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Current mouse and cell models in prostate cancer research

Xinyu Wu[1], **Shiaoching Gong**[2], **Pradip Roy-Burman**[3], **Peng Lee**[1,4,5,6], and **Zoran Culig**[7]

[1]Department of Pathology, New York University School of Medicine, New York, NY

[2]the Rockefeller University, New York, NY

[3]Department of Pathology, University of Southern California Keck School of Medicine, Los Angeles, CA

[4]Department of Urology, New York University School of Medicine, New York, NY

[5]NYU Cancer Institute, New York University School of Medicine, New York, NY

[6]New York Harbor Healthcare System, New York, NY

[7]Department of Urology, Innsbruck Medical University, Innsbruck, Austria

Abstract

Mouse models of prostate cancer (PCa) are critical for understanding the biology of PCa initiation, progression, and treatment modalities. Here, we summarize recent advances in PCa mouse models that led to new insights into specific gene functions in PCa. For example, the study of transgenic mice with TMPRSS2/ERG, an androgen regulated fusion protein, revealed its role in developing PCa precursor lesions, prostate intraepithelial neoplasia (PIN) but not sufficient for PCa development. Double deficiency of Pten and Smad4 leads to a high incidence of metastatic PCa. Targeted deletion of *Pten* in castration-resistant Nkx3-1-expressing cells (CARNs) results in rapid carcinoma formation after androgen-mediated regeneration, indicating progenitor cells with luminal characteristics can play a role in initiation of PCa. Transgenic mice with activated oncogenes, growth factors, and steroid hormone receptors or inactivated tumor suppressors continue to provide insight for disease progression from initiation to metastasis. Further development of new PCa models with spatial and temporal regulation of candidate gene expression will likely enhance our understanding of the complex events that lead to PCa initiation and progression, thereby invoking novel strategies to combat this common disease in men.

Introduction

Prostate cancer (PCa) is the most common male specific cancer in most western countries including the US. An important cause of morbidity and mortality in PCa is skeletal metastasis as the pathogenesis of bone metastasis is poorly understood due to the lack of relevant animal models. Androgens play crucial roles in PCa oncogenesis and progression, hence, androgen ablation therapy (surgical or medical castration) is the standard of treatment. However, most PCa cases eventually become castration-resistant PCa (CRPC), which remains as the primary cause of PCa-related death. Therefore, continued generation of new PCa animal models is necessary to enhance our understanding of PCa development and progression to metastasis.

Corresponding authors: Peng Lee MD, PhD, New York University School of Medicine, 423 E. 23rd Street, Room 6139N, New York, NY 10010, peng.lee@nyumc.org; Zoran Culig, PhD, Innsbruck Medical University, Anichstrasse 35, A-6020, Innsbruck, Austria, zoran.culig@i-med.ac.at.

Prostate Tumor Growth in Xenograft Models

Cell Lines Commonly Used in Xenograft Models

The most commonly used cell lines for prostate xenograft models are LNCaP, PC3 and DU145. The LNCaP cell line is androgen sensitive human PCa cell line derived from lymph node metastasis. LNCaP cells are known to have a mutated androgen receptor (AR) (T877A) (Veldscholte, et al. 1990). Although its tumorigenicity proved rather poor in athymic nude mice, the LNCaP cell line has been used to create LNCaP sublines which can be grown *in vivo* after subcutaneous or orthotopic inoculation (Lim, et al. 1993; Pettaway, et al. 1996)., including LNCaP-Pro3-5 and LNCaP-LN3-4. Subsequent to implantation into the prostate, LNCaP-LN3 cells produced a higher incidence of regional lymph node metastases compared to LNCaP. After intrasplenic implantation, LNCaP-LN3 cells also yielded experimental liver metastases. The metastatic LNCaP-LN3 cells exhibited clonal karyotypic abnormalities, were less sensitive to androgen (*in vitro* and *in vivo*), and produced high levels of prostate-specific antigen (PSA).

The PC3 cell line was originally derived from a bone metastasis of human prostatic adenocarcinoma origin (Kaighn, et al. 1979). Intravenous injection of PC3 (Pettaway et al. 1996; Shevrin, et al. 1988) has led to the establishment of lymph node metastases. Stephenson et al reported that orthotopic implantation of PC3 cells can also yield lymph node metastases (Stephenson, et al. 1992). Some sublines from PC3 have been generated that have increased metastatic ability (Pettaway et al. 1996). PC-3M cells are metastasis-derived variant of PC3. Tumors from the prostate or lymph nodes were harvested after intraprostate growth, and cells were reinjected into the prostate. This cycle was repeated three to five times to yield cell lines PC-3M-Pro4, and PC-3M-LN4. PC-3M-LN4 cells produced enhanced regional lymph node and distant organ metastasis. After i.v. or intracardiac inoculation, PC-3M-LN4 cells produced a higher incidence of lung metastasis and bone metastasis, respectively (Pettaway et al. 1996). As PC3 is negative for AR expression, PC3-AR, clonal PC3 cell line stably transfected with AR, has been used in various studies. The DU145 cell line, which has less metastatic potential compared with PC3 cells, was derived from a brain metastasis of human prostatic adenocarcinoma origin (Stone, et al. 1978).

PC3 and DU145 cells are androgen-independent PCa cells, however neither cell line express AR. Since most human androgen-independent PCa maintain AR expression, efforts have been focused on developing AR positive androgen-independent PCa cell lines. LNCaP-abl cell line was established by Culig et al in 1999 by culturing androgen-sensitive LNCaP cells in androgen-depleted medium for 87 passages. The LNCaP-abl cells express high levels of AR and displayed a hypersensitive biphasic proliferative response to androgen until passage 75. Growth of LNCaP-abl xenografts in nude mice was stimulated by bicalutamide and repressed by testosterone (Culig, et al. 1999). IL-6 reportedly has divergent effects on the growth of the androgen-responsive cell line LNCaP. By using prolonged treatment with this cytokine a subline, LNCaP-IL-6+, was generated that does not show the growth-inhibitory response in spite of upregulated expression of endogenous IL-6. LNCaP-IL-6+ cells grow more rapidly in nude mice than do their counterparts, LNCaP-IL-6-, which were established after serial passaging in the absence of IL-6 (Steiner, et al. 2003). In LNCaP-IL-6+ cells, there is an upregulation of cyclin-dependent kinase 2 and reduced expression of the tumor suppressors pRb and p27.

Thalmann et al. (Thalmann, et al. 1994) developed the androgen-independent subline, LNCaP C4-2. This LNCaP subline was able to metastasize to bone, however the frequency was low (2 of 20). Sublines derived from C4-2, designated B2, B3, B4, and B5, were established and have a higher propensity to metastasize to bone and cause osteoblastic

lesions (Thalmann, et al. 2000; Wu, et al. 1998). The LNCaP-AI cell line is an androgen-independent prostatic carcinoma derived from the androgen-dependent LNCaP-FGC cells. LNCaP-AI cells express higher level of AR compared to LNCaP cells and retain sensitivity to androgen stimulation (Lu, et al. 1999).

VCaP cell line was derived from a vertebral bone metastasis of a hormone-refractory prostate tumor. This cell line expresses high levels of prostate specific antigen (PSA), prostatic acid phosphatase (PAP), cytokeratin-18 and the wild type AR (Korenchuk, et al. 2001). Loberg et al (Loberg, et al. 2006) generated androgen-independent PCa cell line by implanting VCaP cells subcutaneously and serially passing in castrated male SCID mice.

MDA PCa 2a and MDA PCa 2b were established in 1997 from bone metastasis of an androgen-independent PCa (Navone, et al. 1997). Both the MDA PCa 2a cell and the MDA PCa 2b cell have two mutations in the AR gene, the T877A found in LNCaP cells and also a substitution of leucine with histidine at position 701 (L701H). This double mutation in these two cell lines leads to decreased androgen sensitivity and altered ligand specificity observed in these cells. (Zhao, et al. 1999; Zhao, et al. 2000).

CWR22 cells originated from a primary tumor and showed secretion of PSA (Wainstein, et al. 1994). Relapsed androgen-independent xenografts can be established with CWR22 cells (Nagabhushan, et al. 1996). Three other CWR xenografts from primary PCa, CWR21, CWR31, and CWR91 were established (Navone, et al. 1998) by using sustained-release testosterone pellets in the host mice to obtain tumor growth. The patients whose primary PCa gave rise to CWR21, CWR31 and CWR22 all had Stage D prostatic carcinoma with osseous metastases. After androgen withdrawal, the human primary PCa xenograft CWR22 regresses markedly, and blood PSA levels fall in the mice. A novel AR mutation characterized by in-frame tandem duplication of exon 3 that encodes the second zinc finger of the AR DNA-binding domain has been detected in the relapsed CWR22 xenograft (Tepper, et al. 2002). CWR22Rv1 is derived by serially passing of CWR xenograft with repeated tumor regression and relapse under castrated condition (Sramkoski, et al. 1999). In addition to AR mutant (H874Y), AR splice variants were initially discovered in the CWR22R xenograft line, and have since been identified in the VCaP cell line, LuCaP xenografts, the Myc-CaP genetically engineered mouse model of PCa and clinical castration resistant PCa. Watson et al (Watson, et al. 2010) found that AR splice variants are able to function independently of full-length AR (ARFL), demonstrated that several AR splice variants remain dependence on ARFL heterodimerization for nuclear translocation and transcriptional activity, and overall AR activity remains sensitive to ligand-binding-domain targeted anti-androgens.

Other cell lines used are the LuCaP23 series, PC-82, PC-295, and PC-310 lines (Jongsma, et al. 1999; Korenchuk et al. 2001; Noordzij, et al. 1996; van Weerden, et al. 1993), as well as androgen-independent PC-346, PC-346C and PC-34 lines. Two other transplantable models, LAPC-3 and LAPC-4, were isolated from different patients with advanced disease. The LAPC-3 tumor is androgen-independent, whereas LAPC-4 is androgen-responsive (Klein, et al. 1997). These cells all express wild type AR.

The xenograft models, in particular LNCaP xenografts, have been of great benefit in establishing our understanding of prostate tumorigenesis, the central role of the androgen receptor and a number of common gene changes in PCa. Although not ideal, it is still used in many pre-clinical and basic science applications *in vitro* and *in vivo* in many labs as a first port-of-call that might inform use of longer timeframe and more expensive transgenic model systems. LNCaP and LAPC-4 xenograft models have been used in drugs studies (Klink, et al. 2012). LNCaP-LN3 orthotopic PCa xenografts were used to evaluate the response of PCa

bone metastasis to anti-VEGF receptor (flk-1) antibody (DC101) treatment (Sweeney, et al. 2002). Kochuparambil et al (Kochuparambil, et al. 2011) found that a PC3 xenograft model performed in nude mice with simvastatin treatment exhibited reduced tumor growth associated with decreased Akt activity and reduced prostate-specific antigen (PSA) levels.

Site of Xenograft

Subcutaneous xenograft—Athymic mice lack mature T-cells, which enable cross-species "xenografted" tissues, including tumor cells, to be tolerated by the immune system of the recipient animal and subcutaneous xenograft of tumor cells has been performed in most preclinical studies to date. The advantages of subcutaneous tumor models are their ease of tumor establishment, management and reproducibility. However, there is no metastasis observed by conventional subcutaneous xenograft (Kerbel 2003).

Subrenal capsular xenograft—Renal grafting is the process of recombining PCa cells with rat urogenital sinus mesenchyme cells and then transplanting this recombinant tissue beneath the kidney capsule in an immunodeficient mouse to assess growth and other phenotypes (Xin, et al. 2003). Another purpose for renal grafting is to determine the physiological significance of genes that cannot be studied via whole body knockouts due to embryonic lethality, for example the Rb-null mouse (Hayward, et al. 2003). This procedure is also used to determine the ability of putative basal-like prostate stem cells from either the normal mouse prostate or mouse PCa tissues to generate prostatic tissue and ducts (Lawson, et al. 2007; Liao, et al. 2010). Similarly, the model has been used to show that castration resistant Nkx3.1-expressing cells (CARNs) are putative prostate luminal epithelial stem cells (Wang, et al. 2009).

Orthotopic transplantion model—In the experiment where prostatic (orthotopic) transplantation of PC-3M and LNCaP cell lines were subjected to male nude mice, lymph node metastases were observed in all mice given an injection of PC-3M cells in the prostate (Stephenson et al. 1992). Rembrink et al. found local growth of LNCaP cells in 7 of 10 animals, and lymph node metastasis in 4 of 10 animals. Significant serum PSA levels and strong androgen receptor expression in primary and metastatic tumor tissues were observed (Rembrink, et al. 1997). Pettaway et al (Pettaway et al. 1996), using orthotopic transplantation, reported on the generation of variants of PC3 and LNCaP with an increased metastatic potential, producing tumors in lung and bone. Thalmann et al. (Thalmann et al. 1994) were able to select a LNCaP subline, C4-2, with increased metastatic potential to lymph nodes and bone.

PC3-GFP (green fluorescent protein) cells were grown subcutaneously and then sectioned into small fragments and surgically implanted into the mouse prostate (Yang, et al. 1999). This experiment yielded extensive bone metastasis in 3 of 5 mice. LNCaP sublines labeled with GFP were also shown to metastasize to mouse bone (Patel, et al. 2000). Castration following orthotopic implantation of xenografts from LuCaP 23.8 and LuCaP (Corey, et al. 2003b) yielded mice that were PSA positive on bone marrow reverse transcriptase polymerase chain reaction (PCR), suggesting the presence of micrometastases after castration (Corey et al. 2003b).

Co-xenograft models—Co-xenograft models are very useful for investigating the role of two or more different factors on PCa as well as tumor microenvironment. Chung et al. co-inoculated NbF-I (fibroblast cell line established from the ventral prostate of Nb rats) and NbE-I (epithelial cell line established from the ventral prostate of Nb rats) subcutaneously into either adult male syngeneic rats or athymic nude mice and induced the development of tumors that resembled carcinosarcoma on histopathologic evaluation (Chung, et al. 1989).

Camps et al(Camps, et al. 1990) reported that co-inoculation of tumorigenic NbF-1 fibroblasts with human PC-3 prostatic carcinoma cells accelerated tumor growth and shortened tumor latency period. Gleave et al co-inoculated LNCaP cells and various non-tumorigenic fibroblasts into athymic mice to evaluate the role of tumor cell-host stromal interaction and stromal specific growth factors in PCa growth and progression. They demonstrated that fibroblasts differed in their ability to promote prostatic carcinogenesis (Gleave, et al. 1991). Craig et al. showed that co-inoculation of male athymic nude mice with PC-3 PCa cells and U937 promonocytic cells enhances tumor growth and increases tumor angiogenesis (Craig, et al. 2008). Li et al (Li, et al. 2008) used stromal-epithelial co-injection xenograft to demonstrate that the expression of stromal AR inhibits PCa growth.

Genetically Engineered Mouse Models

To generate a mouse model that can better recapitulate genetic events occurring in human PCa, many genes, including oncogenes, growth factors and growth factor receptors, steroid hormones, homeobox genes, and cell-cycle regulators, as well as pro and anti-apoptotic genes and tumor suppressors, have been engineered to generate gain- or loss-of-function mouse models. These mouse models are powerful tools to study tumor biology, the molecular mechanisms and tumor progression. For example, transgenic mouse models have been designed to elucidate the series of events of progression from PIN, a precursor of PCa, to carcinoma in situ, to invasive carcinoma and further progression to metastasis.

Models with Viral Oncogenes

TRAMP/*LADY* models—The first transgenic adenocarcinoma of the mouse prostate (TRAMP) model utilized expression of viral oncogenes in the prostate epithelium (Gingrich, et al. 1997; Greenberg, et al. 1995). In this model, expression of both the large and small SV40 tumor antigens (T/tag) was regulated by the prostate-specific rat probasin promoter (PB). The hemizygous TRAMP mice develop progressive forms of PCa with distant site metastasis and exhibit various forms of disease from mild intraepithelial hyperplasia to large multinodular malignant neoplasia. TRAMP hemizygotes can exhibit PIN by 12 weeks of age and adenocarcinoma can arise by 24 weeks of age, mostly in the dorsal and lateral lobes of the prostate. This model was also the first to display castration-resistant disease. Castration of mice at 12 weeks of age did not affect primary tumor development or metastasis in the majority of TRAMP mice. Recently, it has been reported that the carcinoma developed from TRAMP mice exhibits mostly neuroendocrine phenotype (Chiaverotti, et al. 2008). While TRAMP may be problematic in studies of oncogenesis, it is and can be used for treatment and prevention studies.

The LADY model is pathologically similar to the TRAMP model. The large PB promoter was used to express only the large-T antigen that led to the development of glandular hyperplasia and PIN by 10 weeks of age, followed by high grade epithelial dysplasia and poorly undifferentiated adenocarcinoma by 20 weeks. There are several LADY model derivatives and some of them show neuroendocrine features (Ishii, et al. 2005; Kasper, et al. 1998). These models differ from human PCa in two key respects: the rapid rate of disease progression and the prevalence of neuroendocrine differentiation of the tumor cells.

Mouse model based on c-Myc oncogene overexpression—Myc oncogene enhances cell proliferation and amplification and/or overexpression of Myc has been detected in up to 30% of prostate tumors. Transgenic mice have been developed that express the Myc transgene at different levels depending on the different promoters in which they were integrated with (Ellwood-Yen, et al. 2003). The Myc transgene controlled by the PB promoter has low level of expression (Lo-Myc) and the Myc transgene controlled by a reconstructed PB promoter, ARR2PB (androgen-responsive regions probasin promoter)

(Zhang, et al. 2000) has high level of expression (Hi-Myc). PIN lesions were detected at 2 weeks in the Hi-Myc mice, and by 4 weeks in the Lo-Myc mice, progressing to invasive adenocarcinoma by 3-6 months in Hi-Myc mice and by 10-12 months in Lo-Myc mice.

Mouse models based on hormone receptors—A transgenic model has been developed that selectively targets the expression of AR to the prostate epithelium under the control of a fragment of the rat PB promoter (Stanbrough, et al. 2001). In this model, mice developed hyperplasia by 1 year of age, and mice older than 1 year developed focal areas of neoplasia resembling human HGPIN. These studies suggest a role for these receptors in early PCa progression; however, they also demonstrate the intricate nature of PCa carcinogenesis requiring more than one gene change. While informative, long latent periods and the absence of metastases have limited the scope of these models, especially for therapy evaluation and for understanding late events in PCa progression. Niu et al (Niu, et al. 2008) identified different roles of AR in prostate stromal and epithelial cells with the inducible-(ind) AR Knockout (ARKO)-TRAMP and prostate epithelial-specific ARKO TRAMP (pes-ARKO-TRAMP) mouse models. The tumors developed in pes- ARKO TRAMP mice were larger with a higher proliferation index compared with ARKO TRAMP tumors where AR is lost in both epithelium and stroma.

Han et al (Han, et al. 2005) demonstrated that expression of an AR E231G mutant led to rapid development of metastatic PCa with 100% penetrance while the AR-T857A mutant did not lead to tumor growth. Their study supported the hypothesis that AR is a proto-oncogene and that abrogation of the classical AR signal pathway by mutation or hormonal perturbation can facilitate the transformed state. This model not only explains the initially dramatic response observed in PCa patients to hormone withdrawal, it also provides a paradigm for the subsequent emergence of hormone therapy-resistant disease.

Albertelli et al (Albertelli, et al. 2008) created mice bearing humanized AR genes (h/mAr) varying in polymorphic N-terminal glutamine (Q) tract length. The polyQ length is related to PCa initiation and androgen-independence in distinct manner, with short 12Q developing palpable tumor faster in intact mice and later in castrated mice.

TMPRSS: ERG fusion gene transgenic mice—The genes, TMPRSS2, which is regulated by the male sex-hormone androgen, and ERG, a potential oncogene, are located close to one another on chromosome 21. When fused, TMPRSS2 drives over-expression of the ERG gene.

Klezovitch et al found that overexpression of ERG in prostate cell lines increased cell invasion and targeted expression of this transcript *in vivo* in luminal prostate epithelial cells of transgenic mice results in PIN (Klezovitch, et al. 2008). Other data has shown that transgenic mouse strain overexpressing ERG, both PIN and invasive cancer developed only when crossed with PTEN deficient mice (Carver, et al. 2009; Tomlins, et al. 2008). This study showed that PCa specimens containing the TMPRSS2-ERG rearrangement (∼40%) are significantly enriched for PTEN loss. These data implicate PTEN loss and ERG rearrangements as associated events that act in tandem to promote PCa progression, potentially by inducing transcription of downstream checkpoint genes involved in promoting cell proliferation, senescence, and survival.

Mouse models based on growth factors and growth factor receptors—A transgenic mouse model of activated HER2/Neu (Erbb2) receptor, a member of the epidermal growth factor receptor (EGFR) family, driven by the mouse probasin gene promoter has been developed (Li, et al. 2006). These mice develop prostatic atypical hyperplasia followed by PIN and invasive carcinoma; immunostaining of which indicates

that the tumors are not neuroendocrine in origin. Microarray and immunophenotyping based expression profiling of these tumors has revealed altered expression of several novel genes (>50) together with increased expression of EGFR, Erbb3, and phosphorylated AR (Li et al. 2006).

Fibroblast growth factor 8-isoform b (FGF8b) showed high expression in human clinical sex-organ related cancers including hormone-refractory PCa. Transgenic mice overexpressing FGF8b under the control of ARR2PB promoter developed multifocal epithelial hyperplasia followed by high-grade PIN, but did not lead to local invasion or metastases (Song, et al. 2002). Transgenic mice overexpressing FGF7 under the control of PB promoter develop prostate epithelial hyperplasia after 12 months of age, which does not progress to invasive carcinoma (Foster, et al. 2002). Similar results have been obtained when FGF receptors are targeted; transgenic mice overexpressing FGFR1 under the control of ARR2PB promoter develop various grades of PIN (Freeman, et al. 2003; Song et al. 2002), and other studies also demonstrated that FGFR1 is the most important FGF receptor in promotion of prostate carcinogenesis (Acevedo, et al. 2007).

Transgenic models based on FGFR2iib, transforming growth factor receptor β (TGFβ), insulin-like growth factor 1 (IGF-1), keratinocyte, and epidermal growth factors have also been developed.

Knockout Mouse Models Based on Tumor Suppressor Genes

Tumor suppressor genes are frequently mutated or lost in human cancer. A number of transgenic models have been generated by altering tumor suppressor genes.

Mice with p53 mutation—Modification of p53 expression in PCa has been performed either by mutation or by loss of one copy of the *p53* gene. Elgavish et al. used a gene encoding a mutant p53, placed under the control of the rat probasin promoter to study the role of p53 mutations in PCa. The resulting transgenic mice exhibited HGPIN lesions (grade III-IV) by 52 weeks of age together with reduced apoptotic potential (Elgavish, et al. 2004).

Loss-of-function of the retinoblastoma protein (pRB) mice—The retinoblastoma tumor suppressor gene, *Rb*, located at 13q has also been associated with PCa, and its mutations can be early events in PCa. A transgenic mouse with a conditional deletion of Rb gene specifically in prostate epithelial cells was generated. Inactivation of pRB family proteins (Rb/p107/p130) in prostate epithelium can induce epithelial proliferation and apoptosis and is sufficient to produce PIN, adenocarcinomas develop in all mice with no evidence of neuroendocrine tumors (Hill, et al. 2005).

PTEN-deficient mice—PTEN is a key tumor suppressor, and its loss has been linked to many cancers, including a strong correlation with PCa. Indeed, loss-of-function in PTEN (phosphatase and tensin homologue deleted from chromosome 10) is found in about 35% of primary PCa and 63% of metastatic tissues. *Pten* knockout mouse was created by generating a null mutation in the *Pten* gene and showed that Pten inactivation enhanced the ability of embryonic stem cells to generate tumors in nude and syngeneic mice (Di Cristofano, et al. 1998).

Wang et al (Wang, et al. 2003) generated the murine PTEN PCa model which recapitulates the disease progression seen in humans: initiation of PCa with PIN, followed by progression to invasive adenocarcinoma, and subsequent metastasis Lloyd et al (Trotman, et al. 2003) generated a hypomorphic *Pten* mouse mutant series with decreasing PTEN activity: *Pten*hy/+ > *Pten*+/− > *Pten*hy/− > *Pten* prostate conditional knockout (*Pten*pc) mutants. They found

that the extent of *Pten* inactivation in dose-dependent fashion determines PCa progression, its incidence, latency, and biology. The dose of PTEN affects key downstream targets such as Akt, p27Kip1, mTOR, and FOXO3.

Nkx3.1 knockout mice—Nkx3.1 is a prostate tumor suppressor gene which is essential for normal prostate function and epithelial proliferation. Mutations of Nkx3.1 are found in 60 to 80% of prostate tumors. Nkx3.1 knockout mice (conventional and conditional) display epithelial hyperplasia and PIN with increasing age. Neoplastic properties are associated with homozygous deletion, but mutants fail to develop invasive carcinoma (Abate-Shen, et al. 2008). This mouse line provides a model for studying early stage disease (Abdulkadir, et al. 2002).

Transgenic Mice with Multiple Genetic Mutations

Targeted deletion of Rb showed that the conditional loss of even a single allele of this gene in the prostate epithelial cells causes focal hyperplasia, providing a model for studies of early stage PCa (Maddison, et al. 2004). In addition, tissue recombination with Rb has revealed that the deletion of this gene may predispose prostate epithelial cells to carcinogenesis (Wang, et al. 2000). Interestingly, in contrast to the conditional silencing of either p53 or Rb, the synergistic inactivation of both genes results in invasive carcinomas (Zhou, et al. 2006). These mice developed highly metastatic tumors that are resistant to androgen ablation and share several molecular features seen in advanced stage human PCa. Zhong et al (Zhong, et al. 2006) generated a new combinatorial mouse model which harbors the Fgf8b transgene and haploinsufficiency in PTEN, both in a prostate epithelium-specific manner. In this model, prostatic adenocarcinoma can be yielded with readily detectable lymph node metastases, whereas single models with each of the defects were shown earlier to progress generally only up to PIN. This study indicated that the cooperation between FGF8b activation and PTEN deficiency is linked to acquisition of additional genetic alterations for the progression of the lesions to primary adenocarcinoma and a complete loss of PTEN function is required in the development of invasive cancer. These studies have reaffirmed the importance of multiple gene changes in the progression of PCa.

PCa Metastasis Models

According to recent literature, about 20-40% of PCa patients with follow-up will experience PSA recurrence after prostatectomy and 30% of these biochemically recurrent cases will develop overt metastasis (Antonarakis, et al. 2011). The metastasis of PCa to bone is the most significant cause of morbidity and mortality in this disease (Logothetis and Lin 2005). Subcutaneous PCa xenografts do not yield metastatic lesion, therefore various other models have been developed to study PCa metastasis.

Intracardiac injection

Intracardiac injection of PCa cells has been a method used to produce skeletal metastases in animals, using Mat-LyLu, PC3M, LNCaP C4-2 and PC3 cell lines for injection (Rabbani, et al. 1998; Shukeir, et al. 2004; Wu et al. 1998). Injected cells via this method are subject to high blood flow and also circumvent the pulmonary clearance of cells compared with the tail vein injection models. LNCaP C4-2 cells showed the highest metastatic capability in SCID/bg mice. Retroperitoneal and mediastinal lymph node metastases were noted in 3 of 7 animals, whereas 2 of 7 animals developed osteoblastic spine metastases. Intracardiac injection of LNCaP C4-2 in athymic hosts produced spinal metastases in 1 of 5 animals at 8-12 weeks post-injection; PC-3 injected intracardially also metastasized to the bone but yielded osteolytic responses. Metastases to the spine and long bones have been observed

using this method, however, the disadvantage of this method is widespread metastasis to soft tissues in a pattern uncharacteristic of PCa.

Intratibial and intrafemoral injections

It is very difficult to establish a mouse model that spontaneously metastasizes to bone, therefore direct injection methods were used to study PCa cell-bone interactions and potential new therapies (Corey, et al. 2003a; Soos, et al. 1997; Soos, et al. 1996). In some cases, these models have been incorrectly referred to as metastasis models, although they are more precisely studies of tumor cell growth in bone. Intratibial injections were first used to compare PCa cell lines relative ability to invade and grow in bone (Fisher, et al. 2002). Femurs are larger than tibias in size and cavity size, so intrafemoral injections can also be used as metastasis models. Fizazi et al. injected human MDA-PCa 2b cells into femurs of SCID mice to study the mechanism of these cells forming osteoblastic lesions in bone (Fizazi, et al. 2003). Intrabone injections represent an important model for the elucidation of the importance of genetic pathways and other factors in PCa metastasis to bone. Intratibial and intrafemoral injections provide a platform for studying bone microenvironment and bone-tumor crosstalk.

Intraprostatic injection

Intraprostatic injection in immunocompromised mice also provides a useful PCa metastasis model. Sato et al. (Sato, et al. 1997) injected LNCaP cells intraprostatically in athymic nude and SCID mice and found that primary tumor incidence after intraprostatic injection was 89% (39 of 44) in SCID mice and 60% in athymic mice. In 10 SCID mice with primary tumors, followed for 12 weeks, retroperitoneal or mediastinal lymph node metastases were found in 100% of mice, and microscopic pulmonary metastases were identified in 40%. Intraprostatic injection provides a useful animal model to investigate mechanisms of metastasis and to evaluate therapies targeted toward inhibiting the metastatic cascade.

Other sites for tumor implantation leading to metastasis includes intra peritoneal injection (Bae, et al. 1994).

Transgenic mouse models for metastasis

There have been a few transgenic mouse models established to study PCa metastasis. Mice carrying mutant N-cadherin that lacks the extracellular domain expressed specifically in the prostatic epithelium have been crossed with the TRAMP model, and the bigenic offspring exhibited significantly faster progression to metastatic disease, as well as a marked increase in the rate of metastatic PCa to bone (Winter, et al. 2003). Further studies indicated that the mouse background can influence the development of bone metastasis (Hotte, et al. 2002; Wang et al. 2003).

Modifications of the mouse prostate reconstitution (MPR) model has produced bone metastases at a rate of 50% to 90% (Shaker, et al. 2000). MPRs were produced by infection of either heterozygous (+/-) or nullizygous (-/-) p53-mutant fetal prostatic epithelial cells with the recombinant retrovirus Zipras/myc 9. Thompson et al. used this model to create prostate tissue in which ras and myc were over-expressed in cells homozygous or heterozygous for p53 deletions (Thompson, et al. 1995). Prostatic cancer was found in 100% of the heterozygous and homozygous p53 mutant MPRs with metastatic deposits in 95% of the mice. The pattern of metastasis was remarkably similar to that in human PCa with gross metastatic deposits in the lung, lymph nodes, bone and liver of many animals. The limitations in studying bone metastases in metastatic CR PCa has been reviewed by Sturge et al. (Sturge, et al. 2011).

SMADs are a class of proteins that function as central effectors of the transforming growth factor-beta (TGFbeta) superfamily (Derynck, et al. 1998). Smad4 was originally identified as a candidate tumor suppressor gene that was somatically deleted/mutated/inactivated in many pancreatic or colorectal tumors (Dai, et al. 1999; Takaku, et al. 1998). *Smad4*^{Lox} allele has been developed by Bardeesy et al. (Bardeesy, et al. 2006). Ding et al. (Ding, et al. 2011) used the conditional *Smad4*^{Lox} strain to study the role of SMAD4 in PCa. They found the functional relevance of SMAD4 was further supported by emergence of invasive, metastatic and lethal PCa with 100% penetrance upon genetic deletion of *Smad4* in the *Pten*-null mouse prostate. Pathological and molecular analysis, as well as transcriptomic knowledge-based pathway profiling of emerging tumor identified cell proliferation and invasion as two cardinal tumor biological features in the metastatic *Smad4*/*Pten*-null PCa model. This model-informed progression analysis, together with genetic, functional and translational studies, establishes SMAD4 as a key regulator of PCa progression in mice and humans.

PCa Mouse Models for Stem Cell Studies

Stem cells can divide and differentiate into diverse specialized cell types and can self-renew to produce more stem cells. In the prostate, basal, secretory luminal, and neuroendocrine cells are all potential targets for cancer initiation (Taylor, et al. 2010). PCa tumors contain a subpopulation of cancer stem cells (Tang, et al. 2007). There is increasing evidence that cell markers such as Sca-1 (stem cell antigen-1), a laminin receptor α6 integrin (CD49f), CD133 (prominin), CD44 and CD117 (c-kit, stem cell factor receptor) can be used to enrich for stem cells of the mouse prostate (Lawson, et al. 2005; Leong, et al. 2008). A single mouse prostate stem cell defined by a Sca-1+ CD133+ CD44+ CD117+ phenotype and implanted under the renal capsule can generate secretion-producing prostatic ducts consisting of basal, luminal and neuroendocrine cells (Leong et al. 2008).

It was reported that introduction of constitutively active AKT in Sca-1 enriched murine prostate epithelial cells resulted in the initiation of prostate tumorigenesis. Moreover, the neoplasms that develop in the $Rb^{-/-}p53^{-/-}$ knockout mouse model express Sca-1 and arise in the proximal region of the gland (Zhou, et al. 2007). First, multiple lines of evidence demonstrate initiation of PCa from luminal (and possibly intermediate) cells, based on targeted gene disruption by Cre-recombinase under the control of the probasin (either probasin or ARR_2/probasin) or PSA promoters that show luminal-cell oriented expression. Second, multiple genetic targets involved, such as *Pten* (Ma, et al. 2005), *myc* (Ellwood-Yen et al. 2003) and *Nkx3.1* (Iwata, et al. 2010), result in tumorigenesis under luminal-specific expression.

Choi et al. (Choi, et al. 2012) showed that prostate luminal cells are more responsive to Pten null-induced mitogenic signaling. However, basal cells are resistant to direct transformation. Loss of PTEN activity induces the capability of basal cells to differentiate into transformation-competent luminal cells. Their study suggests that deregulation of epithelial differentiation is a critical step for the initiation of PCa of basal cell origin.

Targeted inactivation of *Pten* tumor suppressor gene with PSA-Cre recombinase (Ma et al. 2005), overexpression of human c-MYC under the control of Probasin and ARR2PB (Ellwood-Yen et al. 2003) and overexpression of human MYC under the control of *Nkx3.1* promoter (Iwata et al. 2010), all result in tumorigenesis under luminal-specific expression. Several recent reports have also shown that progenitor cells with luminal characteristics can initiate PCa. Targeted deletion of *Pten* in castration resistant Nkx3.1-expressing cells (CARNs) results in rapid formation of carcinoma after androgen-mediated regeneration (Wang et al. 2009). These data indicate that luminal cells, including the CARNs as luminal stem cells, represent a potential PCa cell of origin. Alternatively, prostate-specific

conditional deletion of *Pten* by a probasin-Cre has been shown to result in a basal cell expansion compared to luminal cells, suggesting disease in these mice is propagated by basal cells (Wang, et al. 2006). More recently it was found that cells expressing the basal cell-specific marker p63 could initiate PCa after deletion of Pten (Liao et al. 2010; Mulholland, et al. 2009). Another group also showed that the basal fraction is an efficient target population for PCa initiation in response to multiple oncogenic events including activation of the PI3K pathway, enhanced AR signaling, and increased expression of the ETS family transcription factor ERG (Lawson, et al. 2010). In addition, lentiviral overexpression of ERG1 in $Lin^{-}Sca\text{-}1^{+}CD49f^{high}$ cells resulted in a PIN phenotype, while co-activation of Akt and AR signaling resulted in adenocarcinoma (Wang and Shen 2011). Lentiviral overexpression of activated Akt and ERG in $CD49f^{hi}Trop2^{hi}$ cells resulted in high-grade PIN, and coexpression of these two genes together with AR resulted in adenocarcinoma with strong resemblance to clinical PCa (Goldstein, et al. 2011).

Kim et al. (Kim, et al. 2009; Kim, et al. 2012) showed that in the prostates of mice with concurrent homozygous deletion of Pten and focal c-MYC activation, cells were of higher grade and proliferated faster than single mutant (Pten-null) cells within the same glands. The p53 pathway was activated in Pten-deficient prostate cells and tissues, but c-MYC expression shifted the p53 response from senescence to apoptosis by repressing the p53 target gene $p21^{Cip1}$.

The cancer stem cells (CSCs) can be purified by taking advantage of surface markers and fluorescence-activated cell sorting (FACS) technique. The CSC properties of these cell populations were primarily demonstrated by colony formation assays and tumor regeneration *in vivo* transplantations in immunodeficient mice. Human PCa stem cells sorted with $CD133^{+}$, $CD44^{+}$, $\alpha_2\beta_1$-$integrin^{hi}$ displays high proliferative potential in colony-forming assays, as well as the ability to differentiate into a luminal phenotype in culture (Collins, et al. 2005). These cells have capacity of colony formation and tumor initiation following subcutaneous injection. Other investigations have demonstrated tumor formation from sub-populations of human prostate cell lines using renal grafting assays (Gu, et al. 2007). Pten deletion of one allele leads to the development of high-grade prostate intracellular neoplasia (Podsypanina, et al. 1999). In the conditional Pten deletion mouse line, Pten is lost in prostate epithelium (Wang et al. 2003). Loss of both alleles in prostate epithelium results in adenocarcinoma beginning at 9 weeks of age and invasive PCa that metastasizes primarily to lymph nodes subsequently (Wang et al. 2003). Flow-sorted $Lin^{-}Sca\text{-}1^{+}CD49f^{high}$ cells from the *Pten* null mouse model have capacity to form tumor-like spheroids *in vitro* and gave rise to carcinoma lesions in the resulting grafts (Mulholland et al. 2009). Using a similar approach but by further enriching for the CSC subpopulation from tumors in the conditional *Pten* deletion model, Liao et al (Liao et al. 2010) showed that a minor population of epithelial cells possess self-renewal and spheroid-forming abilities along with multipotentiality for differentiation *in vitro* and the ability to form tumor-like grandular structures in renal grafts. Moreover, the study of Liao et al (Liao et al. 2010) identifies that the stem cell activity of the CSCs could be positively influenced by the presence of the cancer associated fibroblasts relative to the normal prostate fibroblasts, implicating a role for a major compartment of the tumor progression of primary or recurrent PCa.

Goldstein et al. showed that basal cells from primary benign human prostate tissue can initiate PCa in immunodeficient mice. The cooperative effects of AKT, ERG, and AR in basal cells recapitulated the histological and molecular features of human PCa, with loss of basal cells and expansion of luminal cells expressing prostate-specific antigen and alpha-methylacyl-CoA racemase. (Goldstein, et al. 2010)

Epilogue

Mouse models are playing an important role in the efforts to elucidate the genetic, biochemical and biological parameters that may distinguish between the primary androgen-dependent growth phase of PCa from those of the CRPC form of the disease. Important clues on the factors that are associated with PCa metastasis and the characteristics of cancer stem/progenitor cells are also being derived from models that await further validation and correlation to human PCa. Multiple immunocompetent mouse models with spontaneous PCa serve as assets in the investigation of disease mechanisms and potential novel therapies. The critical question remains to be asked is why mouse PCa cells, irrespective of the nature or combination of genetic mutations either fail to or turn inefficient in forming skeletal lesions in the host animals of the corresponding spontaneous tumor model. It seems that mouse bones, as compared to human bones, are not significantly permissible to the homing or growth of PCa cells. The underlying differences may relate to the nature of the species or to the sets of genetic mutations introduced to date in the spontaneous models. Perhaps, critical genetic aberrations in the tumorigenic cells that propel bone metastasis in man remain to be identified and recapitulated in the mouse PCa models. Given the heterogeneity nature, both within the tissue and between individuals, of PCa, it is very reasonable that there is no single model that recapitulates all features of PCa from initiation to progression including metastasis to bone, a clinically most significant attribute of human PCa. Therefore, further refinements of current available models and/or development of new PCa models with novel technology to overcome the limitations of current models will be necessary to study the biology of PCa, metastasis and new therapies.

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Table 1
The Characteristics of Cell Lines Commonly Used in Xenograft Models

Cell line	Advantage	Disadvantage
LNCaP	LNCaP sublines can be grown in vivo LNCaP-LN3 cells produced a higher incidence of regional lymph node and liver metastases.	Mutated androgen receptor Tumorigenicity is poor in athymic nude mice
PC3	Derived from bone metastasis high metastatic potential	AR negative no response to androgens, glucocorticoids, or epidermal or fibroblast growth factors.
DU145	Derived from brain metastasis	AR negative Less metastatic potential than PC3
C4-2B	Readily forms tumors in intact hosts Androgen independent growth	
MDA PCa 2a/2b	Double AR mutation: L701H and T877A Derived from bone metastases Androgen-sensitive Retain functional differentiation (express PSA, PAP)	Bax is positive in MDA PCa 2a cells but is negative in MDA PCa 2b cells
LAPC-4	Hormone-responsive Wild type AR	Can progress to androgen independent when grown in female or castrated male mice
VCaP	wild type AR Derived from a vertebral bone metastasis of a hormone-refractory prostate tumor express PSA, prostatic acid phosphatase, cytokeratin-18	Difficult to study TMPRSS2-ERG rearrangement *in vitro* due to the presence of wild-type TMPRSS2 and ERG gene
LNCaP-abl	Express high level AR Basal AR transcriptional activity is 30-fold higher in LNCaP-abl than in LNCaP cells	
LNCaP-AI	AR positive at high level Express high level Bcl-2 p16 expression is reduced	
CWR22	Expresses PSA and AR. Growth is stimulated by epidermal growth factor	Slightly stimulated by DHT;

Table 2
Transgenic Models of PCa Generated with the Prostate-Specific Probasin Promoter

Model	Invasive carcinoma	Metastasis	Purpose	Reference
PB-Large-T/ small-t (TRAMP model)	>12 weeks	Lung, liver, kidney, salivary gland, lymph node, bone; exhibits neuroendocrine features	To study the molecular mechanism of normal prostatic cells and the factors influencing the progression to PCa.	(Gingrich and Greenberg 1996; Greenberg et al. 1995)
Lady model	20 weeks	No	To study the sequential mechanisms in multistep tumorigenesis	(Kasper, et al. 1998b; Masumori, et al. 2001)
PBECO:RI	24 months	No	To study the mechanisms of early stages prostate carcinogenesis	(Voelkel-Johnson, et al. 2000)
PB-fos	No	No	As PBECO:RI	(Voelkel-Johnson et al. 2000)
PB-bcl2	No	No	To study the bcl-2 effect on PCa	(McDonnell, et al. 2000)
PB-mAR	1 animal	No	To assess preventative hormonal therapies	(Stanbrough et al. 2001)
LPB-SV40 Large-T antigen line 12T-10 (Lady model)	6–12 months, adenocarcinoma, with neuroendocrine features	Lymphnode, liver, lung, spleen, kidney, bone	As Lady model	(Masumori et al. 2001)
PB-FGF7 (PKS)	No	No	To study the effect of FGF-7 signaling on interaction between the prostate epithelial – stromal	(Foster et al. 2002)
PB-FGFR2iiib (KDNR)	No	No	To study the emergence of neuroendocrine phenotype in prostate glands	(Foster et al. 2002)
PB-Large-T/small-t (Rat model)	30–35 weeks	Neuroendocrine, into surrounding tissues	A rat model for the PCa	(Asamoto, et al. 2002)
ARR(2)PB-FGF8b	No	No	To investigate the mechanism of development and progression of prostatic hyperplasia and preneoplastic lesions.	(Song et al. 2002)
MPAKT model	No	No	To study the role of Akt in prostate epithelial cell transformation and in the discovery of molecular markers	(Majumder, et al. 2003)
PB-Myc-PAI (Lo-Myc model)	>12 months	No	To study the relevance of mouse models for human disease.	(Ellwood-Yen et al. 2003)
ARR2PB-myc-PAI (Hi-Myc model)	>6 months	No	As PB-Myc-PAI	(Ellwood-Yen et al. 2003)
PB-IGF1	No	No	To study the effect of Insulin-like growth factors IGF-I on PCa	(Konno-Takahashi, et al. 2003)
ARR(2)PB-SKP2	No	No	To study the function of the F-box protein SKP2 on PCa	(Shim, et al. 2003)

Model	Invasive carcinoma	Metastasis	Purpose	Reference
PB-RAS	No	No	To elucidate the mechanisms and potential PCa relevance of intestinal metaplasia.	(Scherl, et al. 2004)
p53/His273 mutant	No	No	To study the relevance of multiple genes invovled in progression of slow growing prostate tumors expressing oncogenes alone to metastatic cancer.	(Elgavish et al. 2004)
ARR2PB-hepsin	No	No	To study the role of Hepsin in metastasis of PCa	(Klezovitch, et al. 2004)

Table 3
Transgenic Models of PCa Generated with Non-Probasin Promoter

Model	Invasive carcinoma	Metastasis	Purpose	Reference
gp91-phox-SV40 Large-T/small-t	Neuroendocrine	Surrounding tissues	To study neuroectodermal malignancies.	(Skalnik, et al. 1991)
MMTV-wap	No	No	To study the function of the whey acidic protein in prostate.	(Hennighausen, et al. 1994)
C3(1)-Polyoma virus middle T	Yes	No	To study the role of the polyomavirus middle T gene in urogenital growth and development.	(Tehranian, et al. 1996)
C3(1)-SV40 Large-T/small t	>28 weeks with neuroendocrine differentiation	No	To study mechanisms of the progression of invasive carcinomas from PIN precursor lesions	(Shibata, et al. 1996)
Fetal gamma globin-SV40 Large-T/small-t	16–20 weeks, epithelial with luminal epithelial and neuroendocrine features	3–4 months, adrenal, lung, lymph nodes, bone, thymus, intrascapular tissue of neck and shoulders	To examine interactions between subtypes of prostatic epithelial cells.	(Perez-Stable, et al. 1996)
MMTV-kgf	No	No	To study the function of kgf	(Kitsberg and Leder 1996)
C3(1)-bcl-2	No	No	To study the effect of bcl-2 on prostate neoplastic development	(Zhang, et al. 1997)
MT-1-rPRL	No	No	To study the effect of overexpressing the prolactin gene on the prostate gland	(Wennbo, et al. 1997)
Cryptdin 2-SV40 Large-T/small-t	12 weeks	16 weeks, lymph nodes lung, liver, bone	To study the significance of neuroendocrine differentiation in PCa	(Garabedian, et al. 1998)

Table 4
Haplotype and Knock-Out Mouse Models of PCa

Model	Invasive carcinoma	Metastasis	Reference
RARγ $^{-/-}$	No	No	(Lohnes, et al. 1993)
p27$^{Kip1\ -/-}$	No	No	(Cordon-Cardo, et al. 1998)
PTEN$^{+/-}$	No	No	(Di Cristofanoet al. 2001; Podsypanina et al. 1999)
Nkx3.1 $^{-/-}$	No	No	(Schneider, et al. 2000)
Stat5a$^{-/-}$	No	No	(Nevalainen, et al. 2000)
PB-Cre4 RXRα $^{f/f}$	No	No	(Huang, et al. 2002)
PSA-CRE Nkx3.1$^{f/f}$	No	No	(Abdulkadir et al. 2002)
PB-Cre4 PTEN $^{loxp/loxp}$	9 weeks	12 weeks, lymph nodes, lung	(Wang et al. 2003)
MMTV-Cre PTEN $^{loxP/loxP}$	3 weeks	No	(Backman, et al. 2004)
Fsp1-Cre Tgfbr2 $^{f/f}$	No	No	(Bhowmick, et al. 2004)
PB-CreAPC $^{flox/flox}$	7 months	No	(Bruxvoort, et al. 2007)

Table 5

Bigenic Mouse Models of PCa

Model	Invasive carcinoma	Metastasis	Reference
PTEN$^{+/-}$ Cdkn1b$^{-/-}$	>12 weeks (25% of mice)	No	(Di Cristofano et al. 2001)
PTEN$^{+/-}$ × TRAMP	Yes	Lung, lymph nodes, kidney, liver, neuroendocrine	(Kwabi-Addo, et al. 2001)
PTEN$^{+/-}$ × Nkx3.1$^{-/-}$	>6 months	No	(Abate-Shen, et al. 2003; Kim, et al. 2002)
TRAMP FGF2-/-	Yes	Rate decreased by approximately 50%	(Polnaszek, et al. 2003)
12T-10 × MT-DNIIR	>6 months	8 months lymph nodes, liver, lung, neuroendocrine	(Klezovitch et al. 2004)
PB-CreFgf8b × PTEN $^{flox/flox}$	9 months	>9 months lymph nodes	(Zhong et al. 2006)