Challenges in Prostate Cancer Research: Animal Models for Nutritional Studies of Chemoprevention and Disease Progression

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ABSTRACT Prostate cancer is the second leading cause of cancer-related death in the United States. The American Cancer Society estimates that there will be over 232,000 new cases of prostate cancer in 2005. Evidence suggests that diet can act as a chemopreventive agent to reduce the incidence of prostate cancer as well as to reduce the mortality of the disease. Epidemiologic studies suggest that diets rich in specific vitamins, grains, fruits, and vegetables may be associated with lower cancer rates than high-fat diets, yet the molecular bases for these positive nutritional actions are largely unknown. The interactions of diet in combination with genetic determinants of disease progression are unclear because prostate cancer is also a disease resulting from abnormal gene expression. Hence, the biology of normal prostate development and the mechanisms underlying the initiation, progression, and metastatic spread of prostate cancer must be understood at the molecular level to develop effective nutritional prevention and intervention strategies to control and treat this malignant disease. However, progress toward understanding the biology of prostate cancer and the development of new therapies has been hampered by the lack of in vivo model systems that adequately recapitulate the spectrum of benign, latent, aggressive, and metastatic forms of the human disease. In this review we discuss the diverse animal models of prostate cancer available and their applicability for nutritional studies of cancer prevention. J. Nutr. 135: 3009S–3015S, 2005.

KEY WORDS: • animal models • prostate cancer • diet

Animal models of prostate cancer are critically important for defining the molecular basis of the disease and are also required to accelerate the development of new chemopreventive approaches and therapies for prostate cancer. Nevertheless, until recently few animal models existed, perhaps reflecting the complexity of events that occur during the disease process and our relative inability to recapitulate these changes through genetic manipulation. Most animals and nonhuman primates do not spontaneously develop prostate cancer. Accordingly, the relevance of any animal model to human disease must still be considered with reservations. Models available today are limited to the few species known to spontaneously develop prostate cancer (rats, dogs, humans) and few human prostate cancer cell lines are available.

Prostate cancer is thought to occur in a sequential series of stages that show increasing pathology ranging from low- and high-grade prostatic intraepithelial neoplasia (PIN) to theophenotypic changes of epithelial cell growth and morphology, including large pleomorphic nuclei and distinct nucleoli that envelop the glandular lumen. Local invasion of the fibromuscular sheath and eventual progression to metastasis occur (Fig. 1). Genetic manipulation of the mouse produced a number of important models to study the defined steps in the progression of prostate cancer from early stage PIN to invasive carcinoma to metastatic disease. In this article, we review the various animal models available; their strengths and limitations; and their relevance to the study of nutritional interventions for prevention of disease, inhibition of disease progression, and enhancement of therapy.

Animal models do not directly mimic all aspects of human prostate cancer

Challenges to the use of animal models for the study of human prostate cancer include the significant anatomical dif-

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5 Abbreviations used: FGF, fibroblast growth factor; IGF-1, insulin like growth factor 1; PIN, prostatic intraepithelial neoplasia; PSA, prostate-specific antigen; SCID, severe combined immunodeficiency; TRAMP, transgenic adenocarcinoma of the mouse prostate.
Diseases that arise in the human prostate vary by zone. Prostate cancer most frequently arises in the peripheral zone whereas human benign prostatic hyperplasia usually involves the transitional zone. In contrast, there are 4 distinct lobes in the mouse and rat prostate (the anterior coagulating gland and the dorsal, lateral, and ventral prostate lobes, which surround the neck of the urinary bladder). The mouse dorsolateral prostate is thought to be most similar to the peripheral zone of the human prostate, but this concept is based upon descriptive information and not molecular characterization (1). Dogs have a single-lobed prostate gland (like humans) but without the anatomical regions seen in the human prostate. The histology varies between species as well. In rodents, compound ductules are present whereas in humans true acini are present in a unique branching pattern. The dog prostate has ducts that arborize into branched alveolar glands (2).

Similarly, prostate tumors that arise in the various species differ in histology and their metastatic potential. In rats and mice the rare tumors that occur spontaneously may be discovered as secondary tumors such as lymphoma. In some strains of rats, prostate tumors develop that are androgen responsive and, similarly to tumors in humans, progress to a state of androgen independence. Metastasis to the lung and lymph nodes occurs. In contrast, prostate cancer in dogs is usually androgen independent (unlike early stages of human prostate cancer), androgen receptor negative, and without the human serum biomarker of prostate cancer, prostate specific antigen (PSA). However, bone metastasis occurs in dogs as it does in humans. The histology ranges from adenocarcinoma to anaplastic carcinoma to transitional cell-like carcinoma (2). Specific animal models are described in more detail below.

**Rat models of prostate cancer**. Rats are one of the few species that spontaneously develop prostate adenocarcinomas. The Dunning rat model (3) is undoubtedly the most common model and is widely used for nutritional studies. The Dunning R-3327 tumor spontaneously developed in an inbred Copenhagen rat and then was transplanted into a syngenic Copenhagen x Fischer F1 hybrid rat. It is a well-differentiated tumor that is nonmetastatic and slow-growing. Sublines with various characteristics were developed from the Dunning R-3327 tumor (3–7). The cell sublines are advantageous in that they exhibit a range of phenotypes that mimic some aspects of human prostate cancer from a slow-growing androgen-responsive tumor to a fast-growing androgen-insensitive tumor. Finally, the Dunning R-3327 MAT LyLu tumor is highly metastatic and metastasizes to the lymph nodes and lung (6,7). These sublines differ in growth regulation, growth rate, histology, and androgen sensitivity as well as metastatic potential. Siler et al. (8) showed that lycopene and vitamin E interfere with the natural autocrine and paracrine loops in prostate cancer using this model. The strengths of this model are that it is widely used to study androgen-independent growth and metastasis: >600 papers using it have been published.

Some strains of rats, such as Lobund Wistar and ACI/Seg, have an increased incidence of prostate cancers in the anterior prostate (9). In some strains, such as Copenhagen and ACI/Seg, PIN is evident but the tumors do not usually progress to clinically relevant disease (2). Other rat models are not commonly used. Some rat models such as those developed by Rowley (10,11) and Chung (12,13) are designed to investigate stromal and epithelial cell interactions in prostate cancer development and offer some advantage for studies specifically focused on the role of androgens.

**Canine models of prostatic carcinogenesis**. Spontaneous carcinoma of the prostate occurs most commonly in dogs. The tumors that form are androgen independent and lack a functional androgen receptor (unlike human prostate cancer). Nevertheless, naturally occurring high-grade PIN and adenocarcinoma are found in aged dogs and this may have relevance for studies of prevention of prostate cancer (14). The canine model is primarily used for studies of metastasis and provides a larger animal model for studies of potential therapies. Interestingly, both normal and malignant canine prostate tissues effectively induce the formation of new bone (2). Metastatic disease in humans represents a failure of previous therapies for prostate cancer and a probable poor outcome for the patient. Thus, the dog provides an important model for advanced disease studies aimed at screening bone-targeted therapies focused on osteoblastic metastasis with no associated bone destruction. Nevertheless, the dogs are an expensive and difficult model for researchers because of their cost, care, and genetic heterogeneity (15).

**Genetically engineered mouse models of prostate cancer**. Mouse reconstitution model. The mouse reconstitution model takes advantage of the ability of the fetal urogenital sinus to differentiate into prostate after the stromal and epithelial cells are transplanted under the kidney capsule of an isogenic male host (16). Transduction of the ras and myc oncoproteins into the 2 cellular compartments (separately or together) induces the development of poorly differentiated prostate cancer at high efficiency (16). A recent variation using a modification of this approach with human prostatic epithelial cells overexpressing MYC examined in a similar tissue recombination model and again resulted in a poorly differentiated prostate carcinoma (17). The former model was used for dietary chemoprevention studies with fenretinide (18) and gene therapy studies (19–21).

**Transgenic mouse models of prostate cancer**. Two general strategies are used to induce prostate cancer development in transgenic mice: expression of viral oncogenes in prostate tissues (such as that first described above in the mouse reconstitution model) and genetic manipulation of pathways implicated in human prostate cancer. Strong prostate-specific promoters such as probasin and PSA are required to drive target gene expression. Target genes that have been manipulated in
either gain- or loss-of-function strategies include those altering expression of growth factors and their receptors, cell cycle regulators, pro- and antiapoptotic genes, steroid hormone receptors, oncogenes, tumor suppressor genes, and homeobox genes. In general, and perhaps not surprisingly, a variety of phenotypes are obtained depending on the specific genetically engineered mouse model, but none exactly mimics the human disease. No single genetic manipulation yields the entire spectrum of human disease. However, specific models have provided some important insights as described below.

Efficient and specific targeting of transgene expression is critical for successful development of an animal model. Promoter elements from the rat probasin gene target transgene expression to the mouse ventral, dorsolateral, and anterior prostate (the anterior to a lesser extent). The transgenic adenocarcinoma of the mouse prostate (TRAMP) model was the first efficient transgenic mouse model developed using the (426+/+28 bp) rat probasin promoter to express the SV40 early genes (T/t antigen:Tag) specifically targeted to the terminally differentiated tall columnar epithelial cells of the mouse prostate (22). Male TRAMP mice develop progressive prostate disease that histologically and pathologically mimics human disease with metastatic spread to distant sites (23,24). High-grade PIN or prostate cancer is first evident by age 12 wk with metastases (predominantly to the lung and lymph nodes) present by 30 wk. Androgen ablation diminishes tumor incidence and promotes progression to androgen-independent disease providing a model for some aspects of human prostate cancer. This model has been used for chemoprevention studies of green tea polyphenols, R-flurbiprofen, genistein, dietary restriction, flaxseed, and other agents because of its high tumor incidence (25–35).

Soon after the TRAMP model, a 12-kb fragment of the prostate-specific probasin promoter was isolated (long probasin promoter) and it elicited a higher level of expression in the mouse prostate that was developmentally and differentially regulated by androgen. This longer construct was used to construct the LADY model when linked to the large-T antigen of SV40 (with a deletion that removes small-T antigen expression) (36,37). The model is advantageous in that expression is high but the disease progression is less aggressive, beginning with low- to high-grade PIN that progresses to early carcinoma with neuroendocrine characteristics (1) but no metastasis. The line permits study of progression of androgen independence. Mouse strain background differences may affect overall progression (1). The LADY model was used to examine the effects of dietary fat levels and antioxidants (selenium, vitamin E, and lycopene) on prostate cancer progression (38). Although both the TRAMP and LADY models offer many advantages, they differ from the human disease in their rapid onset and metastatic potential, and have been termed preclinical models.

ANIMAL MODELS FOR PROSTATE CANCER

Gene expression of oncogenes is another strategy; c-myc is elevated in about 30% of human prostatic adenocarcinomas and overexpression of Myc results in PIN type lesions that progress over time to adenocarcinoma in the mouse (45). Early studies in the rat suggested that Ras might be implicated as well, but overexpression of Ras resulted in epithelial hyperplasia and stromal proliferation (46,47). Similarly, fos overexpression targeted to the prostate did not result in progression beyond low grade PIN (48), suggesting that only Myc may be clinically relevant.

Some transgenic mouse models targeted steroid hormone receptors. Early-stage prostate cancer is androgen-responsive and androgen-deprivation therapy, first proposed by Charles Huggins in 1941, remains the gold standard of care for men with metastatic disease. Thus, a focus on the androgen receptor that mediates the actions of testosterone and dihydrotestosterone on target tissues is relevant. A transgenic model was developed that targeted expression of the androgen receptor under the regulation of the probasin promoter (−426+/+28 bp) to the epithelial cells of the prostate (creating dysplasia but no metastasis) (49). Other receptors were considered as well. Although prostate-specific deletion of the estrogen receptor is not available, estrogen receptor-α knockout mice develop enlarged prostates but no real pathology (50). Deletion of estrogen receptor-β resulted in either hyperplastic foci or no phenotype change (51,52). Targeted deletion of retinoid acid receptor-γ resulted in prostatic squamous metaplasia (53) and later studies using a prostate specific deletion of retinoid X receptor-α implicated retinoid X receptor receptor in prostate growth initiation and neoplastic transformation but not invasive carcinoma and metaplasia (54).

Growth factors are critical in tumorigenesis and have been another target for prostate cancer models. These include animal models that overexpress fibroblast growth factor (FGF, overexpressed in human tumors and associated with progression and metastatic potential) or express a dominant negative FGF2ii receptor, but only hyperplasia or dysplasia were observed (55,56). This may prove to be a useful model for chemoprevention of early-stage disease. Overexpression of FGF8b resulted in development of high grade PIN and prostate hyperplasia (57). An interesting approach for studying the effects of FGF receptor 1 expression in normal prostate developed by Spencer and co-workers (58) resulted in hyperplasia and then high-grade PIN, providing a model for studies of early-stage cancer of the prostate. Various models of insulin-like growth factor 1 (IGF-1) overexpression, constitutively active IGF-1 receptor, and other interactive models with androgen receptor cross-talk and a bovine keratin 5–IGF-1 transgenic model again provide models for high-grade PIN and perhaps the development of a neuroendocrine phenotype (59–61). Deregulated overexpression of IGF-1 leads to neoplastic changes and tumor promotion (62,63) Similarly transforming growth factor-β receptor inactivation in the prostatic stroma is associated with development of PIN (64).

Genes that play an important role in the regulation of the cell cycle and apoptosis are thought to control cell fate. However, targeted deletion of a key protein, such as p53 in the mouse, has a surprisingly benign phenotype (65). Tissue recombination studies with the retinoblastoma gene (RBl, known to be expressed in prostate cancers), showed that loss of this gene in the prostatic epithelium but not the stroma predisposes the prostate to carcinogenesis (66).

Inactivation of the phosphatase PTEN (phosphatase and tensin homolog deleted on chromosome 10) prevents activation of AKT and apoptosis resulting in embryonic lethality (67). However, haploinsufficiency leads to early stages of carcinogenesis in the prostate. Tissue-specific deletion showed that homozygous loss of PTEN in the prostate led to most developmental stages of carcinogenesis in the prostate (68). Another model of loss of PTEN expression using a different Cre mouse resulted in high-grade PIN by age 2 wk, and this difference was thought to reflect the relative Cre promoter choice. Not surprisingly, deficiency of other downstream targets of the PTEN/AKT pathway result in similar phenotypes.
(42). Because this pathway can be modulated by dietary agents such as genistein from soy, these models can provide new insights into the mechanism of action of chemoprevention (69–72).

Loss of the Nkx3.1 homeobox gene results in the initiation of age-related dysplasia and hyperplasia, and haploinsufficiency resulted in a low-grade PIN phenotype, suggesting again that this might be a useful model for early-stage disease (73,74).

A mouse model of prostatic genomic instability was developed. Targeting expression of EcoR1 to the prostate of the mouse resulted in the development of high-grade PIN and local invasion but no further progression (48).

A variety of mouse models with multiple (2 to 5) genetic hits shows the relative complexity of events required for the progression of prostatic disease from early-stage PIN along the complete disease spectrum to metastatic disease. Clearly, a series of genetic events is necessary for prostate cancer to develop. A detailed review of these models is found in Kasper (36,42) and shown in Figure 2. There are multiple routes to the development of specific prostatic phenotypes in genetically engineered mouse models (such as low- and high-grade PIN, invasive carcinoma, androgen-responsive and androgen-independent disease, as well as metastatic disease) and the events required for development of advanced disease are complex and multifactorial. Given this complexity, the development of models spanning all stages of the disease becomes even more challenging.

**Xenograft models of prostate cancer.** In immunodeficient mice, tumor growth occurs after implantation of human cell lines or xenografts with no evidence of graft-versus-host response. In 1966 Flanagan (75) proposed that immunodeficient rodent models could be used for prostate cancer studies—specifically, the nude mouse model. The advantage of this approach over an in vitro study is obvious: a 3-dimensional structure complete with angiogenesis, paracrine and hormonal factors, stromal interactions, and metastasis. Schroder et al. (76) first began attempts to xenograft human prostate cancer tissues in the 1970s. Since these initial studies, their group and others developed a number of cell lines that exhibited a spectrum of prostate cancer characteristics when transplanted into immunodeficient mice (77). Subsequently, in 1983, Bosma et al. (78) described the severe combined immunodeficiency (SCID) mouse model; the SCID mutation results in a lack of T- and B-lymphocyte function. However, normal natural killer cells and myeloid function are present and may influence initial tumor growth and metastatic spread after implantation (79). Orthotopic implantation of human tumor cell lines into the prostate (as compared with subcutaneous injections) of an immunodeficient mouse allowed investigators to examine both tumorigenicity and the incidence of metastasis in an in vivo model (80). In 1995 Shultz et al. (81) described a new immunodeficient mouse model obtained by crossing the SCID and nonobese diabetic (NOD) mouse strains. The NOD strain is characterized by a functional deficit in natural killer cells, an absence of circulating complement, and defects in the differentiation and function of antigen-presenting cells. The NOD-SCID model combines multiple functional defects of adaptive and innate immunity and thus offers specific advantages for the development of a xenograft mouse model for prostate cancer. The NOD-SCID mouse strain provided the basis for the development of an orthotopic animal model for subcutaneous and orthotopic implantation of human prostate carcinoma cell lines (PC-3 and DU145) (82). The efficacy of tumor take was excellent at 100% for subcutaneous implantation and 83% for orthotopic implantation for both cell lines and thus this is a relevant preclinical animal model. The advent of the xenograft model to study human cancer tissue in a mouse revolutionized cancer research.

Some xenograft models result in metastasis to the bone after intracardiac injection of bone cells under the assumption that the cells will go to and survive in a niche with the correct microenvironment optimal for their colonization and expansion. Nevertheless, intracardiac injections are not ideal and so other investigators focused on xenografts of human tissue to orthotopic sites such as directly to the prostate. The success rates vary depending on the host mouse strain, source of the tissue, and use of testosterone or Matrigel (83) to provide the required growth factors and scaffold for subsequent cell proliferation in vivo.

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**FIGURE 2** Model of progression of prostate cancer with mouse models of genetic manipulations resulting in phenotypic prostate cancer. Adapted from Kasper (42). The results of numerous studies with either targeted deletion or overexpression of specific genes was shown to induce phenotypic changes in the mouse prostate that resemble various stages of human prostate cancer. Investigators are beginning to identify both tumor suppressor pathways that block tumorigenesis as well as pathways involved in the carcinogenic process. It is noteworthy that for evidence of advanced stages of prostate cancer, multiple genetic events are required in combination. The events required for progression of prostate cancer to the advanced form of the disease are both complex and multifactorial.

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**SUPPLEMENT**

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Another innovative approach pioneered by Nemeth et al. (84) involves the direct injection of prostate cancer cell lines into human bone implants or into circulation allowing the cancer cells to go to specific tissues in immunodeficient mice. Most cells formed osteolytic tumors in human fetal bone (LNCaP were both osteoblastic and osteolytic). This model showed species- and tissue-specific enhancement of human prostate cancer growth in bone.

Novel uses of the genetically engineered mouse models for bioluminescent imaging

Tumor imaging in small animal models revolutionized the ability of researchers to follow disease progression in preclinical studies of human cancer (85). One of the most useful of these techniques uses bioluminescent imaging to noninvasively monitor the growth of luciferase-expressing carcinoma cells in vivo (86–90). Bioluminescent prostate carcinoma cell lines in xenograft models were monitored for the growth of androgen-independent tumors and developing metastatic lesions in vivo (91–94). Various sublines of the LNCaP human line manipulated to develop bioluminescent models allow temporal, noninvasive imaging of primary tumor growth and metastasis in vivo at real time (91). Bioluminescent imaging is also used to monitor the localization of a luciferase-expressing adenovirus to androgen-dependent LAPC4 human prostate cancer xenografts in gene therapy models (95).

Relevance of animal model systems to preclinical prevention studies

Chemoprevention, using either natural or synthetic agents, can slow, inhibit, or reverse carcinogenesis. A natural approach using dietary agents is preferable over an approach using synthetic agents. The ideal chemopreventive agent will either inhibit or slow tumor growth with a low level of toxicity or side effects. Administration of the agent should be simple and the mechanism of action must be defined. Given the long latency of prostate cancer development, this disease is an ideal target for chemoprevention. Early approaches focused on agents with significant anticarcinogenic activity, but later studies focused on more complex interactions of combinations and their efficacy even when neoplastic lesions are already present. Some of the agents used for chemoprevention in animal models described above are shown in Table 1.

The ideal animal model for the study of nutritional agents for chemoprevention of prostate cancer should meet the following criteria: all stages of initiation, promotion, and progression should be present and readily identifiable; cancer should develop from precursor lesions such as PIN and progress to higher-grade, more aggressive forms (i.e., adenocarcinoma); the model should demonstrate not only histological but molecular identity to human prostate cancer; early-stage disease should exhibit androgen-responsive growth with a slow rate of proliferation; tumors should progress to a state of androgen-independence with androgen ablation; the incidence should occur with sufficient frequency to permit analysis of carcinogenic inhibition; and the animal should have an intact immune system.

Prevention of prostate cancer should be a primary research goal, but human studies of nutrition and dietary agents are limited by the long latency periods and challenging epidemiological considerations. Genetically manipulated mice and other animal models offer researchers an opportunity to identify chemopreventive agents; however, none of the available models described here truly mimic the human situation. Sev-

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LITERATURE CITED


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