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Modeling prostate cancer in mice: Something old, something new, something premalignant, something metastatic

Shazia Irshad and **Cory Abate-Shen**
Departments of Urology and Pathology & Cell Biology and Herbert Irving Comprehensive Cancer Center, Columbia University College of Physicians and Surgeons, New York, New York, United States of America

Abstract

More than 15 years ago the first generation of genetically-engineered mouse (GEM) models of prostate cancer was introduced. These transgenic models utilized prostate-specific promoters to express SV40 oncogenes specifically in prostate epithelium. Since the description of these initial models, there have been a plethora of GEM models of prostate cancer, representing various perturbations of oncogenes or tumor suppressors, either alone or in combination. This review describes these GEM models, focusing on their relevance for human prostate cancer and highlighting their strengths and limitations, as well as opportunities for the future.

Introduction

Prostate cancer is now the most prevalent cancer in aged men (reviewed in [1, 2]). Although the majority of newly diagnosed prostate cancers are relatively indolent and have good prognosis, a subset will progress to highly aggressive disease, which is usually lethal. Distinguishing prostate tumors that will progress to lethality from those that will remain indolent represents a major clinical challenge. Another major challenge is to understand the role of androgen receptor signaling in prostate tumorigenesis, which has proven to be much more complex than initially anticipated. Indeed, because prostate cancer is dependent on androgen receptor signaling, a prevalent treatment is androgen deprivation therapy. However, while androgen deprivation initially leads to tumor regression, ultimately the tumors recur and most continue to be dependent on androgen signaling, which is thereby is referred to as “castration resistant” disease [3]. A third major clinical challenge has been the propensity of prostate cancer to metastasize to bone, a primary contributor to its morbidity and mortality; however bone metastases have been exceedingly difficult to reliably model in mice.

These clinical challenges have provided the impetus for development of a plethora of genetically engineered mouse (GEM) models of prostate cancer. This review describes these genetically engineered mouse models, beginning with the “first generation” transgenic models and ultimately the “newest generation” inducible models (Table 1). These genetically engineered mouse models recapitulate the spectrum of prostate cancer phenotypes, ranging from those that display premalignant lesions to those that exhibit aggressive adenocarcinoma including, castration-resistance and metastases (Figure 1). Many of these GEM models are based on perturbations of genes or molecular pathways that are of known importance for human prostate cancer (reviewed in [1, 2]), which has enhanced their relevance for the disease. This review discusses the relationship of these models to human

Correspondence to: Cory Abate-Shen, Herbert Irving Comprehensive Cancer Center, Columbia University College of Physicians and Surgeons, New York, New York, United States of America, cabateshen@columbia.edu, Phone: 212-851-4731, Fax: 212-851-4787.

prostate cancer, highlighting their strengths and limitations, as well as opportunities for identifying biomarkers of disease progression and their use as preclinical models for therapeutic intervention. We provide a somewhat historical perspective, emphasizing models that have been most widely utilized and/or are most promising for biomarker discovery or preclinical applications. Primary references are provided mainly for the GEM models rather than for the molecular pathways that they are based on; for more extensive discussion of the molecular mechanisms of prostate cancer the reader is referred to our previous comprehensive reviews of this area [1, 2].

Prostate biology in mice and man

A major consideration in "credentialing" GEM models is how closely they resemble important features of the human prostate gland and human prostate cancer. In humans, the prostate is a walnut-sized tissue surrounding the urethra at the base of the bladder, which produces important components of seminal fluid. The human prostate lacks discernible lobular structure, although it has a zonal architecture, corresponding to central, periurethral transition, and peripheral zones [4–7]. The outermost peripheral zone harbors the majority of prostate carcinomas, while benign prostatic hyperplasia (BPH), a common non-malignant condition, arises from the transition zone.

Unlike the human prostate, the mouse prostate consists of multiple lobes, corresponding to the ventral, lateral, dorsal, and anterior lobes, which have distinct patterns of ductal branching, histological appearance, gene expression, and secretory protein expression [8]. It is often asserted that the mouse dorsolateral lobe is most analogous to the human peripheral zone with respect to prostate cancer; although there is limited evidence to support this conclusion overall, gene expression profiling data supports this relationship [9].

At the histological level, both the mouse and human prostate contains three differentiated epithelial cell types: luminal, basal, and neuroendocrine. The luminal epithelial cells form a continuous layer of columnar cells that produce protein secretions; basal cells are located beneath the luminal epithelium, while neuroendocrine cells are rare cells of unknown function that express endocrine markers. Notably, most human prostate cancers are adenocarcinoma in origin and have primarily luminal features. However, neuroendocrine tumors may become increasingly more relevant in the most advanced tumors following clinical interventions.

"First generation" transgenic models expressing SV40 oncogenes

In the early days of modeling prostate cancer in mice, a major focus was based on expressing the human Simian Virus 40 (SV40) early genes, large T and small t-antigens specifically in the mouse prostatic epithelium using prostate-specific (or other) promoters. SV40 has been expressed in at least 10 prostate cancer transgenic mouse models. Here we will focus on those that have made the most impact in the field.

The TRAMP Model

Since its generation in 1996, the Transgenic Adenocarcinoma of the Mouse Prostate (TRAMP) mouse model has been one of the most widely used mouse in prostate cancer research [10–12]. This model represents a transgene comprising the minimal probasin promoter (−426 /+28) (Table 2) driving viral SV40 large-T and small t antigen antigens, which leads to prostate-specific inactivation of *pRb* and *p53*, specifically in the prostatic epithelium [11, 13]. TRAMP mice develop prostatic intraepithelial neoplasia (PIN) by 6 weeks, which progresses to high grade PIN by 12 weeks, and to poorly differentiated and invasive adenocarcinoma by 24 weeks of age with nearly 100% penetrance. Additionally,

this model displays prevalent metastases to distant organs [10], although rarely to the skeleton. Notably, the lack of reliable metastases to bone represents a major limitation of most GEM models.

A major limitation of the TRAMP mice is the inherent use of the probasin promoter (Table 2), which itself is regulated by androgens; in fact, this issue affects the many models driven by the probasin promoter. Therefore, interpreting the consequences of castration is confounded by the possibility that observed androgen sensitivity is due to the down-regulation of transgene expression.

Another concern regarding the TRAMP mice is that a majority of tumors are neuroendocrine in origin [14] and therefore this model is most likely to be relevant to the sub-population of patients with neuroendocrine disease [15]. Interestingly, the neuroendocrine phenotype of the TRAMP mice is suppressed by in mice lacking the ubiquitin ligase Siah2 via regulation of HIF-1alpha availability [16], which underscores the emerging significance of neuroendocrine phenotypes in prostate cancer. Furthermore, while a criticism of the TRAMP (and similar) models is the use of the non-physiological SV40 T antigen; prostate-specific inactivation of *pRb* and *p53* (which are the major targets of SV40 T antigen) also leads to aggressive neuroendocrine tumors with metastases to distant organs [17]. Thus, the phenotype of the TRAMP mice may reflect the consequences of *RB* and *p53* pathway inactivation.

Models combined with TRAMP

The TRAMP model has made a significant impact in our understanding of the molecular mechanisms of prostate cancer, in part because it has been extensively used to evaluate other molecules/pathways of interest. Below we discuss some of the molecular factors that have been investigated in the context of the TRAMP model.

BCL-2—Among the various mouse alleles that TRAMP mice have been crossed with is the anti-apoptotic gene, Bcl-2. The corresponding PB-BCl-2/TRAMP mice have an accelerated prostate cancer phenotype, but do not have increased incidence of metastases [18]. Although up-regulation of Bcl-2 has been associated with androgen-independent prostate cancer in humans, castration of the PB-BCl-2/TRAMP mice resulted in loss of Bcl-2 expression presumably because of the androgen dependence of the probasin promoter, which precluded the ability to assess whether Bcl-2 accelerated hormone-independent tumor growth.

Caveolin-1—Caveolin-1 is a major structural protein of the caveolae critical for mediating molecular transport and signal transduction and associated with advanced metastatic prostate cancer [19]. Although *Caveolin-1* null mice do not display an evident prostate phenotype, when crossed with the TRAMP mice, cancer progression was accelerated in terms of the extent of tumor burden as well as metastases [20], supporting a tumor suppressor role for Caveolin-1 in prostate.

EGR-1—The early growth response protein 1 (EGR1) transcription factor is over-expressed in a majority of human prostate cancers and has been implicated in the regulation of several genes important for prostate tumor progression. The consequences of its deficiency were studied by combining the *Egr1* null mice with the TRAMP or with an alternative SV20 model (CR2-T-Ag, discussed herein) [21]. Deletion of *Egr1* in these contexts resulted in significantly delayed progression from PIN to invasive carcinoma, suggesting a role for Egr1 in the transition from localized to invasive carcinoma.

IGF-1—The role of insulin-like growth factor I (IGF-I) in prostate cancer has been controversial. Some studies have suggested that elevated serum IGF-I levels are associated with increased risk of developing prostate cancer [22], while other studies were not confirmatory [23]. Crossing TRAMP mice with mice that lack growth hormone-releasing hormone (GHRH) receptor function, which thereby have low levels of both serum growth hormone (GH) and IGF-I, results in significantly slower progression to prostate cancer [24]. Subsequent studies to evaluate the consequences of loss of IGF receptor or its forced expression in the prostate in combination with the TRAMP mice [25, 26] further support the role of IGF levels in prostate tumorigenesis.

SRC-3—The steroid receptor coactivator-3 (SRC-3) is a coactivator of the androgen receptor that is expressed primarily in basal and stromal cells, although TRAMP mice have increased expression of *SRC-3* in luminal cells during cancer progression [27]. Combining the TRAMP mice with *SRC-3* null mice resulted in a marked delay in tumorigenesis, with these mice exhibiting only PIN and early stage carcinomas [28], suggesting that SRC-3 contributes to tumor progression by influencing proliferation and survival of prostate cells.

The LADY Model

To circumvent the problem of variable expression levels of the initial version of the probasin promoter, the LADY series utilized a larger fragment of the probasin promoter (LPB; −11,500/+28) (Table 2) driving large-T antigen but lacking small-t antigen [29, 30]. Seven transgenic lines were derived, which form 3 groups based on the stage of the neoplasia and the timing of the initial occurrence of tumor phenotypes. Each of these lines develops hyperplasia, progressing to dysplasia, high-grade PIN and ultimately adenocarcinoma, and thereby models the various stages of prostate cancer. 12T-7f LADY mice are initially responsive to androgen, since tumors regress upon castration, while administration of androgens restores the epithelial/stromal cell ratio and tumor growth.

Involvement of distant organ metastases is a rare event in the LADY series. The exception is line 12T-10, which exhibits a neuroendocrine phenotype and develops prevalent metastasis by 9 months, although not to bone [31]. However, a double transgenic model with a probasin-driven Hepsin crossed with the LADY 12T-2f model (such that both the large T antigen and the hepsin were expressed specifically in the prostate) was reported to exhibit increased metastatic potential including to the skeleton [32]. However, since this initial report, there has been no further information on this model.

Another interesting application of the LADY model was to assess its collaboration with TGF-β signaling, which was achieved by crossing transgenic mice expressing a dominant negative TGFβ Receptor II with the LADY mice [33]. Although the prostate tumor phenotype was not affected by attenuation of TGF-β signaling, the mice displayed increased incidence of metastases. These findings support a role for TGF-β signaling particularly in prostate cancer metastases.

Other transgenic models expressing SV40 oncogenes

The T121 Model—A series of transgenic mouse lines were developed using a second-generation probasin promoter, the ARR_2PB promoter, which has higher-level expression in prostatic epithelium [34] (Table 2). The ARR_2PB promoter was used to express a truncated SV40 large-T antigen (T121), which inactivates pRb, without affecting the functionality of p53 or other T antigen targets [35]. These TgAPT121 mice develop PIN by 2 months of age, which by 4 months progresses to microinvasive and well-differentiated prostate adenocarcinoma [35]. Notably, as is the case for various other mouse alleles (discussed herein), this phenotype is accelerated by the *Pten* loss of function [35]. Given that

dysfunctional pRB signaling is observed with high frequency in human prostate cancer [1, 2, 36], the TgAPT121 model may be useful to investigate the function of the RB pathway in prostate tumorigenesis.

The PSP94-TGMAP Model—Prostate secretory protein of 94 amino acids (PSP94) is one of the three abundant secretory proteins in the prostate. A 3.8 kb fragment of the PSP94 promoter region was used to drive SV40 large and small T-antigen expression in prostate epithelium, and the resulting mice developed hyperplasia by 10 weeks and well-differentiated adenocarcinomas by 24 weeks [37]. To overcome inherent variability of the transgenic approach, a knock-in model was generated by inserting the SV40 T-antigen coding sequence to the PSP94 locus (PSP-KIMAP) giving rise prostate cancer with higher tumor penetrance and rare occurrence of neuroendocrine differentiation [38]. These models have not been widely utilized, although this promoter may be beneficial for knock-in of genes other than SV40.

SV40 models that do not use prostate-specific promoters

Several models have been generated using promoters that are not prostate specific, although the resulting mice have prostate cancer phenotypes. Most of these express SV40 antigens and are discussed in this section. An example of one that does not express SV40 is the transgenic model expressing mutated B-Raf^{V600E} [39], which is discussed in a subsequent section.

CR2-Tag—The CR2-Tag model expresses SV40 T antigen under the control of the *cryptidine-2* (CR2) promoter, which was generated to study intestinal epithelium, but unexpectedly the male mice developed prostate cancer [40]. Prostate cancer in the CR2-Tag mice exhibits PIN by 12 weeks of age, and invasive cancer with neuroendocrine features by 24 weeks with metastases to lymph node, liver, and lung.

C3(1)SV40—The C3(1)SV40 T mice were generated using a 4.5 Kb of rat [C3(1)] promoter to drive expression of SV40 T antigen [41]. These mice develop low-grade PIN by 2 months of age and high-grade PIN by 5 months, which eventually progresses to locally invasive adenocarcinoma.

Globin-SV40—Transgenic mice having the fetal globin promoter driving SV40 T-antigen expression develop prostate tumors that metastasize to lymph nodes and distant sites [42]. These tumors are resistant to castration and exhibit both neuroendocrine and epithelial phenotypes.

Proto-oncogene activation

An important category of genetically-engineered mouse models are those with activation of key oncogenic pathways of known relevance for human prostate cancer. In general, the phenotypes of these mice are less aggressive than the SV40 models, but have the important advantage that they tend not to develop neuroendocrine tumors. These transgenic models have been developed using variations of the probasin promoter (Table 2) to drive the transgene expression. Notably, in some cases, their phenotype is not as robust as more recent models using conditional activation of oncogene expression or deletion of tumor suppressors driving their activation (described herein).

c-Myc—A majority of human prostate cancers exhibit amplification and/or overexpression of c-Myc ([36] and reviewed in [1, 2]). To investigate its role in disease progression, transgenic mice were engineered to express human c-Myc under the control of a minimal

(weaker) probasin promoter, called the Lo-Myc mice, or the stronger probasin promoter (ARR_2PB; Table 2), called the Hi-Myc mice [43]. Both the Hi- and Lo-Myc transgenic models develop PIN that progresses to locally invasive adenocarcinoma by 3–6 or 10–12 months, respectively. The initial phenotypic observations of these Lo- and Hi-Myc models were further supported and extended by subsequent analyses [44]. Furthermore, castration of Hi-Myc mice at 2 months, but not at 8 months, causes tumor regression. This suggests that these early but not later tumors are more resistant to castration, although this interpretation is complicated by the dependence of the probasin promoter on androgen signaling. The castration-resistance of this Myc model was also shown by the collaboration of Myc with NF-kappaB signaling [45].

Notably, crossing the Hi-Myc mice with the ARR2PB-hepsin transgene (similar to the strategy used for the LADY model discussed above) resulted in tumors without metastases [46], raising concerns about the interpretation that hepsin is associated with bone metastases [32]. An important feature of the Myc models is that develop adenocarcinoma rather than neuroendocrine tumors. Additionally, expression-profiling analyses of the Myc mice was one of the first examples of cross-species analyses from GEM models to human prostate cancer to provide biomarkers of disease progression [43], a strategy that continues to be extremely valuable.

TMPRSS2-ERG—An important recent discovery was the observation that a majority of prostate cancers have translocations of members of the *ETS* gene family fused with the TMPRSS-2 promoter [47]. The functional role of these translocations has been evaluated in various transgenic mouse models. In an initial report, the ARR2PB promoter (Table 2) was used to express a fusion of the noncoding exon 1 of TMPRSS2 with two *Ets* genes, *ERG* or *ETV1*; these mice were reported to develop PIN by 12–14 weeks of age [48, 49]. The conclusion that overexpression of ETS genes lead to PIN was supported by another study of an independent study [50]. However, subsequent reports using analogous transgenic approaches concluded that mice expressing *ETS* genes do not develop PIN, although they do so when combined with loss of function of *Pten* [51, 52]. Given the presumed importance of *ETS* gene translocations for prostate tumorigenesis, this issue of their functional role in prostate cancer needs to be further addressed, potentially using alternative approaches to conditionally express *ETS* genes in the prostate.

Akt—Based on the considerable importance of activation of mTOR/Akt signaling for prostate tumorigenesis, a transgenic mouse was developed that expressed a constitutively activate form of Akt in the prostate using a probasin promoter [53]. These mice develop PIN but, interestingly, the severity of the phenotype is modest relative to that of *Pten* loss of function (described herein), despite the fact that Akt deficiency is sufficient to suppress tumor development in *Pten* null mice [54]. Subsequent analyses of these Akt transgenic mice revealed cooperativity with *p27* via relief of senescence [55], which is notable since *Pten* loss also cooperates with loss of *p27* (described herein).

Vav3—Based on its overexpression in prostate cancer, Vav3 function was investigated in GEM models, using the ARR_2PB promoter to express the constitutively-active protein [56]. These mice display PIN by 3 months of age, which progresses to adenocarcinoma [56]. Interestingly, these Vav3 transgenic mice developed nonbacterial chronic prostatitis, which is of interest since prostatitis is thought to predispose to prostate cancer. Thus, these Vav3 transgenic mice may enable the study of prostatitis for prostate cancer development.

Her-2/neu—The role of the Her2/neu oncogene has been investigated in prostate cancer using the long probasin promoter (as used for the LADY series) to express a constitutively

active Neu in prostate [57]. These mice develop hyperplasia or varying degrees of PIN, without invasion or metastases.

Ras/Raf—Ras and Raf signaling pathways are frequently deregulated in lethal tumors [36]. Transgenic mice expressing a mutated H-Ras (Ras^{Val12}) driven by a minimal probasin promoter (PB-Ras) develop PIN that does not progress to cancer [39]. However, these PB-Ras transgenic mice have interesting differentiation defects, as they develop multifocal intestinal metaplasia with vacuolated cells, resembling goblet cells that resemble cells found in the intestinal epithelium. The role of *Ras* pathway activation in prostate cancer has been investigated the context of *Pten* loss of function in more robust knock-in models (described herein).

An oncogenic mutation in the B-Raf protooncogene ($B\text{-}Raf^{V600E}$), which was engineered to express in the skin using a tyrosinase promoter, was found to develop prostate hyperplasia with progression to adenocarcinoma [39]. A limitation of this model is that the cell type in which the transgene is expressed in prostate is not known. The role of *B-Raf* activation in prostate cancer has been further investigated in collaboration with *Pten* (described herein).

Developmental and signaling pathways

In addition to evaluation of tumorigenic pathways, analyses of molecular pathways that are biologically relevant for the development and maturation of the prostate have provided insights into the role of essential pathways in prostate cancer, as well as important mouse models. Notably, these studies have also provided innovative and more sophisticated approaches for gene targeting in the prostate. These include models that perturb homeobox transcription factors that function in prostate development, and models that perturb key signaling pathways in the epithelium or stroma.

Homeobox genes and other transcription factors

Nkx3.1—The unique relationship of the *Nkx3.1* homeobox gene as a regulator of prostate development whose loss of function is associated with prostate cancer initiation [58] has merited generation of both conventional and conditional knockout mouse models, as well as the development of new alleles to achieve targeted gene deletion in the prostate. Therefore, germline loss of function of *Nkx3.1* leads to prostate epithelial defects, including aberrant branching morphogenesis and defective protein secretions [59, 60]. These mice develop dysplasia by 3 months, which becomes progressively more severe with increasing age, culminating in PIN by 1 year [59, 60]. A similar phenotype is observed in mice having conditional deletion of *Nkx3.1* in the prostate [61]. However, neither the germline nor the conditional mice progress to invasive prostate cancer, suggesting that *Nkx3.1* loss is not sufficient for cancer progression. Notably, *Nkx3.1* heterozygotes display a similar, although less severe, phenotype as their null counterparts, indicating that *Nkx3.1* is haploinsufficient and providing a model to evaluate the interplay between transcription factor dosage and tumor initiation.

Nkx3.1 has been shown to be expressed in a rare population of luminal epithelial stem cells called castration resistant Nkx3.1-expressing cells (CARNs) [62], and has therefore been important in studying prostate stem cells as well as cell or origin of prostate cancer. Furthermore, these analyses have been facilitated by the generation of an inducible Cre allele, $Nkx3.1\text{-}Cre^{ERT2}$, which has a Cre-ERT2 cassette knocked in to the *Nkx3.1* gene (Table 2) and thereby enables gene deletion in adult prostate epithelium following delivery of tamoxifen [62]. This $Nkx3.1^{CreERT2}$ allele provides a valuable means to inactivate tumor suppressors or activate oncogene function in the prostate (see below). However, similar to probasin, the *Nkx3.1* promoter is also dependent on androgens.

HoxB13—Another homeobox gene that is important in both prostate development and cancer is *HoxB13.* Unlike *Nkx3.1*, *HoxB13* is not androgen regulated [63] while similar to *Nkx3.1,* the germline mutant mice display defects in prostate differentiation [64]. Recent studies showing the association of human *HOXB13* with genetic risk of developing prostate cancer [65] have led to a resurgence of interest in its function in prostate cancer. Notably, the promoter region of *HoxB13* required for expression in the prostate has been defined [66] (Table 2), which may be a meaningful alternative to other prostate specific promoters that are androgen regulated. Furthermore, analyses of the *HoxB13* promoter has led to the development of a tetracycline-regulated mouse model for regulated gene expression in the prostate [67], which is of considerable importance since previous attempts to develop such Tet-regulated models for prostate have largely failed.

Sox9—The transcription factor Sox9 is expressed in the epithelia of all mouse prostatic lobes from the initial stages of their development. Using a conditional approach with mice expressing Cre recombinase under the control of Nkx3.1 regulatory sequences revealed a lack of ventral prostate development and abnormal anterior prostate differentiation and an early loss of expression of genes specific to the prostate epithelia such as Nkx3.1 [68]. Conversely overexpression of Sox9 accelerated the consequences of *Pten* loss of function in cancer progression [69].

Growth Factor Signaling

FGF—Based on studies that have implicated FGF signaling in prostate cancer (reviewed in [2, 70]), transgenic mice were generated expressing FGFR1 in the prostatic epithelium, using a novel dimerization approach to inducibly and reversibly activate receptor activity specifically in prostate [71]. The resulting mice engineered to express the modified FGFR1, referred to as JOCK1, exhibit hyper-proliferation and PIN, while aged JOCK1 mice progress to invasion and metastatic disease [72]. In addition to providing models to study FGF function in prostate cancer, this approach may provide a means to study “oncogene addiction” in prostate cancer, as there are currently few other suitable alleles that allow for inducible and reversible gene induction. Notably, in an alternative approach to evaluate FGF signaling in prostate, transgenic mice expressing FGF8b in the prostate together with *Pten* loss of function were shown to display poorly differentiated adenocarcinoma [73], which further underscores the significance of FGF pathway activation as well as its interaction with *Pten* loss of function.

Wnt/β-Catenin pathway—Canonical Wnt/β-catenin signaling plays a key role in prostate tumorigenesis, and particularly for advanced disease. Perturbation of Wnt/β--catenin signaling in the prostate has been investigated using a probasin-driven Cre allele (PB-Cre4; Table 2) to achieve conditional deletion in prostate epithelium [74]. This PB-Cre4 allele was crossed with a floxed allele of the adenomatous polyposis coli gene (APC), which is a negative regulator of the Wnt/β-catenin pathway. The corresponding *PBCre4-APC*$^{f/f}$ mice develop locally invasive adenocarcinoma without distant metastases by 7 months [75]. Castration of mice harboring advanced tumors result in partial regression, indicating that tumors in the *PBCre4-APC*$^{f/f}$ mice may be partially castration resistant. An alternative model to activate Wnt/B-catenin signaling used the *PBCre4* combined with conditional deletion of exon 3 ofβ-catenin, in which the resulting *PBCre4- β-Catenin*$^{f/f}$ mice display progression from hyperplasia to high-grade PIN, with castration-resistance [76]. Given the importance of aberrant Wnt/β-catenin signaling in human prostate cancer, these models may help to investigate the role of this signaling pathway in cancer progression.

TGF-β—Analyses of the role of TGF-β signaling for prostate tumorigenesis using mouse models has provided insights into the role of stroma in cancer progression and the

interaction of the epithelium and stroma for these processes [77, 78]. Notably, targeted disruption of TGF-β receptor II in mouse fibroblasts (although not specifically in to prostate) was found to result in PIN, which was accelerated when crossed with mice having cooperation with defects in the prostatic epithelium. These findings emphasize the importance of interactions of the epithelium and stroma for prostate tumorigenesis.

Androgen Receptor Signaling

Androgen receptor signaling is essential for all aspects of prostate development as well as prostate cancer (reviewed in [2, 70]). In fact, perturbation of androgen receptor signaling is arguably the most significant driving event in prostate tumorigenesis, and a critical target for new therapeutic approaches for treatment of prostate cancer. Therefore, a clear understanding of the role of androgen signaling in prostate cancer is essential; however, such analyses has been complicated by the fact that androgen receptor signaling occurs in both the epithelium and the stroma, where its functions in these compartments have been reported to be distinct [79, 80].

Androgen receptor function in the prostate epithelium has been evaluated using the minimal probasin promoter to express androgen receptor [81]. The resulting *PB-mAR* mice develop hyperplasia that progresses to microinvasive high-grade PIN, supporting the idea that AR is a positive regulator of prostate tumorigenesis. Moreover, an alternative approach in which AR was conditionally activated in the prostate also resulted in a PIN phenotype [82]. However, an alternative using the probasin promoter to express either wild-type androgen receptor or a prevalent mutated form (AR-E231G) in prostatic epithelium reported the rapid development of PIN with progression to invasion only in mice that expressed the mutated androgen receptor [83]. Conversely, inactivation of androgen receptor expression in the prostatic epithelium via conditional deletion using PB-Cre4 resulted in prostate differentiation defects as well as increased epithelial proliferation [84]. Furthermore, the phenotypic consequences of this androgen receptor knock-out were accelerated in the context of the TRAMP mice [79].

Furthermore, various lines of evidence suggest that androgen receptor, which is expressed in the prostatic stroma as well as epithelium, has different roles in these compartments, which is not unexpected from the classic work of Cunha and colleagues (reviewed in [1, 2]). In particular, deletion of both stromal and epithelial androgen receptor in the context of the TRAMP mice, result in reduced proliferation and abrogation of tumor defects [79]. Furthermore, conditional deletion of androgen receptor in fibroblasts or smooth muscle cells result in distinct defects in prostate differentiation or tumor growth [85, 86], suggesting a role for stromal AR not only in normal prostate development but also in prostate cancer. The complexity and significance of androgen receptor signaling warrants further study using systematic approaches to target androgen receptor in the epithelium or stroma in various normal and tumor contexts.

Pten and other tumor suppressor models

Of the many gain of function transgenic mouse models of prostate cancer, most transgenic models with the notable exception of the Myc transgenic model [43], are limited in terms of the robustness of their phenotype or their relevance to human prostate cancer (*i.e.*, neuroendocrine features). Considerably more robust and versatile are GEM models based on loss of function of tumor suppressor function, and most notably, those based on *Pten* loss of function. Indeed, *PTEN* is of considerable importance for prostate cancer because of its relevance for regulation of androgen receptor signaling [1, 2, 87]. Reflecting its broad significance, the consequences of loss of function of *Pten* have been investigated in a variety of different types of GEM, including germline, conditional and inducible ones, in which the

consequences of *Pten* loss have been investigated alone or in conjunction with loss of function of various tumor suppressors or with conditional activation of various oncogenes. These have enabled a progressive series of models to study prostate tumorigenesis (Figure 1).

Interestingly, as already introduced for the combination of *Pten* loss with *TMPRSS-ERG* (described above), many of these other alleles, including both gain and loss of function, have limited or minimal phenotypes on their own, while their consequences for tumorigenesis are unleashed in combination with *Pten* loss. It is likely that models based on *Pten* loss of function, and particularly the more sophisticated conditional or inducible models, will be a primary focus in future studies, particularly those focused on using GEMs for preclinical applications.

Germline deletion—Since *Pten* null homozygosity results in early embryonic lethality, the consequences of its germline loss of function have been studied in the heterozygotes, which are viable and display a broad range of cancer phenotypes [88, 89]. In particular, *Pten* heterozygotes display prostate hyperplasia with PIN, although they do not progress to cancer [88, 89]. Notably, an important observation from analyses of its germline deletions is that *Pten* loss is sufficient for hormone independence at least [90].

Conditional deletion—Although these initial models established a critical role for *Pten* in prostate cancer, and particularly for castration resistance, analyses of germline *Pten* loss is complicated by the other cancer phenotypes, most of which emerge much earlier than their prostate cancer phenotypes [88, 89]. Thus, various groups have investigated the consequences of *Pten* loss of function in prostate cancer via its conditional deletion of a floxed allele using the probasin PB-Cre allele [91, 92] or alternative approaches [93]. Although one initial study claimed to observe metastases following conditional *Pten* loss [92], this was not observed in the other initial reports [94, 95], nor have there been any subsequent reports showing that *Pten* loss alone leads to metastases. Further analyses have shown that conditional loss of *Pten* in the prostate leads to PIN that progress to high grade PIN with areas of microinvasion. Importantly, these conditional *Pten* mice develop senescence, which precludes their advancement to overt adenocarcinoma [96].

Inducible deletion—A primary limitation of the *Pten* conditional models described above is that the PB-Cre4 allele induces *Pten* deletion prior to complete differentiation of the prostate and is not restricted to the prostatic epithelium (M. Shen and C. Abate-Shen, unpublished observations), both of which confound the interpretation of the prostate phenotypes. To circumvent this problem, alternative Cre alleles have been developed which enable inducible deletion of *Pten* specifically in the epithelium of adult (fully mature) prostate [97] [98]. These models use, alternatively, a PSA-CreERT2 allele or the Nkx3.1-CreERT2 allele to achieve inducible expression following tamoxifen induction. Another inducible Cre allele has been described using the ARR_2PB promoter, although not crossed with *Pten* [99].

The phenotypes of mice having inducible *Pten* deletion are similar to the conditional alleles in that they develop PIN that progresses to microinvasion but not metastases. However, the phenotype of the inducible deletion models is generally more severe than the constitutive deletion model (M. Shen and C. Abate-Shen, unpublished observations). Interestingly, the $Nkx3.1^{CreERT2}; Pten^{flox/flox}$ mice develop castration-resistant prostate tumors, which in contract to the non-castrated (intact) counterparts having robust senescence, virtually lack a senescence phenotype [98]; this suggests that castration-resistance promotes cancer progression by bypassing senescence.

Other PI3 Kinase pathway perturbations—The significance of *Pten* loss and concomitant activation of PI3 kinase pathway activation for prostate tumorigenesis is further underscored from analyses of other GEM models having perturbations of alternative components of this signaling pathway. In addition to the Akt transgenic mice described in the preceding section, alternative GEM models of the PI3 kinase signaling pathways include constitutive activation in the prostate of p110β, a downstream effector of *Pten* [100] and prostate-specific loss of function of the AKT-inactivating phosphatase PHLPP1 [101].

Pten combined with other genes

Combined with germline *Pten* loss—Several models have examined the consequences of germline loss of function of *Pten* together with loss of other tumor suppressor genes, which typically result in more aggressive prostate cancer phenotypes. In particular, analyses of germline loss of function of *p27*, a tumor suppressor involved in the in G1/S transition, has a modest phenotype on its own [102, 103], but together with *Pten* loss displays cooperativity resulting in PIN and progression to microinvasion [103]. Similarly, compound germline loss of *Pten* and *Nkx3.1* results in high grade PIN that ultimately progresses to adenocarcinoma in aged mice, and castration-resistance following surgical depletion of androgens [104, 105]. Notably, the aged (>12 month) *Nkx3.1; Pten* compound mutant mice develop rare metastases to lymph node and lungs [105], which is a considerable progression from the germline *Pten* single mutants. Furthermore, analyses of PIN in the *Nkx3.1; Pten* compound mutant mice also led to a classification of PIN in genetically engineered mice that is still widely utilized [106]. Finally, combining the *Pten, Nkx3.1* and *p27* germline alleles led to the identification of haploinsufficiency of *p27*, which was not evident in the individual combinations [102].

In addition, combining Myc gain of function with germline loss of *Pten* revealed a complementary function of these key pathways, that together with loss of *p53*, drives prostate tumorigenesis [107, 108]. Another interesting combination is that of loss of function of *Par,* a negative regulator of *NFκb*, combined with loss of *Pten*, whose progressive defects accentuate the significance of *NFκb* activity for prostate tumorigenesis [109]. These examples underscore the value of analyzing molecular pathways of cancer in the context of *Pten* loss of function.

Combined with conditional or inducible *Pten* loss—Particularly informative have been a series of models having conditional or inducible deletion of *Pten* combined various alleles that result in deletion of tumor suppressors or activation of oncogenes. Analyses of these models have provided important insights regarding the molecular pathways of prostate tumorigenesis, as well as essential models of lethal, metastatic prostate cancer.

The role of loss of function of *p53* in prostate cancer has been investigated in collaboration with *Pten* following their combined conditional deletion using the probasin Cre allele [96]. This important study showed that although senescence precluded the development of more aggressive phenotype in the *Pten* model, loss of function of *p53* overcame this halt in tumor progression. The resulting compound mutant mice developed lethal prostate tumors that did not display senescence, although they were also not metastatic. This is an excellent example in which analyses of the prostate phenotype of mutant mice led to the identification of a key molecular pathway that influences prostate tumorigenesis. Furthermore, accelerating the phenotype of the *Pten* and *p53* mice by perturbing telomerase function resulted in lethal prostate tumors that are locally invasive to bone [110]

SMAD4 was identified as a gene of interest in prostate cancer based on its expression in the *Pten* conditional mice as well as in human prostate cancer [111]. Its functional consequences for prostate tumorigenesis were investigated in mice having conditional loss of *SMAD4*

together with *Pten* driven by the probasin Cre allele [111]. The resulting mice develop lethal prostate tumors that are adenocarcinoma in origin; however, unlike the lethal tumors in the *Pten; p53* conditional mice, the *Pten; SMAD4* conditional mice are reported to also have metastases to distant organs in ~12% of the cases, although not to the bone. Additionally, analyses of the gene signature of these lethal tumors revealed a 4-gene signature of aggressive prostate tumors that is being evaluated for its prognostic significance for human prostate cancer [111].

As noted above, the *RAS* and *RAF* signaling pathways are activated in a high percentage of lethal prostate tumors in humans [36], and translocations of both *RAS* and *RAF* have been reported to occur in aggressive prostate tumors [112, 113]. Although transgenic models having activation of *RAS* or *RAF* have phenotypes that were either modest or difficult to analyze (discussed above), mice having conditional activation of either *Braf* or *Kras* in the mouse prostate together with loss of function of *Pten* have promising phenotypes. In particular, a conditionally activatable *Braf* allele [114] combined with inducible deletion of *Pten* using the *Nkx3.1-CreERT2* allele resulted in lethal adenocarcinomas, which display metastases to distant organs in ~30% of the cases [115]. Interestingly, these mice display activation of Myc as well as MAP kinase signaling.

Finally, combining a conditionally activatable *Kras* allele [116] with inducible deletion of *Pten* using the *Nkx3.1-CreERT2* allele results in lethal prostate tumors that display metastases to distant organs in 100% of the cases [117]; a similar model has been developed using the constitutive probasin Cre allele [118]. In addition to *Pten*, activated *Kras* also cooperates in tumorigenesis in combination with the Scribbled (SCRIB), which is involved in establishing and maintaining epithelial polarity [119], as well as with activation of Wnt signaling [120]. Despite its robust phenotype and prevalence of distant metastases, activation of *Kras* together with loss of *Pten* does not result in bone metastases. Although it is an important advance to have a model that displays full penetrance of metastases, it is intriguing, and at the a same time disappointing, that this very aggressive mouse model fails to display metastases to bone, which is the major site of metastases in humans.

Conclusions and perspectives

The reader of this review with its intentionally historical perspective will undoubtedly be impressed by the remarkable evolution of GEM models of prostate cancer in the short span of ~15 years. These models originated from simple transgenic ones that established the feasibility of studying prostate cancer phenotypes in mice. Now, the current standard is conditional or inducible models having 2 or more molecular perturbations. These models have not only evolved in terms of their sophistication, but also in terms of the sophisticated information that has been learned from their analyses, which is immediately relevant for understanding the pathogenesis of human prostate cancer, and the potential for impacting cancer treatment and diagnosis by through the identification of prognostics signature and the preclinical evaluation of new therapeutic approaches.

Yet, we still have a ways to go to develop the “ideal” GEM model. First of all, while most current models feature molecular pathways that are relevant for human prostate cancer, many of the actual mouse alleles do not accurately represent the precise molecular events that occur in humans. For example, few human prostate cancer has loss of both *Pten* alleles, yet this is the most frequently used GEM model. Secondly, we are limited by the availability of relatively few reporter CRE drivers, none of which is optimal either because of their early expression pattern, their dependence of androgens or, in the case of the tamoxifen regulated promoters, the potential impact from even short doses of tamoxifen. Finally, while we have advanced considerably in developing models of metastatic prostate cancer, the remaining

challenge is to develop models that reliably target bone. So, while we have come a long way so far, we still have some significant challenges ahead.

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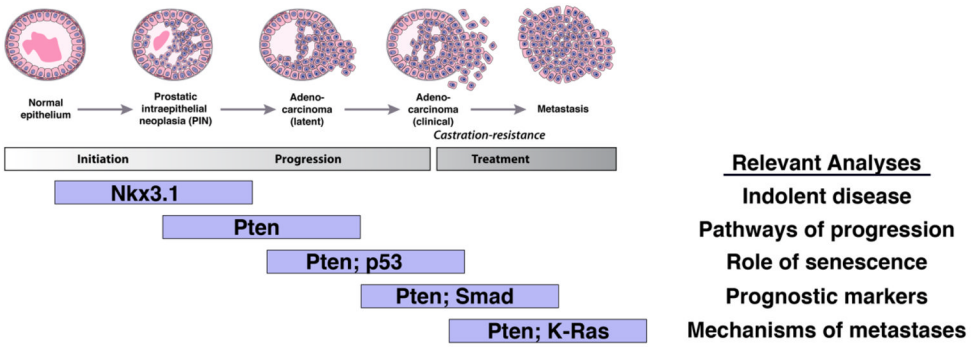

Figure 1.

Representative models for specific stages of prostate cancer, indicating the types of analyses that can be addressed with each. Note that since these models are intended to be representative, rather than inclusive, they are biased towards *Pten* models.

Table 1

Representative genetically-engineered mouse models of prostate cancer

Mouse Model	Strengths	Limitations	References
Group 1: SV40 transgenic models			
TRAMP model Minimal probasin promoter driving SV40 large and small T antigens	• One of the first prostate cancer models developed • Prostate specific phenotype • Progression from PIN to cancer and metastases • Displays castration- resistant disease • High penetrance and predictable tumor growth • Crosses with many other models has informed on disease mechanisms	• Tumors primarily neuroendocrine in origin • Promoter dependent on androgens • Rarely metastasizes to bone • Relatively short kinetics differ from the characteristically slow development in humans	[10–12, 121]
LADY model Large probasin promoter driving SV40 large T antigen	• Promoter has higher expression levels • Progression from PIN to carcinoma • Progression slower than TRAMP mice	• Distant metastases are rare • Primarily PIN, although 12T-10 line develops neuroendocrine phenotype	[31, 122]
T121 Model: Minimal probasin promoter driving SV40 small T antigen	• Provides insight on pRB function • Phenotype accelerated when combined with Pten	• Does not model metastases	[35]
Group 2: Oncogene transgenic models			
c-Myc models Probasin promoter driving c-Myc *Lo-Myc Hi-Myc*	• Adenocarcinoma rather than neuroendocrine tumors • Progression from PIN to invasive adenoarcinoma	• Promoter dependent on androgens • Does not metastasize	[43]
TMPRSS-ERG models: Probasin promoter driving expression of ERG or ETV1	• Functional analyses of a key translocation event • Cooperates with *Pten* loss of functions	• Different phenotypes observed merits further clarification	[48–52]
Akt model Probasin promoter driving activated form of Akt	• Models Akt activation	• Relatively weak phenotype compared with other models of this pathway	[53]
Group 3: Developmental pathways and androgen receptor signaling			
Nkx3.1 Germline loss of function; conditional loss of function	• Models prostate cancer initiation • Cooperates with *Pten* loss	• Does not progress to cancer	[59, 61, 123]
Wnt/β-Catenin Probasin Cre to drive conditional inactivation of APC or β-Catenin	• Modeling Wnt signaling in mice	• No distant metastases	[75, 76]

Mouse Model	Strengths	Limitations	References
FGFR1 Chimeric protein having dimerization motif attached to FGFR1	• Models cancer progression • Allows for inducible and reversible expression	• May be limited to receptor activation	[71]
TGFβ Stromal-Cre to conditionally inactivate TGFβ	• Model of stromal interactions in prostate cancer	• Stromal promoters not specific for prostate	[78]
Androgen receptor Probasin promoter to express wild-type or mutated androgen receptor	• Model to study androgen receptor function in prostate epithelium	• Conflicting reports need further clarification • Need to distinguish from stromal from epithelial role of androgen receptor	[81, 83]
Group 4: Pten tumor suppressor models			
***Pten* germline**	• Principal driving event in prostate cancer in mice • Displays cancer progression • Cooperates with various other factors in cancer progression	• Other cancer phenotypes confound analyses of prostate phenotype	[88, 89]
***Pten* germline** combined with: • Nkx3.1 • p27 • Myc	• Cooperative phenotypes in cancer progression • Molecular insights into pathways of progression • Rare metastases in aged mice	• Other cancer phenotypes confound analyses of prostate phenotype	[102–104, 107]
***Pten* conditional** Probasin Cre to conditionally inactivate *Pten* in prostate	• Principal driving event in prostate cancer in mice • PIN to microinvasive phenotypes • Has been crossed with many other alleles to investigate their contribution to disease progression	• Develop senescence that limits cancer progression Do not develop metastases	[92, 95]
***Pten* inducible** Nkx3.1^{CreERT2} or PSA ^{CreERT2} to inducibly inactivate *Pten* in prostate	• Regulated expression in adult prostate • PIN to microinvasive phenotypes • Castration alleviates senescence phenotype	• Do not develop metastases • Tamoxifen induction may affect prostate phenotype	[97, 98]
***Pten; p53* conditional** Probasin Cre to conditionally inactivate *Pten* and *p53* in prostate	• Lethal prostate tumors • *p53* loss overrides senescence phenotype	• Do not develop metastases	[96]
Pten; Smad4 Probasin Cre to conditionally inactivate *Pten* and *SMAD4* in prostate	• Lethal prostate tumors with metastases • Signature of prognostic value	• Low penetrance of metastases	[111]
Pten; Braf Nkx3.1^{CreERT2} to inducibly inactivate *Pten* and activate Braf in prostate	• Lethal prostate tumors with metastases • Myc pathway activation	• Does not develop metastases to bone	[115]

Mouse Model	Strengths	Limitations	References
Pten; Kras Nkx3.1^{CreERT2} to inducibly inactivate *Pten* and activate Kras in prostate	• Lethal prostate tumors • Fully penetrant metastatic phenotype	• Does not develop metastases to bone	[117]

Table 2

Promoter elements used to develop mouse models of prostate cancer

Promoter/Cre Allele	Relevant mouse models
Rat Probasin (PB): **Minimal PB** **Expression cassette carrying 426 basepairs of the rat probasin (PB) promoter and 28 base pairs of 5′-untranslated region (−426/+28)**	**PB-T/tag(TRAMP)** **TRAMP crossed with BCL2 transgene** **TRAMP crossed with EGR1 null mice** **PB-mAR** **PB-Akt**
Large PB (LPB) A large (L) fragment of the PB promoter (from 11 500 to +28) that retains some enhancer elements to facilitate high gene expression	LPB-Tag (LADY) LARGE T ANTIGEN
ARR_2PB A composite probasin promoter with androgen and glucocorticoid, prostate-specific, and achieves high levels of transgene expression	ARR_2PB-myc ARR_2PB-FGFR1 (JOCK1) T121 $ARR_2PB^{CreERt2}$
PB-Cre/PB-Cre4 PB-Cre4 Cre gene under the control of the ARR_2PB	$PB\text{-}^{Cre4}$; $Pten^{flox/flox}$ $PB\text{-}^{Cre4}$; $Pten^{flox/flox}$; $p53^{flox/flox}$ $PB\text{-}^{Cre4}$; $Pten^{flox/flox}$; $SMAD4^{flox/flox}$ $PB\text{-}^{Cre4}$; $Apc^{flox/flox}$
Prostate Specific Antigen (PSA) PSA-CreERT2 6-kb fragment of the human PSA promoter driving expression of the nuclear-targeted modified Cre (tamoxifen-inducible)	PSACreERt2; $Ptenflox^{/flox}$
Nkx3.1 Prostate-specific homeobox gene regulated by androgen $Nkx3.1^{CreERt2}$–modified Cre knocked into the *Nkx3.1* gene to express in prostate; nuclear-targeted modified Cre (tamoxifen-inducible)	$Nkx3.1^{CreERt2}$; $Ptenflox^{/flox}$ $Nkx3.1^{CreERt2;}$ $Pten^{flox/flox}$; $Braf^{LSLflox/+}$ $Nkx3.1^{CreERt2;}$ $Pten^{flox/flox}$; $Kras^{LSLflox/+}$
HoxB13 Prostate-specific homeobox gene not regulated by androgen Hoxb13-rtTA–tetracycline regulator driven by HoxB13 promoter	Hoxb13-rtTA/TetO-H2BGFP