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)5 to phenotypic 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 surrounded by the anterior fibromuscular covering. Histologically, this is evident from the normal prostate present in young men with evidence of atypical cell growth followed by clearly evident low-grade PIN that is thought to progress to high-grade PIN that is diagnosed by prostate biopsy. Locally invasive disease is thought to develop from high-grade PIN. Less commonly, it may have evidence of a neuroendocrine pathology (NE). Either form of locally invasive carcinoma can progress to metastasize outside the prostatic capsule and spread to organs such as bone.

**FIGURE 1**  A multistep model of prostate cancer progression. Current research suggests that prostate cancer develops over time. Histologically, this is evident from the normal prostate present in young men with evidence of atypical cell growth followed by clearly evident low-grade PIN that is thought to progress to high-grade PIN that is diagnosed by prostate biopsy. Locally invasive disease is thought to develop from high-grade PIN. Less commonly, it may have evidence of a neuroendocrine pathology (NE). Either form of locally invasive carcinoma can progress to metastasize outside the prostatic capsule and spread to organs such as bone.
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 present by 30 wk. Androgen ablation diminishes tumor incidence 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). Deregressed 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 (RB1, 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 protein 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.
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 mice 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.
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 in 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 antitumor 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 a state of androgen responsiveness; tumors 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 carcinogenesis 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. Several loss- and gain-of-function models show features of PIN and early-stage disease but do not progress to become invasive or metastatic. Others such as TRAMP and LADY form invasive tumors but the tumors have a predominantly neuroendocrine phenotype (1). Although several models develop invasive adenocarcinoma, none represents the full spectrum of the disease. Indeed, molecular analysis of these mouse genetic manipulation models suggests that at least 2–5 genetic events are required to promote metastasis. No animal model reproducibly develops bone metastasis at a rate similar to that in human prostate cancer. Future studies will require additional models to define each step in the progression of the disease and the ability of agents to impair or retard this progression.

LITERATURE CITED


TABLE 1

Examples of agents evaluated for prevention of prostate cancer

<table>
<thead>
<tr>
<th>Category</th>
<th>Agent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamins</td>
<td>Vitamins A, D, and E; folate</td>
</tr>
<tr>
<td>Minerals</td>
<td>Selenium, zinc</td>
</tr>
<tr>
<td>Aniandrogen</td>
<td>Bicalutamide, hydroxyflutamide, nonsteroidal</td>
</tr>
<tr>
<td>selective androgen response modulators</td>
<td></td>
</tr>
<tr>
<td>Flavonoids</td>
<td>Genistein, quercetin</td>
</tr>
<tr>
<td>Carotinoid</td>
<td>Lycopene</td>
</tr>
<tr>
<td>Green tea</td>
<td>Polyphenolic compounds</td>
</tr>
<tr>
<td>Chinese herb</td>
<td>Emodin</td>
</tr>
<tr>
<td>Fats</td>
<td>Fatty acids, ω-linoleic acid</td>
</tr>
<tr>
<td>Flaxseed</td>
<td>N-3 fatty acids</td>
</tr>
<tr>
<td>Anti-inflammation</td>
<td>Cyclooxygenase-2 inhibitors, celecoxib, fish oil</td>
</tr>
<tr>
<td>Others</td>
<td>Curcumin, oltipraz</td>
</tr>
</tbody>
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adenocarcinoma in transgenic mice carrying a rat C3(1) simian virus 40
dependence of prostate tumors in probasin-large T antigen transgenic mice: a
An investigation of the effects of late-onset dietary restriction on prostate cancer
protein 70 increases the secretion of Hsp70 and provides protection against
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