease.

**FGFRs**

The topic of changes in the FGFR expression patterns with prostate cancer progression is of considerable interest (Foster et al. 1998, Huss et al. 2003). The role of the FGFR signaling axis, specifically related to certain FGFRs, has recently been evaluated in transgenic models (Freeman et al. 2003b). The motivation to select targeted expression of FGFR1 and FGFR2 was directly based on a previous report that FGFR2 may limit, while FGFR1 may accelerate, tumorigenesis of prostate epithelial cells in an experimental system (Feng et al. 1997). Transgenic mice were generated with ARR2PB-driven expression of either ligand-inducible FGFR1 or FGFR2. Recognizing that growth factor receptors like FGFRs (Schlessinger et al. 2000) are naturally activated by oligomerization, a chemically induced dimerization technology was cleverly employed to achieve temporal control of the FGFR axis in the transgenic animals (Freeman et al. 2003b). While activation of FGFR1 triggers hyperplasia followed by mPIN lesions, such activation of FGFR2 in these mice does not appear to elicit any observable changes in the prostate even after 12 weeks of treatment with the inducer. The effect of FGFR1 expression is highly penetrant, as 100% of these transgenic mice treated for 12 weeks with the inducer show mPIN in virtually every acinus. By timed removal of FGFR1 signaling, the investigators show that induced hyperplasia is reversible until intraductal vascularization, after which the hyperplastic cells become independent of FGFR1 signaling for their maintenance. These interesting models highlight the physiological differences between the FGFR1 and FGFR2 receptor functions in the prostate epithelium. Similar to what is described for FGFR8 and FGF7 transgenic mice, these studies reemphasize the specificity and potency of individual players in the FGF–FGFR signaling, which could potentially be exploited for targeted intervention of prostate tumorigenesis.

In another study, a dominant negative, truncated FGFR2 ‘b’ splice form was targeted to the prostate epithelium using the minimal rat probasin promoter to functionally abrogate the endogenous signaling through this receptor isoform (Foster et al. 2002). As with the overexpression of FGFR2 in the prostate, interference of signaling by the dominant negative mutant also does not yield gross changes in the prostate except that the size is smaller than the littermate controls. Histologically, blocking the FGFR2 ‘b’ signal appears to induce some disorganization of the prostatic ducts, and areas of hyperplastic stroma in the transgenic mice. Moreover, a trend in the emergence of epithelial–neuroendocrine transition is noted in the prostate gland in the absence of mPIN or tumor lesions.

**SKP2**

The ubiquitin–proteasome pathway of protein degradation is an important control in maintaining appropriate amounts of short-lived regulatory proteins in the cell (Hershko & Ciechanover 1998). Protein ubiquitylation for proteolysis is a complex process involving a series of components, both invariable and variable, that is targeted towards a specific protein. A ubiquitin E3 ligase, the SCF SKP2 complex, consisting of SKP1, Cull, Rbx1 and the F-box protein SKP2, mediates the polyubiquitination of the CDK inhibitor p27 Kip1 (Carrono et al. 1999, Sutterluty et al. 1999, Tsvetkov et al. 1999). In this complex, SKP2 serves as a substrate-targeting subunit that binds to the phosphorylated p27. In many cancers, increase in SKP2 is inversely correlated with low expression of p27 (Gstaiger et al. 2001, Hershko et al. 2001, Kudo et al. 2001, Chiarle et al. 2002), and there is evidence that SKP2-deficient mice have excess cellular accumulation of p27 (Nakayama et al. 2000). There may also be an interesting link between SKP2, p27 and the PTEN tumor suppressor. It seems that PTEN-mediated accumulation of p27 could be caused by the specific inhibition of SKP2 expression through down-regulation of the phosphatidylinositol 3-kinase (PI3K) pathway (Mamillapalli et al. 2001). Targeted overexpression of SKP2 in the T-lymphocyte lineage in mice results in increased susceptibility to lymphoma (Lmates et al. 2001).

Based on the knowledge of inverse relationship between the levels of SKP2 and p27, and on the general observation that low or lack of p27 expression may be associated with human cancers including the malignancy of the prostate (Cordon-Cardo et al. 1998, Lloyd et al. 1999, Slingerland & Pagano 2000), transgenic mouse lines were generated that express SKP2 under the control of the ARR2PB promoter in the prostate epithelium (Shim et al. 2003). It is demonstrated that such overexpression of SKP2 in the prostate gland induces downregulation of p27 and is associated with development of epithelial hyperplasia and dysplasia up to mHGPIN lesions in both VP and DLP glands. While it is very likely that SKP2 acts as an oncoprotein in the mouse prostate through
induction of ubiquitin-dependent degradation of p27, there may be other mechanisms yet to be defined. For example, although hyperplasia and dysplasia are prominent by 3-7 months of age in SKP2 transgenic mice, only hyperplasia but not dysplasia develops in p27-deficient mice at the advanced age of 14 months (Cordon-Cardo et al. 1998, Di Cristofano et al. 2001). These discrepancies may be related simply to differences in the mouse strains used or, potentially more interestingly, to differential levels of p27. The p27 protein, besides being a major CDK inhibitor in the cell cycle, may also function in the assembly of the cyclin D/CDK4 or CDK6 complex (Cheng et al. 1999). It is speculated that if SKP2 expression fails to completely abolish p27 levels, as in p27 null mice, the residual p27 may be critical in sustaining the assembly role (Shim et al. 2003). Still another possibility could be that SKP2 may promote earlier onset of hyperplasia and subsequent progression to dysplastic lesions by not only targeting p27 but, perhaps, other cell cycle regulators for proteolysis. The increased latency period for hyperplasia and absence of dysplasia in the p27 null mice thus offer an interesting model for a comparative scrutiny of the other factors or parameters responsible for the relative severity of lesions in the SKP2 model.

AKT

The cellular oncogene c-Akt was originally identified as a homologue of the viral oncogene v-akt (Staal et al. 1977, Staal amp; Hartley 1988). Activity of AKT (a serine/threonine kinase involved in cell growth and survival) is positively regulated by PI3K (Franke et al. 1977). Activated AKT kinase further controls multiple signaling pathways, including cell survival and cell proliferation. Cells or tumors lacking the function of PTEN tumor suppressor gene (see below), a phosphatase which antagonizes the PI3K pathway, display dramatically increased AKT activity, suggesting that upregulation of AKT signaling pathway may be a crucial step in cancer progression, including prostate cancer formation (Stambolic et al. 1998, Wu et al. 1998, Sun et al. 1999).

To determine whether AKT activation is sufficient for the transformation of normal prostatic epithelial cells, a transgenic mouse line was generated in which a constitutively activated form of AKT (myr-Akt, MPAKT) is spatially overexpressed in the murine VP, starting as early as postnatal day 2 (Majumder et al. 2003). The resulting animals developed hyperplastic/dysplastic lesions with severe atypia, histopathological features consistent with mPIN. Although Kaplan–Meier analysis showed minimal overall differences in survival between transgenic and non-transgenic littermates, the older MPAKT mice developed a protuberant abdomen as a result of a bladder outlet obstruction, which warranted their killing. This resulted in a decrease in survival at later time points. No invasive carcinoma and metastasis were observed in the MPAKT mice, suggesting that activation of AKT alone may not be sufficient for prostate cancer formation (see Pten conditional knock-out model).

As a result of myr-Akt expression and activation of downstream signaling molecules, such as p70S6K, increases in both cell number and cell size were observed in the VPs of MPAKT animals, consistent with the transgene expression. Additionally, AKT activation led to changes in gene expressions that are also known to occur in human prostate cancers. Among the most significantly upregulated genes is PSCA (prostate stem cell antigen), a gene that is expressed in prostate ductal tips during prostate development (Reiter et al. 1998). In human prostate cancers, PSCA is expressed in almost all cases of high-grade PIN and is overexpressed in approximately 40% of local and as many as 100% of bone metastatic prostate cancers (Gu et al. 2000). Moreover, PSCA induction has been reported in PTEN+-/- mice as well (Dubey et al. 2001). The gene for osteocalcin (or gla protein), an elevated expression of which has been reported in primary prostate cancer (Levedakou et al. 1992) and in the serum of men with metastatic prostate cancer (Coleman et al. 1988), is also strongly upregulated in the expression profiles of the prostate from the MPAKT mice.

Angiogenin-3 is a member of a family of secreted proteins that induce angiogenesis. Angiogenin-3 is induced 32-fold in MPAKT prostate, together with upregulation of other family members, such as angio- genin-related protein and angiogenin-1. Additional proangiogenic factors or hypoxia-induced genes that are strongly induced in the MPAKT prostate include FGF-BP1, endothelin-1, NIP3 and hypoxia-induced gene 1. Consistent with the gene expression profiling analysis, CD31 staining reveals an extensive vasculature in the VP of MPAKT mice compared with non-transgenic controls (Majumder et al. 2003). Thus, MPAKT mice may serve as a useful model to test novel inhibitors which specifically target AKT or downstream molecules, such as angiogenins.

c-MYC

The transcription factor, c-MYC is a nuclear protein which generally functions as a positive regulator of cell proliferation. Like many other human cancers, c-MYC gene copy number or expression is also known to be increased in up to 30% of prostate tumor, often in lesions as early as PIN (Fleming et al. 1986, Jenkins et al. 1997, Qian et al. 1997, Nesbit et al. 1999, Sato et al. 1999). The c-MYC protein, in some systems or under certain conditions, may be a useful model to test novel inhibitors which specifically target AKT or downstream molecules, such as angiogenins.
conditions could induce an effect opposite to cell proliferation. For example, it can act as a pro-apoptotic factor when serum or other survival factors are compromised (Evan et al. 1992, Ahmed et al. 1997, Prendergast 1999). It is believed that in some physiological settings, secondary events triggered to rescue c-MYC-induced apoptosis, may turn to favor transformation over cell death (Dang 1999, Pelengaris et al. 2002a,b). To determine the consequence of c-MYC overexpression in the prostate, transgenic mice were generated in which the transgene is expressed from two different strength prostate epithelium-specific promoters (Ellwood-Yen et al. 2003).

Based on the levels of transgene expression, the minimal probasin promoter-driven and ARRP2PB-driven c-myc transgenic mice were designated as low expresser (Lo-Myc) and high expresser (Hi-Myc) respectively. Several interesting observations were made from the examination of these two model systems (Ellwood-Yen et al. 2003). Multifocal proliferative lesions develop in both Lo-Myc and Hi-Myc mice in VP and DLP, and as expected from the promoter activity, to a lesser extent in AP. At 4 weeks of age mPIN lesions are seen in both lines, although they appear as early as 2 weeks in Hi-Myc mice. The mPIN lesions appear to progress to invasive adenocarcinomas by 3−6 months in the Hi-Myc mice and by 10-12 months in the Lo-Myc mice. These results imply that the dosage of c-MYC may affect the rate of disease progression. Invasion noted to date is mostly local as determined from the penetration through the fibro-muscular layer, and in some cancers, with detection of foci indicative of lymphovascular invasion. Since the observed penetrance of mPIN and adenocarcinoma is practically 100%, and their occurrence is delayed in Lo-Myc relative to Hi-Myc mice, these systems should be valuable in the scrutiny of progression from mPIN to cancer or for comparative preclinical evaluation of therapeutic regimens. The role of c-MYC in both prostate proliferation and apoptosis is evident. The proliferation index is increased by 7- to 8-fold in mPIN and tumor lesions in the transgenics compared with wild-type samples. A simultaneous increase in apoptosis is also induced by c-MYC, although it appears that proliferation may outpace apoptosis to some extent. From the rapid appearance of mPIN lesions in these mice, it may be implied that, in addition to proliferation advantage, survival signals operative in the prostate may be responsible for the rescue of much of the gland from c-MYC-induced apoptosis.

Microarray-based expression profiling and subsequent investigation of some of the clues have been productive from the study of these animals. For example, it is found that while NKX3.1 protein expression is detected in the mPIN lesions at variable levels, it is not detected in the cancers in the c-myc transgenic mice. Thus, c-MYC expression may be a causal effect on NFX3.1 downregulation and cells lacking NKX3.1 expression may have increased propensity to progress to cancer. Alternatively, c-MYC expression and NKX3.1 loss may be distinct events in time in the mPIN/cancer transition. Another interesting finding is that PIM1, a serine/threonine kinase, which can cooperate with c-MYC in murine lymphomagenesis, is also overexpressed in tumors in the c-myc transgenic mice. The relevance of this information to human cancer is that PIM1 expression is increased in a subset of human prostate cancers that also display poor clinical outcome (Dhanasekaran et al. 2001). Considering these issues along with identification of some other c-myc gene signature clusters, the expectation is raised that murine and human cross-species comparisons could indeed be valuable in better understanding of the prostate cancer progression.

**Mouse models with loss of single gene function**

Loss of function of several genes has been implicated in human prostate cancer. One approach to study the role of a single gene in tumorigenesis is to disrupt the gene activity in a suitable animal model. There are three different ways this could be approached. First and the conventional method has been genome-wide knock-out of the gene in mice (Capicchi 1994). This standard gene-targeting strategy has shed light on various parameters about the function of many known genes during development. However, this approach does not permit direct evaluation of a lack of gene function in cancer if its disruption leads to embryonic lethality or premature death. Furthermore, since conventional gene knock-outs provide animals that inherit genetic deletions in all cell types, it is often difficult to exclude the possibility that abnormal phenotypes observed in adult animals arise indirectly from an underlying developmental defect (Copp 1995), or to define in which tissue the targeted gene acts.

The second approach, which is based on conditional gene knock-out techniques, provides a means to circumvent some of the limitations of conventional gene knock-out. One such technology, commonly referred as Cre-loxP recombination, has become a powerful tool for conditional, cell-type and tissue-specific deletion of genes (Nagy 2000, Kwan 2002). This tool for prostate cancer research is now available. Although three reports have recently appeared describing prostate-specific Cre systems (Maddison et al. 2000, Wu et al. 2001, Abdulkadir et al. 2002), the usefulness of the PB-Cre4 line (Wu et al. 2001) has been fully documented. In the PB-Cre4 line,
nuclear-localized Cre recombinase is expressed from the improved prostate-specific ARR2PB promoter. In order to validate the extent and specificity of Cre expression and its recombination capability, the PB-Cre transgene was crossed with a conditional reporter gene called R26R (Soriano 1999). The R26R gene with the potential for β-galactosidase gene sequence is constitutively expressed in every cell of the embryo and adult, but in the absence of recombination does not encode a functional LacZ product. The R26R allele is, therefore, an extremely useful reporter allele for validating the expression pattern of Cre-expressing transgenes, as the detection of LacZ activity is a precise and measured readout of Cre activity. The analysis of the pattern of LacZ expression in urogenital tissue of the PB-Cre4; R26R hybrid mice at several time points has conclusively documented that Cre expression is postnatal and prostate epithelium-specific (Wu et al. 2001). Although the Cre recombination is detected in all lobes of the mouse prostate, there is a significant difference in expression levels between the prostatic lobes. For example, at 2 months, while the staining in LP is near complete (95%), the recombination efficiency in VP is about 50%, and much lower in DP and AP (about 10 and 5% respectively). However, Cre-mediated recombination being a unidirectional process, the recombination frequency in all the lobes is likely to increase over time, approaching 100%. In fact, in more recently analyzed mice, in 8-month-old males, the extent of recombination in LP is essentially 100%, and in VP and DP it is approximately 95% as well. Recombination in AP at 8 months is much higher than at 2 months (35 vs 5%), although clearly not comparable in extent with that in the other lobes (Powell et al. 2003). Besides the prostate gland, no other tissues of the adult PB-Cre mice show significant Cre expression, with the exception of limited focal Cre activity in the stroma of seminal vesicles, seminiferous tubules and the ovary. This PB-Cre4 line is currently being used by a large number of laboratories for seminiferous tubules and the ovary. This PB-Cre4 line is currently being used by a large number of laboratories for

The third approach concerns the idea of inducing Cre-mediated gene disruption in not only a tissue-specific but also time-controlled fashion (Feil et al. 1996, Indra et al. 1999, Jonkers & Berns 2002). For example, mice expressing fusion of Cre-recombinase and a mutated hormone-binding domain of the human estrogen receptor, Cre-ERT, under transcriptional control of a ubiquitously expressed locus can be induced to undergo recombination in vivo at the site of topical application of 4-hydroxytamoxifen (Bex et al. 2002). A combination of ligand-dependent Cre recombination and tissue-specific Cre expression will provide exciting opportunities to faithfully mimic extent or timing of gene disruptions in the multi-step tumorigenesis. Although at this time such inducible Cre-loxP systems are lacking in the field of prostate cancer research, they are likely to emerge soon to, perhaps, supersede the utility of the other two approaches.

NXX3.1

The loss of activity of tumor suppressor genes, by either deletion, mutation or silencing, has long been known to be associated with the development of tumors in humans and animals. A homeobox transcription factor, NXX3.1, which displays prostate-specific expression, is a candidate tumor suppressor in its properties. A region of chromosome 8p21, which undergoes allelic deletion in about 80% of prostate cancers, includes the locus in which the NNX3.1 is mapped (Dong 2001). Unlike the conventional inactivation of the remaining allele of a tumor suppressor gene by mutation, epigenetic modifications, yet to be clearly documented, may be involved in loss of NNX3.1 protein expression in prostate cancer (Voeller et al. 1997, Bowen et al. 2000). Both conventional and conditional knock-outs of the Nkx3.1 gene in mice were attempted to define the role of NNX3.1 in prostate tumorigenesis (Bhatia-Gaur et al. 1999, Schneider et al. 2000, Tanaka et al. 2000, Abdulkadir et al. 2002). All of these models point to NNX3.1 as a regulator of branching morphogenesis, secretory phenotype of the glandular epithelium, and when lost, as a factor in the development of mLGPIN by 1 year age, largely in AP. Mice with extended observation of up to 2 years of age reveal progression but not beyond mHGPIN (Bhatia-Gaur et al. 1999). In order to extend the ‘life’ of these lesions, Kim et al. (2002a) used tissue recombination techniques to determine if in vivo passing of the lesions would allow for further progression. Combinations of rat urogenital sinus mesenchyme and NNX3.1 knock-out prostate tissue were implanted under the renal capsule and lesions allowed to grow through a serial transplantation/tissue recombination approach (Cunha et al. 2002). The lesions did progress to a more advanced stage of mPIN, but did not convert to invasive cancer (Kim et al. 2002a). This implies that NNX3.1 alone is not sufficient to produce prostate cancer and may require other secondary events for progression. There are two observations that are noteworthy in this matter. Since allelic deletion of NNX3.1 is frequently present in human PIN (Dong 2001), and targeted deletion of Nkx3.1 in both homozygous and heterozygous mutants results in mPIN as a consequence of aging (Bhatia-Gaur et al. 1999, Kim et al. 2002a), reduced NNX3.1 expression may be directly related to initiation of preneoplastic lesions in the
RXRα

Actions of retinoids are mediated either by their nuclear receptors or through receptor-independent mechanisms. There are two families of RA receptors, RARs (α, β and γ) and RXRs (α, β and γ) (Leid et al. 1992, Chambron 1996). The physiological consequences of RXR and RAR inactivation have been investigated via conventional knock-out technology (Chambron 1996). RARγ null mutant mice develop squamous metaplasia of the prostate (Lohnes et al. 1993). Since mice lacking both RXRβ and RXRγ are normal in terms of prostate morphology and function (Krezel et al. 1996), and considering that the active RA receptor is mostly a heterodimer of one RAR and one RXR (Mangelsdorf et al. 1994), the critical RXR in prostate biology appears to be RXRα. Moreover, to mediate multiple signaling pathways in the prostate, RXRα may also partner with other nuclear receptors, such as peroxisome proliferator-activated receptor γ and vitamin D receptor, whose ligands have been shown to inhibit prostate cancer cell growth (Kubota et al. 1998, Peehl & Feldman 2003). The human chromosomal region, 9q34.3, in which RXRα gene is mapped, is characterized by a high rate of recombination (Almasan et al. 1994), and the incidence of loss of heterozygosity at this locus has been reported to be 20% in prostate cancer (Ruijter et al. 1999). The expression of RXRα in human prostate cancers has been examined (Kikugawa et al. 2000, Lotan et al. 2000), although such analyses are usually complicated because of post-translational protein modifications (e.g. phosphorylation, ubiquitination, truncation, mislocalization, etc.), which contribute to regulation of retinoid receptor function (Chambron 1996, Delmotte et al. 1999, Matsushima-Nishiwaki et al. 2001). More recently, it has been demonstrated that nuclear expression of RXRα is generally downregulated in human prostate cancer cell lines and specimens, and that manipulated overexpression of just RXRα subtype in prostate cancer cells can significantly induce cell death by apoptosis (Pandey & Batra 2003, Zhong et al. 2003).

To elucidate the mechanism of action of RXRα in the context of the prostate, the PB-Cre4/loxP system was used to disrupt RXRα gene specifically in the prostatic epithelium of the mouse (Huang et al. 2002). This approach is necessary because conventional disruption of the RXRα gene is embryonically lethal (Kastner et al. 1994, Sucov et al. 1994). No compensatory changes in the expression of any of the other RXR or RAR receptor family members are detected in the prostate of conditional RXRα homozygous-deficient mice. Developmentally, prostatic branching is increased from the loss of RXRα function. There is also a significant change in the profile of secretory proteins in the RXRα mutant prostate relative to littermate controls with intact RXRα allele. Histopathologically, homozygous RXRα-deficient prostates show multifocal hyperplasia as early as 4 months of age. Lesions, which could be described as mLGPINs, are detected after 5 months. Subsequently, beginning at about 10 months, mHGPINs develop in some animals, and could be present in any of the lobes. The incidences of mLGPIN and mHGPIN among the animals 10–15 months of age are 62 and 17% respectively (Fig. 8). The development of progressive lesions is likely to be a multi-step process that requires cumulative changes in gene expression. Thus, it seems that loss of RXRα function may make some of the affected cells escape negative growth control mechanisms, leading to hyperplasia. Increased proliferation is likely to enhance the probability to acquire additional genetic alterations to produce a higher degree of dysplasia. At this time, there is no direct evidence to support that mLGPIN actually progresses to mHGPIN, or even whether specific defined areas of hyperplasia turn to mLGPIN, although detection of mPINs, in general, is always associated with hyperplasia, as mHGPINs with mLGPINs but not vice versa. While studying biallelic inactivation of RXRα gene, animals with monoallelic deletion were also accumulated. Similarly to homozygous mice, the monoallelic mice appear to develop hyperplasia, mLGPIN, and mHGPIN in a temporal fashion, except that the incidence is substantially delayed by several months (Fig. 8). Thus, it appears that haploinsufficiency of RXRα could be a factor in prostate disease, the reduced production or delayed accumulation of which might promote a positive environment for proliferation and transformation to preneoplastic lesions. All in all, the model with the conditional disruption of the RXRα allele in the prostate epithelium documents for the first time that a major component of retinoid action in the prostate is mediated by a retinoid receptor, RXRα, the inactivation of which in the prostatic epithelium leads to the development of preneoplastic lesions (Huang et al. 2002). Furthermore, the conditional RXRα mutant mice produced should be a valuable resource to examine the specifics of RXRα signaling in relation to the prostate, because they might be useful in turn, in studies of therapeutic regimens or chemoprevention of the beginning stages of prostate tumorigenesis.
PTEN

The phosphatase and tensin homologue deleted on chromosome 10 or PTEN is mutated in a variety of sporadic cancers and in two autosomal dominant hamartoma syndromes (Simpon & Parsons 2001). The second messenger, phosphatidylinositol (3,4,5) triphosphate (PIP3), is produced from PIP2 via activated PI3K. While PIP3 is necessary for activation of AKT, PTEN acts in opposition to PI3K by dephosphorylating PIP3 to PIP2. Some recent studies also suggest that, in addition to the AKT activation, PTEN may function through some AKT-independent mechanisms (Gao et al. 2000, Weng et al. 2001, Freeman et al. 2003a). A central role for PTEN in prostate cancer has been implicated. The gene is frequently lost in prostate cancer cell lines and xenografts (Vlietstra et al. 1998). While the homozygous deletions in primary prostate tumors may not be high (Wang et al. 1998), various alterations appear to be more frequent in metastatic prostate cancers (Suzuki et al. 1998). Generally, abnormalities in the majority of advanced prostate cancer have been ascribed to a mode of PTEN gene inactivation through loss of expression (Whang et al. 1998, Abate-Shen & Shen 2000). PTEN is biologically an essential gene function since its conventional knock-out leads to early embryonic lethality in homozygous mutants (Di Cristofano et al. 1998, Podyspanina et al. 1999). The heterozygous mutants (Pten+/−), however, show a broad spectrum of spontaneous tumors with propensity towards those of intestines, and

Figure 8 Incidence and onset of prostate lesions in mice with monoallelic or biallelic inactivation of RXRα in the prostate epithelium. (A) Homozygous mutant prostate displays mLGPIN after 5 months, while mHGPIN begin to appear after 10 months. (B) The heterozygous mutant mice also develop similar phenotypes, but in a delayed manner, implying a dose effect (from Huang et al. 2002).

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lymphoid, mammary, thyroid, endometrial and adrenal glands (Di Cristofano et al. 1998, Suzuki et al. 1998, Podsypanina et al. 1999, Stambolic et al. 2000). Different rates of prostatic hyperplasia and cancer have also been reported in the above studies. In a more recent study, \( Pten^{+/−} \) male mice on the Balb/c/129 genetic background have been observed to develop mPIN lesions with near 100% penetrance with a long latency, and the lesions do not progress to invasive adenocarcinoma (DJ Freeman & H Wu, unpublished observations).

To assess the role of prostate-specific \( Pten \) deletion, mice harboring floxed alleles of \( Pten \) (Lesche et al. 2002) were crossed with the PB-Cre line (Wu et al. 2001). Cohorts of littersmates, heterozygous or homozygous for \( Pten \) prostate-specific deletion, from 4 to 29 weeks of age, were compared with the wild-type controls and to each other (Wang et al. 2003). Deletion of both alleles of \( Pten \) leads to progressively enlarged prostate glands. Multifocal hyperplasia is evident starting from DLP and VP from 4 weeks and later reaching AP. All of these mice (100%) with homozygous deletion develop mPIN at 6 weeks and prostate adenocarcinoma at 9 weeks. The latency for mPIN formation is shorter than heterozygous animals, which display these lesions from 8 to 10 months. While heterozygous mice progress only up to mHGPIN in their late life (DJ Freeman & H Wu, unpublished observations), mice with homozygous \( Pten \) deletion display invasive adenocarcinoma as early as 9 weeks. Practically all lobes are involved, and there is subsequent progression to metastatic carcinoma. From 12 weeks, approximately half of the \( Pten \) null mice appear to have cancerous cells migrating through compromised basement membrane and invading lymph and blood vessel system. Some of the cancer cells do survive in the circulation as they seed into distant sites such as subcapsular sinus of lymph nodes and the lung. The metastatic tumor cells remain AR-positive and null for PTEN immunostaining. Thus, the conditional \( Pten \) null mouse represents the first animal model in which deletion of a single endogenous gene leads to metastatic prostate cancer.

As summarized in Fig. 9, the \( Pten \) prostate cancer model mimics the course of human prostate cancer formation. There is progression from hyperplasia to mPIN, then to invasive adenocarcinoma followed by metastasis. Androgen ablation on these mice reveals that the cancer cells do respond to the treatment as there is a significant reduction of prostate volume. However, even though the survival of \( Pten \) null prostate cancer cells appears to be androgen-dependent, their proliferation is not sensitive to androgen withdrawal. This property of androgen-independent growth observed in \( Pten \) null prostate cancers may contribute to hormone-resistant prostate cancer formation.

The distant colonization of metastatic cells in the model is noteworthy. Since most of the metastatic lesions are very small, the actual rate of metastasis could be higher than has been identified histologically to date. It is also noted that a majority of human prostate cancers

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**Figure 9** A summary of onset and progression of prostate cancer development in mice with homozygous \( Pten \) deletion in the prostate epithelium (from Wang et al. 2003).
metastasize to the bone and cause osteoblastic lesions (Harada et al. 1992, Bubendorf et al. 2000, Cher 2001, Mundy 2002). Even though prostate cancer cells in the bone of Pten null mice remain to be identified, some dramatic bone remodeling activity in aged homozygous deleted mice has already been observed. Further studies, using sensitive imaging methodologies and prostate-specific markers are likely to be productive in this mouse model.

In this Pten null prostate mouse line, as expected, the AKT serine/threonine kinase, one of the primary targets of the PTEN-controlled signaling pathway, is activated. Pten null, AKT-activated prostate epithelial cells are also larger than the wild-type or heterozygous control cells, consistent with the role of PTEN in controlling cell size (Backman et al. 1992, Groszer et al. 2001). While AKT activation is likely to be an important mechanism in this model, there must be other AKT-independent processes that contribute to the full disease spectrum. In this regard, interesting clues are emerging from the global assessment of molecular changes caused by homozygous Pten deletion in the prostate (Wang et al. 2003). For example, instead of gradual and cancer stage-dependent NKX3.1 downregulation observed in the c-myc model (Ellwood-Yen et al. 2003), in the Pten model NKX3.1 loss can be directly correlated with PTEN loss and AKT activation. Another molecule, PSCA, is found to be upregulated in the prostate of the Pten null mice. PSCA is expressed in almost all cases of HGPIN and is overexpressed in about 40% of local and as many as 100% of bone metastatic prostate cancers (Gu et al. 2000). It would, however, be critical to distinguish whether PSCA serves as a marker for a unique subpopulation of prostate epithelial cells associated with PTEN deficiency-controlled cell transformation, or acts as an important player in the process of prostate cancer progression caused by Pten loss. Still another robust upregulated gene in Pten null prostate is clusterin. Clusterin is a highly conserved glycoprotein which is reported to have a protective function against apoptosis and may be involved in the development of androgen independence and resistance to chemotherapy (Steinberg et al. 1997, Lakins et al. 1998, July et al. 2002). Another molecule, osteopontin, which is known to be associated with the presence of bone metastases of prostate cancer (Thalmann et al. 1999, Hotte et al. 2002), is found to be increased in the metastasis deposits in the Pten null prostate mice (Z Song and P Roy-Burman, unpublished observations). It is, thus, predicted that continued analysis of molecular changes in this model in relation to disease progression would shed light on those ‘signature’ genes, which are also associated with human prostate cancer metastasis.

Mouse models with compound mutations

Since carcinogenesis is a multi-step process involving a number of genetic changes or aberrations, it is realized that successive development of increasingly complex mouse models with respect to these aberrations should be valuable in better understanding of the disease progression. To date, a few models have been developed incorporating dysregulation of up to two gene functions in a single system for prostate tumorigenesis, although most of them may be lacking the desired degree of tissue-specificity. As loss of expression of PTEN and p27<sup>kIP1</sup> is a frequent observation in human prostate cancer, one strategy was to attempt concomitant inactivation of one Pten allele and one or both p27 alleles (Di Cristofano et al. 2001). The logic behind the combination is that regulation of p27 levels through the control of AKT kinase activity may be a mechanism by which PTEN could control cell-cycle progression. While most of the Pten<sup>+/−</sup> heterozygous mice die within 8 months from massive lympho-splenomegaly, long-term survivors develop a spectrum of epithelial tumors with a propensity for those of adrenal, endometrium and thyroid tissues. They also manifest a high incidence of mPIN after 9 months (Di Cristofano et al. 1999, 2001). Mice null for p27 alleles do not display enhanced spontaneous tumors (Kiyokawa et al. 1996, Nakayama et al. 1996). In this regard, it is remarkable that when Pten<sup>+/−</sup> mice are produced in the background of p27 null genotype, the compound mutants develop prostate carcinoma within 3 months postnatally with complete penetrance along with tumors of various other histological origins (Di Cristofano et al. 2001). The prostate cancer is formed in DLP and AP but not in VP and is described to be locally invasive as rupture of the basal membrane of the epithelium is frequently seen. These results implicate a cooperative role of PTEN and p27 in tumor progression in epithelial tissues including the prostate.

A similar cooperativity, but to a much lesser degree, is observed between PTEN and INK4a/Arf in the context of the mouse prostate. The Ink4a/Arf gene encodes two distinct tumor suppressors, p16<sup>INK4a</sup> and p19<sup>ARF</sup>, which are regulators of the pRB and p53 pathways respectively (Sharpless & DePinho 1999). Inactivation of INK4a/Arf is known to be associated with a variety of human cancers (Sharpless & DePinho 1999, Burri et al. 2001). From the study of Ink4a/Arf gene dose in the Pten<sup>+/−</sup> background, a deficiency of INK4a/ARF could be directly related to a shortened latency and even an expanded spectrum of malignancies. In the prostate tissue, mPIN manifests earlier in Ink4a/Arf<sup>+/−</sup>/Pten<sup>+/−</sup> mice than the Pten<sup>+/−</sup> controls and the onset is further accelerated in Ink4a/Arf<sup>+/−</sup>/Pten<sup>+/−</sup> compound mice (You et al. 2002). However, progression to invasive adenocarcinoma is not
observed in this model. *Pten* heterozygous mice, when crossed with the TRAMP model, lead to compound mutants in which loss of *Pten* heterozygosity in the prostate tumors is noteworthy. The consequent enhancement of the malignancy shortens the life span of TRAMP mice significantly (Kwabi-Addo et al. 2001).

The conventional knock-out mice for *Nkx3.1* and *Pten* alleles were crossbred to examine progression of prostatic lesions. The latency of mPIN development in the Nkx3.1 insufficiency background is reduced by half in the *Nkx3.1*+/−; *Pten*−/− mice (Kim et al. 2002b). Furthermore, evidence is obtained for the loss of NKKX3.1 protein as well as the loss of the second allele of *Pten* in focal areas of tumors formed in the prostate of these compound mutants carrying each of the two genes as heterozygous alleles. The malignant potential of the preinvasive lesions in *Nkx3.1*; *Pten* compound mutants has been further investigated using a serial tissue recombination/transplantation assay or as a consequence of aging beyond 1 year of life (Abate-Shen et al. 2003). Such prolonged observation or propagation appears to conduces development of invasive adenocarcinoma, which is also frequently accompanied by metastases to lymph nodes. These results are quite consistent with the observations made with the conditional inactivation of *Pten* alleles in the prostate, in which there is also concomitant decrease in NKKX3.1 protein levels (Wang et al. 2003).

To date very little has been accomplished in modeling the potential of cooperativity between target genes in a strict prostate-specific manner. It is, however, anticipated that important information on this issue will now be rapidly forthcoming as the reagents and appropriate mouse resources are already available. In this regard, one study, which is yet to be completed, concerns the influence of RXRα inactivation in the prostate epithelium with FGF8b overexpression (Song et al. 2002). Early results appear to indicate a genetic synergy between loss of RXRα function and activation of FGF8b in terms of extent of prostatic enlargement, and earlier onset of extensive preneoplastic lesions (P Roy-Burman, unpublished observations).

**Epilogue: current status and perspectives**

There is now ample evidence that dysregulated expression of endogenous genes in the mouse prostate can lead to stages of prostate tumorigenesis which are dependent on the selection of the gene target. Since mice do not spontaneously develop cancer of the prostate gland, a lingering question has been whether the mouse prostate is excessively resistant to this disease even when endogenous genes of relevance are genetically manipulated. The threshold that constrains some of the earlier models to premalignant lesions has been broken recently by a generation of mouse models that mimic most of the characteristics of the human cancer. A major factor in this progress is the availability of a robust promoter which is specific for the prostatic secretory epithelium of the mouse. The power of this promoter has allowed scrutiny of the prostate disease almost exclusively in the absence of significant involvement of other tissues of the animal.

New promoter design coupled with attention to activation of a strong proto-oncogene like *c-myc* or disruption of the master tumor suppressor gene *Pten* has rendered models which rapidly develop invasive adenocarcinoma of the prostate with 100% penetrance. The same promoter that drives *c-myc* expression is also the choice in the Cre-LoxP mediated inactivation of the *Pten* alleles. Evidence is already accumulated that the cancer in the *Pten* null prostate further progresses to distant metastases into lung and lymph nodes, and possibly also to the bones, for which compelling data are, however, yet to be produced. These new models with aggressive and metastatic disease are complemented by other ‘natural’ models, namely FGF8b, RXRα, NKKX3.1, SKP2 and others, that display a stochastic pattern of increasing degree of phenotypic abnormalities of lesions, beginning with epithelial hyperplasia followed by presentations of mLPINs and then mHGPINs. Together the models represent an almost complete spectrum of the progression of the prostate tumor, albeit currently short of clear evidence for skeletal deposits. While the adenocarcinomas in the advanced disease models do appear to respond to castration through increased apoptosis, the cancer cells survive and proliferate eventually. In due course of time, it will be also critical to know whether the ability to proliferate in the absence of androgen could precede the development to hormone-refractory prostate cancer as happens in humans after prolonged androgen-Ablation therapy.

Mouse models of prostate tumorigenesis are beginning to shed light on the molecular features of the disease. Global assessment of molecular changes caused by either *c-myc* overexpression or *Pten* homozygous deletion have already identified key genes known to be relevant to prostate cancer. Other models with less severe histopathological lesions are also targets for microarray-based analyses for assessment of molecular expression changes that are associated with progression from one early step to the next in time. Similarly, analyses of primary adenocarcinoma with metastatic deposits or with androgen-independent tumors will be informative on the identification of ‘signature’ genes that determine the nature of the advanced lesions. The clues obtained from the models could then be examined for their correlation or validity in the context of appropriate or late stages of human prostate carcinogenesis. Such integrated and
parallel investigations are likely to continue to yield new insights into the pathogenesis of this common disease, and in the process, reveal new molecular targets which could be exploited for prostate cancer prevention and therapy. In this regard, the models, depicting the natural and tissue-specific disease process in immunocompetent animals should be ideal for initiating the essential preclinical tests of potential clinical utility of new therapeutics.

It is likely that effort will be continued to generate mouse models of prostate cancer to recapture eventually all of the pathophysiological characteristics of the human disease. The models that favor the genesis and progression of the tumor in the DLP would be of more relevance for the pathology that is frequently seen in the PZ of the human prostate. The multi-step process in cancer is linked to multiple genetic aberrations; thus, there is a strong justification for the current emphasis on the models with compound mutations. The theme of compound mutants is also being refined by the requirement of introducing somatic mutations in not only tissue-specific manner but also in a time-controlled fashion. It is predicted that the third-generation models of prostate cancer, which are founded on prostate-specific, regulatable expression of appropriate proto-oncogenes in combination with conditional as well as temporal inactivation of relevant tumor suppressor genes, will be forthcoming not too far in the future. There is also ongoing effort in incorporating prostate epithelium-specific activation of a reporter gene to allow monitoring tumor metastases in a living mouse by a sensitive and non-invasive imaging system. These future complex models should mimic the sporadic prostate cancer better than any existing models and should provide ideal opportunities to gain novel insight into the initiation, progression and treatment of human prostate cancer.

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