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Modeling Prostate Cancer in Mice: Limitations and Opportunities

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Abstract

The complex dynamics of the tumor microenvironment and prostate cancer heterogeneity have confounded efforts to establish suitable preclinical mouse models to represent human cancer progression from early proliferative phenotypes to aggressive, androgen-independent, and invasive metastatic tumors. Current models have been successful in recapitulating individual characteristics of the aggressive tumors. However, none of these models comprehensively mimics human cancer progression, establishing the challenge in their exploitation to study human disease. The ability to tailor phenotypic outcomes in mice by compounding mutations to target specific molecular pathways provides a powerful tool toward disruption of signaling pathways contributing to the initiation and progression of castration-resistant prostate cancer. Each model is characterized by unique features contributing to the understanding of prostate tumorigenesis, as well as limitations challenging our knowledge of the mechanisms of cancer development and progression. Emerging strategies utilize genomic manipulation technology to circumvent these limitations toward the formulation of attractive, physiologically relevant models of prostate cancer progression to advanced disease. This review discusses the current value of the widely used and well-characterized mouse models of prostate cancer progression to metastasis, as well as the opportunities begging exploitation for the development of new models for testing the antitumor efficacy of therapeutic strategies and identifying new biomarkers of disease progression.

Keywords

Castration-resistant prostate cancer (CRPC); knockout; transgene; metastasis; prostatic intraepithelial neoplasia (PIN)

Prostate cancer is the second leading cause of noncutaneous cancer deaths in men in the United States. With approximately 1 in 6 men diagnosed during their lifetime, there were an estimated 190 000 new diagnoses made in the United States in 2010 (Merkle and Hoffmann, 2011). Several treatments have proved efficient in the treatment of localized disease, including radical prostatectomy, radiotherapy, and hormone therapy (androgen ablation or castration). Unfortunately, no treatment is currently available for the management of metastatic prostate cancer (Hill and Kyprianou, 2002), which is clinically significant as cancer metastasis is the leading cause of cancer-related deaths (Hsu et al, 2011). Moreover, the shift from androgen-sensitive prostate tumors (treated with hormone therapy) to castration-resistant prostate cancer (CRPC) presents an increasingly complex target for optimizing the therapeutic efficacy of antitumor modalities. Efforts to establish suitable animal models to physiologically mimic the tumor microenvironment have met with varying degrees of success because of obstacles in genetic manipulation.

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The use of mammalian models to study tumorigenesis requires the physiological relevance to human disease. The challenge specifically relating to prostate cancer stems from the heterogeneity of prostate tumors. In vitro studies utilizing immortalized human prostate cancer cell lines such as DU-145, PC-3, and LNCaP (reviewed in Sobel and Sadar, 2005) have been successful in characterizing intracellular mechanisms involved in various stages of tumor progression, but lack in a systemic sense the complex paracrine signaling of the tumor microenvironment. Prostate cancer does not stem from a single focus of cells; rather it tends to develop multifocally in nearly 60%–90% of patients (Andreoiu and Cheng, 2010). Prostate cancer progresses from prostatic intraepithelial neoplasia (PIN) to confined androgen-dependent carcinogenic cells to androgen-independent metastatic cells, no longer under endogenous control of the cell cycle or programmed cell death mechanisms (Ibrahim et al, 2010; Sakamoto and Kyprianou, 2010). The ability of prostate cancer to metastasize to the bone and influence hematopoietic lineages is seen in greater than 80% of prostate cancer–related deaths (Ibrahim et al, 2010) and must be accounted for in an efficient in vivo model. The vast heterogeneity of prostate tumorigenesis at the pathological and molecular levels has exhausted efforts to establish mammalian models that mimic the onset, behavior, and progression of metastasis and CRPC.

Mammalian genome manipulation has been exploited to ectopically model human prostate cancer onset, progression, and metastasis in a highly reproducible and tissue-specific manner. Mario Capecchi received the 2007 Nobel Prize in Medicine for his development of gene-specific modification in mice using embryonic stem cells (Thomas and Capecchi, 1987). More sophisticated techniques have resulted from technological advances, including transgene expression, conditional knockouts, inducible mutations, and xenograft transplantation. These have added temporal and spatial regulation to gene targeting. The use of traditional gene targeting to knock out tumor suppressor genes has been successful in recapitulating many characteristics of prostate cancer. Transgenic expression of oncogenes can functionally stimulate prostatic stromal cell hyperplasia, effectively modeling human benign prostatic hyperplasia (BPH). In other models, genetic manipulation causes epithelial proliferation as an effective model of human PIN (Kasper, 2005). Both BPH and PIN are inflammatory precursors to prostate cancer and are targets for early diagnosis and intervention in the progression of the disease.

In 1971, Dr Alfred Knudson's groundbreaking discovery of the retinoblastoma gene led to a revolutionary concept that cancer development correlates with DNA mutation frequency. A mutation in a tumor suppressor gene during early embryogenesis is propagated to the progeny during cell division, eventually creating a mosaic array of somatic expression of the particular mutation. Loss of heterozygosity (LOH) mutations in the remaining functional allele render progeny with no functional gene expression, which becomes phenotypically manifested in tumorigenesis (Knudson, 1985). In certain cases, simply mutating 1 allele can result in an insufficient expression of protein required for proper function, a phenomenon known as haploinsufficiency. At least 25 genes have been identified whereby haploinsufficient expression leads to tumor development, including mutations in *Pten* and the DNA repair enzyme *Nbn* (Smilenov, 2006). Each of these tumor suppressor genes has potential value in the development of knockout models to study human carcinogenesis. Diverse efforts have focused on breeding a variety of null alleles to both *Pten* and *Nkx3.1* mutants. Compound mutants expressing mutations in the cyclin-dependent kinase inhibitor *p27^{kip1}* or its substrate *Cdkn1b* have been shown to enhance carcinogenesis via an increased proliferative index (Di Cristofano et al, 2001; Gao et al, 2004).

This review discusses the current use of mouse models to study prostate cancer progression to advanced metastatic disease (summarized in the Table), including the limitations posed by each, efforts directed at circumventing such limitations, and the translational significance of

interrogation of molecular pathways toward construction of clinically relevant *in vivo* models of prostate cancer for therapeutic targeting and biomarker validation.

Current Models

Germline Knockouts

Tumor suppressor genes function to maintain a homeostatic balance between cell proliferation and cell death. Mutations resulting in haploinsufficiency of tumor suppressors are frequently encountered in a variety of human cancers, resulting in uncontrolled proliferation (Berger and Pandolfi, 2011). These same mutations can be exploited within the genomes of model organisms to recapitulate phenotypic characteristics and gain a better understanding of the underlying molecular mechanisms toward the development of a platform for therapeutic strategies. Creation of a gene knockout requires the germline mutation of an exon corresponding to a domain essential to the functional integrity of a protein (ie, a kinase domain). Germline mutations are globally expressed at the beginning of embryogenesis, thus posing the risk of premature lethality prior to onset of prostate cancer because of the essentiality of the targeted gene in early development (Chaible et al, 2010; Gama Sosa et al, 2010). Several knockout models have made significant contributions to prostate cancer research.

The Pten knockout

It was in the late 1990s when Parsons and his team at Columbia University first mapped homozygous deletions in human chromosome 10 to identify a putative phosphatase frequently mutated in human cancer (Li et al, 1997). *PTEN* (phosphatase and tensin homologue deleted on chromosome 10) is a tumor suppressor gene that is commonly inactivated because of LOH. Indeed, mutations in *PTEN* occur frequently in human cancer, with homozygous mutations detected in up to 44% of glioblastomas, 50% of endometrial cancers, 43% of malignant melanomas, 6% of breast cancers, and 35% of metastatic prostate cancers (Podsypanina et al, 1999).

In vitro studies have elucidated that Pten acts as a phosphatase, removing a phosphate from the 3 position of phosphatidylinositol 3,4,5-triphosphate (PIP_{3,4,5}). This dephosphorylation inhibits the ability of PIP_{3,4,5} to activate the antiapoptotic AKT, thus suppressing tumorigenesis (Maehama and Dixon, 1998). Ectopic expression of PTEN in breast cancer cell lines upregulates apoptosis machinery and decreases AKT activation, whereas a constitutively active AKT rescues these cells from PTEN-mediated apoptosis (Li et al, 1998; Stambolic et al, 1998). In prostate cancer, *PTEN* mutations can confer chemoresistance (Priulla et al, 2007), radioresistance (Anai et al, 2006), and recurrence following prostatectomy (Bedolla et al, 2007). Since its initial identification, *Pten* has been assigned a critical role in the progression of cancer cells to androgen independence and metastasis (Shen and Abate-Shen, 2007), 2 properties that have made the targeting of this gene translationally valuable in a mouse model. Mice expressing null mutations were engineered with a truncated exon 5, rendering the phosphatase activity of Pten nonfunctional. Mutations in *Pten* exploit the AKT-signaling axis as a mechanism of tumorigenesis (Figure). Increased activation of AKT initiates a series of intracellular signaling cascades that contribute to proliferative and morphological cellular phenotypes (Manning and Cantley, 2007). The AKT-mediated phosphorylation and activation of the androgen receptor (AR) induces its nuclear localization and transcriptional regulation of AR target genes (Ghosh et al, 2003).

Homozygosity for this null mutation results in embryonic lethality around embryonic day 9.5 whereas nearly half of heterozygotes die within 1 postnatal year as a result of lymphosplenomegaly (Stambolic et al, 1998; Di Cristofano et al, 1999; Podsypanina et al, 1999). Considering that an identical coding sequence exists between the mouse gene and its

human homologue with the exception of a single amino acid substitution (Steck et al, 1997), and because engineered mutations in mouse *Pten* are modeled from those expressed in human carcinogenic tissue, mice initiate tumorigenesis in a functionally parallel fashion to human disease. *Pten*^{+/-} mice develop epithelial hyperplasia and multifocal PIN, but fail to progress to metastasis (Podsypkina et al, 1999). Considering that *PTEN* is the most frequently mutated gene in early prostate cancer (Di Cristofano and Pandolfi, 2000), the aberrant regulation of cell survival pathways conferred by the *Pten* mutant provides a suitable molecular platform for the development of a mouse model of disease progression and implementation of targeted therapies for CRPC.

The Nkx3.1 knockout

Nkx3.1 is a member of the NK family of homeobox transcription factors. First characterized by its role in *Drosophila* development, Nkx3.1 is involved in early embryonic patterning and organogenesis in many species (Kim and Nirenberg, 1989). This gene is the earliest known marker of murine prostate development, as its expression delineates the prospective prostate epithelium prior to its specification, with continual expression throughout prostate development and maturation (Bhatia-Gaur et al, 1999; Abate-Shen et al, 2008). Aside from its role in prostate development, Nkx3.1 is essential for normal prostate function and epithelial proliferation. Null mutations in mice disrupt normal prostate protein secretion and ductal morphogenesis (Bhatia-Gaur et al, 1999). Mouse and human homologues share complete conservation within the functional homeodomain, and *Nkx3.1* mutant mice have been adopted as a classical model for PIN (Abate-Shen et al, 2008). Comparable to mutations in *PTEN*, mutations resulting in LOH at the *NKX3.1* locus on human chromosome 8 are prevalent in 60% to 80% of prostate tumors (Vocke et al, 1996; Bhatia-Gaur et al, 1999). Null mutants were generated by deletion of the homeodomain, resulting in a LOF protein that contains no intrinsic transcription regulatory ability (Bhatia-Gaur et al, 1999). Protein expression of Nkx3.1 in heterozygote mutants is comparable to expression seen in human prostate cancer patients (Asatiani et al, 2005). These mice exhibit severe epithelial defects, including hyperplasia and dysplasia, that increase in severity with age. Neoplastic properties are associated with homozygous deletion, but mutants fail to develop invasive carcinoma (Bhatia-Gaur et al, 1999; Abate-Shen et al, 2008). Xenograft transplantation of prostatic tissue from null mutants into wild-type prostates recapitulates PIN lesions that continue to progress histopathologically via the host microenvironment following transplantation. This effectively models early stages of disease progression and creates a model in which to study paracrine signaling in the tumor microenvironment (Kim et al, 2002a).

The *Nkx3.1* gene may functionally contribute to prostate tumor suppression by providing protection against oxidative stress and cellular damage. Reactive oxygen species (ROS) are cycled intracellularly as a byproduct of mitochondrial respiration. Increased ROS above homeostatic levels has long been implicated in carcinogenesis (Ouyang et al, 2005). Gene expression analysis has revealed a down-regulation of antioxidant and DNA repair enzymes and a corresponding increase in oxidation-mediated DNA damage in *Nkx3.1* mutants (Ouyang et al, 2005). Considering that the formation of PIN has been previously associated with oxidative DNA damage, this offers a mechanism by which LOF mutations result in an increased susceptibility to ROS-mediated DNA degradation and disease progression (Ouyang et al, 2005). Autophagic catabolism of a cell's own components as a protective mechanism has also been correlated to ROS in prostate cancer cells (Karna et al, 2010). This model can be used to study the effects of DNA damage and autophagy stimulation on prostatic disease and could provide insight into the efficacy of antioxidant therapies in the treatment of prostate cancer.

Another mechanism via which *Nkx3.1* can promote prostate development and maintain normal organ homeostasis is that of engaging the AR. During embryogenesis *Nkx3.1* expression precedes the expression of *AR* and topological distribution in the prostatic epithelium. However, as *Nkx3.1* functions in the mature prostatic epithelium to control epithelial cell proliferation, its expression is dependent on androgen signaling (Bhatia-Gaur et al, 1999), with significantly attenuated expression following castration (in vivo) and androgen deprivation (in vitro) (Bieberich et al, 1996; Prescott et al, 1998). Reciprocal transcriptional regulation may add another layer of complexity, as it is proposed that the mouse *AR* contains a consensus binding site for *Nkx3.1*, further complicating redundancy in gene regulation in the prostate (Magee et al, 2003; Lei et al, 2006).

Phenotypes develop in prostates of *Nkx3.1* mutants with increasing severity over time and in a gene-dosage manner, with heterozygotes developing similar, but less severe, proliferative phenotypes (Bhatia-Gaur et al, 1999). *Nkx3.1* maintains prostatic epithelial cells in a transiently proliferative state, and LOH of one or both alleles results in uncontrolled cell cycle progression (Abdulkadir et al, 2002). Arguments propose that it may take a single cell at least 4 mutations to escape endogenous apoptotic/cell cycle control mechanisms to gain the proliferative advantage required for uncontrolled cell growth (Spencer et al, 2006). A single mutation in a cell cycle regulatory gene such as *Nkx3.1* results in clonal expansion of daughter cells. This event further increases the chance of mutagenicity, reinforcing the 60% to 80% of human prostate cancers that exhibit LOH in *Nkx3.1* (Vocke et al, 1996; Magee et al, 2003). Loss of *Nkx3.1* may be an early event in prostate cancer, acting to promote genetic mutations that result in tumorigenesis. Tumor heterogeneity resulting from multifocal accumulation of mutations is indeed a source of persistent clinicopathological challenge in the diagnoses and therapeutic response in subsets of cells (Michor and Polyak, 2010).

Compound mutants

The use of compound mutants adds yet another element to modeling disease progression. Although mutations in specific genes confer unique prostatic phenotypes, one could easily argue that when used in combination, models can more accurately and completely recapitulate human prostate carcinogenesis from onset (epithelial hyperplasia and PIN) to terminal impact (androgen independence, castration-resistant recurrence, and metastasis). Loss of *Nkx3.1* characterizes initiation of prostate cancer, but such an alteration is not independently sufficient for development of mature disease (Bhatia-Gaur et al, 1999). Loss of *PTEN* has been implicated at later stages of tumor progression in patients, and heterozygous deletion in mice leads to dysplasia and carcinogenesis of multiple tissues (Podsypanina et al, 1999). Compounding these mutations (*Nkx3.1*^{-/-}, *Pten*^{+/-}) within mice has a more translationally significant synergistic effect on the prostatic phenotype. By 6 months of age, mice develop high-grade PIN lesions (Kim et al, 2002b) with progression to adenocarcinoma and invasive metastasis into the lymphatic system and distant organs (Abate-Shen et al, 2003). Significantly, analogous to human tissues, these cancerous lesions are multifocal and are poorly differentiated (Park et al, 2002). Interestingly, this concerted loss of 2 tumor suppressor genes has prostate-specific synergism, as no additional phenotypes have been characterized in the compound mutants beyond those in independent mutants (Abate-Shen et al, 2008). Of major translational significance is evidence that compound mutant mice develop androgen-independent prostate cancer following androgen ablation therapy, reinforcing this model as a viable one in which to test novel therapeutic efficacy in targeting CRPC (Abate-Shen et al, 2003). Castration-induced androgen ablation-based approaches can be used to attenuate progression, but therapeutic resistance to these treatments and ultimate disease recurrence to CRPC is yet to be overcome.

Conditional Knockouts and Transgenics

The use of transgenics to conditionally knock out genes is particularly useful when studying genes that result in embryonic lethality upon germline mutation (Chaible et al, 2010). Genes involved in vital organ development and early tissue specification may need to be specifically and exclusively deleted from select tissues to study their role in postembryonic development. Conditional mutations delete gene expression in a temporal and spatial manner within the organism (Chaible et al, 2010). Cre recombinase, an enzyme isolated from phage P1, catalyzes site-specific recombination in genes that are engineered with flanking (floxed) synthetic 34 base pair sequences, known as loxP sites (Abremski and Hoess, 1984). Highly regulated expression of *Cre* makes this transgenic system of gene targeting so functional. In mice, *Cre* is recombined into the genome as a transgene under the control of a tissue-specific, typically endogenous promoter. This promoter activity is driven by transcription factors that are expressed in the desired tissue at a specific stage in the organism's development (Wu et al, 2001). Genes are targeted via homologous recombination to orient loxP sites on either side of a functionally essential exon. Breeding schemes combining these 2 genetic manipulations result in tissue-specific activation of the transgene promoter, driving Cre expression and catalyzing the recombination (knockout) of targeted genes (Chaible et al, 2010). Several genes implemented in prostate carcinogenesis have been conditionally targeted with a variety of tissue-specific promoters (reviewed in Kasper, 2005).

Genetic manipulation toward creation of prostate-specific promoters

To efficiently target deletion of genes within the prostate (and only in the prostate), promoter sequences from genes with high endogenous prostatic expression are exploited to drive Cre recombinase (or oncogene) expression. The rat probasin (PB) promoter has been extensively used, and its role in prostate transgenics has been well characterized (Kasper, 2005; Zhang et al, 2010). Two consensus AR binding sites within the promoter (−236 to −223 and −140 to −117), referred to as the androgen response region (ARR), are required for full promoter activation (Kasper, 2005). Reporter analysis reveals high levels of PB-driven expression in all lobes of the murine prostate using both elements in the ARR (Gingrich et al, 1996). Additional interrogation of the PB promoter resulted in isolation and utilization of a larger 12-kb fragment (−11 500/+28, large PB [LPB] promoter) that resulted in significantly higher expression of transgene specific to the luminal epithelium. Elevated androgen levels during prostate maturation compounds this increased activity in the LPB promoter (Yan et al, 1997). An even more prostate-specific, enhanced promoter was created by adding 2 ARRs to the LPB promoter, creating the synthetic ARR2PB promoter. This was used to drive Cre recombinase within the prostate (PB-Cre4), resulting in high luminal epithelium-specific expression (Wu et al, 2001). A portion of the human prostate-specific antigen (PSA) promoter has also been used to express Cre in postpubescent murine prostates (Abdulkadir et al, 2002). Elements from the rat C3(1), cryptdin-2, and fetal Gγ-globin promoters have been used to drive transgene expression of carcinogenic antigens specifically within the prostate microenvironment; these will be discussed in subsequent sections.

Use of the Cre-loxP system: lessons from the prostate

Homozygous lethality resulting from germline mutations in *Pten* (*Pten*^{−/−}) has been circumvented via conditional mutations. Breeding the PB-Cre4 transgene to floxed *Pten* alleles (*PB-Cre4* × *Pten*^{loxP/loxP}) has permitted the study of a *Pten*-deficient prostate. This genotype results in significantly decreased latency of PIN relative to *Pten*^{+/-} mice, with progression to metastasis of lymph nodes and lungs (Wang et al, 2003; Backman et al, 2004), a phenotype more closely resembling the progression in human disease. *Nkx3.1*^{−/−} mice develop prostatic hyperplasia and neoplasia resulting from deregulation of the cell

cycle. However, mice homozygous for this mutation have severe developmental defects in the prostate that undermine the ability for these specimens to be properly studied during carcinogenesis. To delete expression subsequent to prostate development, the *PSA-Cre* transgene was bred to mice expressing floxed alleles of *Nkx3.1* (*PSA-Cre* × *Nkx3.1^{loxP/loxP}*). This loss of Nkx3.1 in the mature prostate captures a phenotype closely resembling human PIN (Abdulkadir et al, 2002). Prior to conditional deletion in the prostate, all prostatic hyperplastic phenotypes of *Nkx3.1^{-/-}* mice were attributed to the role of Nkx3.1 in early prostate development. This phenotype can now be attributed to the role of Nkx3.1 in the predisposition of prostate cancer in addition to its role in the development of the prostate gland.

Transgenic expression of oncogenes

In 1989, Varmus and Bishop were awarded the Nobel Prize in Medicine/ Physiology for their seminal discovery that genes within normal cells may become mutated to initiate cancer (Stehelin et al, 1976). These *oncogenes* have been ectopically exploited to induce tumorigenesis in murine prostate cancer models using prostate-specific promoters. Proto-oncogenes remain quiescent when expressed in an endogenous manner. Gain of function mutations or overexpression of these genes via aberrant regulatory control or aneuploidy can confer cell viability and proliferative advantages (Gonzalzo and Isaacs, 2003; Levy, 2008). Ideal transgenic models for prostate cancer reproduce tumorigenesis while maintaining immune system integrity and without transgene disruption of endogenous epigenetic controls (Jeet et al, 2010).

The human simian virus 40 (SV40) is defined as a DNA tumor virus. The small genome size of the virus prohibits it from encoding its own DNA polymerase and other proteins needed for DNA replication. SV40 must “hijack” host replication machinery to facilitate viral infection (Pipas, 2009). SV40 transduction stimulates DNA synthesis through the expression of 2 viral proteins, the large-T and small-t antigens. Large-T binds to cell cycle regulatory tumor suppressors within the host cell, including p53 and pRb (retinoblastoma 1). Binding and subsequent stabilization of T antigen to both of these proteins is required for the transforming ability of the virus (Chen and Paucha, 1990; Zhu et al, 1991) and leads to genetic and cell cycle instability. Small-t disrupts the phosphatase activity of protein phosphatase 2A, resulting in constitutive mitogenactivated protein kinase activity and promoting tumor cell survival (Sontag et al, 1993). In 1984, in a historic study, the T antigen was the first viral oncogene expressed in transgenic mice, resulting in neoplastic brain tissue (Brinster et al, 1984). Since then, the SV40 antigen has been expressed in at least 10 prostate cancer transgenic lines to efficiently promote tumorigenesis (Kasper, 2005; Jeet et al, 2010).

The TRAMP modeling

The transgenic adenocarcinoma of the mouse prostate (TRAMP) mouse model is one of the most widely used models to investigate prostate tumorigenesis in vivo since its inception in 1996. Its “construction engineering” required that the SV40 large-T and small-t antigens were placed under control of the rat PB (−426/+28) promoter to drive viral transgene expression specifically to the prostatic epithelium (PB-SV40 T or PB-Tag). TRAMP mice develop progressive prostatic neoplasia, with hyperplasia seen as early as 10 weeks of age (Gingrich et al, 1996). The PB-Tag transgene expression augments with increasing age (correlating to positive androgen regulation at the ARR) as invasive adenocarcinoma is initiated as early as 18 weeks and becomes fully developed by 6 months (Gingrich et al, 1996). Metastasis to the lymph nodes, lung, bone, kidney, and adrenal glands pathologically resembles human prostate cancer metastasis (Jeet et al, 2010). Despite several limitations and heavy criticisms, the TRAMP mouse model is currently considered the best-

characterized model for prostate cancer, having been exploited to study prevention, treatment, and progression to metastasis.

The transgene expression of PB-Tag initiates around 4 to 6 weeks of age. A succession of phenotypes typically progresses to poorly differentiated adenocarcinoma with nearly 100% penetrance by 24 weeks of age (Winter et al, 2003). However, any model utilizing the PB promoter is not an ideal system in which to study hormone dependence during tumorigenesis. The PB promoter is regulated by androgens, but tumors derived from transgenic expression of the SV40 antigens are androgen independent (Green et al, 1998). Thus, early castration or hormone ablation strategies in these models may create a false refractory period in tumorigenic progression, not because of a positive therapeutic response by the tumor, but rather as a consequence of down-regulation of transgene expression (Green et al, 1998). When animals are castrated at the age of 12 weeks, only 80% of mice develop prostate cancer (compared to nearly 100% in uncastrated animals). However, one must recognize the immense molecular and biological complexity associated with the emergence of CRPC, occurring despite the presence of the AR within prostate cancer cells. When cancer does develop following castration in this model, tumors are more poorly differentiated, are pathologically aggressive, and exhibit a 2-fold increase in their metastatic potential (Green et al, 1998).

Skeletal metastasis is characteristic of greater than 80% of prostate cancer-related deaths and is highly correlated to poor diagnosis and mortality (Ibrahim et al, 2010). Therefore, the need for integrating the metastatic process in a suitable model of prostate cancer progression is significant. Upon its development, the TRAMP transgene was recombined into a pure C57Bl/6 genetic background. Interestingly, skeletal metastases were only present in a C57Bl/6 $\times$ FVB inbred background and not in the pure C57 animals (Gingrich et al, 1996). The incomplete penetrance of a skeletal metastasis phenotype in TRAMP mice is a limitation for the use of this model. However, identification of a potential modifier gene endogenous to the FVB genome may prove fundamental to the current understanding of prostate cancer metastasis to bone.

The TRAMP model has been used extensively in the development of chemotherapeutic agents in the targeted prevention and disruption of prostate cancer progression (Wang et al, 2009). The focus on the progression of precancerous PIN lesions to metastasis demands a model in which lesion development strictly mimics human disease (Gupta et al, 2001). Interestingly enough, the development of PIN and morphological manifestation in both humans and TRAMP mice is characterized by similar histopathological and molecular phenotypes, including growth factor receptor deregulation and altered AKT activation (Klein, 2005). PIN lesions develop and progress to metastatic carcinoma in all male mice (Kaplan-Lefko et al, 2003). The high reproducibility and relevant molecular activity of the TRAMP tumor creates an advantageous model to test preventative agents. Chemoprevention studies have included, green tea, polyphenols, R-flurbiprofen, toremifene, genistein, and celecoxib (Klein, 2005; Sargeant et al, 2007). Because tumor progression in TRAMP mice has been highly characterized and documented, precision in targeting various temporal stages of cancer can be achieved.

A LADY becomes a model

The LPB promoter driving the large-T antigen (LADY) model is pathologically similar to the TRAMP model. The LPB promoter (−11 500/+28) was used to drive the large-T antigen in the LADY mouse model for prostate cancer, creating the LPB-Tag transgene (Kasper et al, 1998). The T antigen genes contain the mutation *d1 2005*, effectively removing the small-t antigen from the construct. PIN and high glandular proliferation develop by 10 weeks of age, followed by high-grade epithelial dysplasia and poorly undifferentiated

adenocarcinoma with neuroendocrine differentiation by 20 weeks. Metastasis to the lymph nodes, liver, and lung is detected in several transgenic founder lines (Kasper et al, 1998; Masumori et al, 2001). LADY mice are intensely studied for their progression from initial androgen-dependent regression to androgen-independent relapse following castration (Kasper et al, 1998).

Although histopathologically similar, the LADY model is less aggressive than the TRAMP model (Klein, 2005), perhaps in an ironic twist resembling the inner core similarities and phenomenological disparities characterizing human social conditions. Studies using the LADY mouse model to explore the dietary effects of fat and antioxidants on prostate cancer progression have established that a high-fat diet increased the frequency of poorly differentiated tumors, whereas the dietary addition of antioxidants decreased the frequency of tumor development (Venkateswaran et al, 2004). Other promoters have been used to direct expression of SV40 antigens to the prostate (reviewed in Jeet et al, 2010). The use of the rat C3(1) promoter results in the development of high-grade PIN and prostate adenocarcinoma by 3 months of age. Use of the cryptidin-2 promoter results in androgen-independent prostate tumors at 24 weeks of age, while utilizing the fetal γ -globin promoter to drive large-T antigen expression results in androgen-independent, metastatic tumors (kidney, lung, bone, adrenal gland, and thymus) and a considerable neuroendocrine phenotype.

Conclusions

Efforts to establish mammalian models that accurately replicate human prostate cancer initiation and progression to metastasis have encountered challenges. Factors act to compound the heterogeneity of the human disease, including race and allelic variation among individuals (Gingrich et al, 1999). In evaluating a clinically relevant model of human prostate cancer, issues such as epigenetic regulation, androgen sensitivity progression, and the immune response must be considered. Because human disease is unique to the mature prostate gland (Simanainen et al, 2007), models cannot progress in disease to the point of preclusion from reaching prostate maturity. This is particularly limiting considering that many influential housekeeping genes and ones involved in embryonic development have become targets for mutational alterations/inactivation. In the final sections, we will discuss the limitations in current preclinical models while highlighting emerging new possibilities for enhancing their physiological relevance.

Limitations—The genetic prospective

The use of transgene expression has inherent implications in the development of the organism and normal homeostasis. Because transgene insertion into the mouse genome is not achieved by homologous recombination, insertional mutations must be accounted for in a genomic context (Chaible et al, 2010). The use of constitutive promoters to drive expression of an exogenous transgene may significantly sequester transcription factors within the cell that would otherwise be allocated to control expression of related genes, potentially disrupting epigenetic regulation of endogenously expressed genes. These issues are not encountered when using homologous recombination to target genes in a site-specific manner (Chaible et al, 2010). However, germline knockouts express mutations in all somatic cells within every tissue (Gama Sosa et al, 2010), but human prostate cancer initiation has been correlated with LOH mutation as an isolated event within a subset of somatic cells (Smilenov, 2006). In addition, simply overexpressing an exogenous protein (SV40 antigen or Cre recombinase) may have cytotoxic effects on endogenous protein-protein and protein-DNA interactions involved in signaling cascades.

The phenotypic prospective

The preclinical models chosen for discussion in this review have been characterized in detail and are among the most widely used to study prostate cancer. Efforts have contributed to creating a universal histological grading system for tumors derived from specific transgenic models (Gingrich et al, 1999; Suttie et al, 2003), leading to a custommade heterogeneous inventory of molecular and physiological phenotypes that, in combination, represent every stage of prostate cancer progression. Nevertheless, there is no single model that encompasses the entire array of molecular and pathological events that constitutes human disease progression. Mice tend to develop cancer of mesenchymal origin, whereas human cancer originates in epithelial tissues (DePinho, 2000). A fundamental consideration is the fact that prostate physiology and basic histopathology/morphology differ significantly between humans and mice. The human prostate is a single-lobular structure surrounding the urethra. The mouse prostate consists of distinct lobes: anterior, dorsolateral, and ventral. Precursor events typically give rise to cancer in the peripheral zone of the human prostate, which is most analogous to the dorsolateral prostate of the mouse (Abate-Shen and Shen, 2002). A primary disadvantage to using transgene expression to induce carcinogenesis is the almost unambiguous use of androgen-sensitive promoters that drive transgene expression (see "Genetic manipulation toward creation of prostate-specific promoters"). Pre-ventative studies targeting the androgen-signaling axis are not viable in these transgenic lines because disrupting androgen expression levels also effects transgene promoter activation and transgene expression (Jeet et al, 2010). The amount of androgen sequestered by regulatory elements of the transgenic promoters may also prove to be physiologically significant, effectively decreasing the endogenous levels of available androgens. The use of conditional knockouts of tumor suppressor genes circumvents this problem, as Cre recombinase transgene activity under the control of an androgen-independent promoter deletes desired genes in an irreversible, tissue-specific manner, making the genetic alterations insensitive to androgen levels and creating a viable model in which to study androgen signaling during cancer progression. The LPB, C3(1), and fetal G γ -globin promoters do not efficiently drive transgene expression within the anterior prostate (Winter et al, 2003; Kasper, 2005). In addition, certain knockout models encounter a paramount obstacle to modeling the initiation of human cancer. To study LOH in genes associated with embryonic lethality, conditional mutations must be implemented, which may not accurately represent the gene dosage effect associated with LOH.

Emerging Technologies

The use of inducible mutations can be a viable alternative to the expensive and time-consuming breeding required to generate biallelic conditional mutants. Furthermore, inducible mutations can be more precisely temporally and spatially regulated (Chaible et al, 2010). Investigative efforts can induce genetic alterations using completely exogenous or synthetic promoters that do not interfere with the homeostatic balance within the organism.

Several novel methods have been pursued to place Cre recombinase under the control of an inducible promoter. One study created a construct that placed the highly specific MerCreMer recombinase under the control of a tamoxifen-inducible promoter. Transgene expression of this construct was induced with consecutive intraperitoneal tamoxifen injections, resulting in high levels of tissue-specific recombination of floxed genes (Birbach et al, 2009). Another study developed a viral vector that placed Cre expression under the control of the endogenous viral promoter, which was injected intraductally into prostatic ducts of mice containing floxed target genes. Highly efficient recombination was also achieved under these conditions (Leow et al, 2005). There have been advances in xenotransplantation and tissue reconstitution as models for prostate cancer (reviewed in Jeet et al, 2010). Tumor tissues and malignant cells can be injected into immunocompromised

and immunocompetent mice to study the effects of the acquired immune system on tumorigenesis. Tissues can be transplanted into organisms that may not express a certain ligand involved in cell motility and invasion, thus facilitating the study of paracrine signaling during the initial stages of cancer. Tissue reconstitution involves the transplantation of mouse epithelial or stromal cells from the fetal urogenital sinus to the host kidney capsule. Entire prostates have been grown in this manner, allowing for tissue-specific gene expression in otherwise wild-type animals (Jeet et al, 2010).

Opportunities and Future Directions

Clinical challenges related to the emergence of CRPC continue to guide research efforts. Perhaps the most viable and currently attainable option to model the entirety of prostate cancer is compounding established mutations by selective breeding. Our lab is currently exploring the effects of compounding the TRAMP model with mutations in poly (ADP-ribose) polymerase (PARP), a ribosyltransferase that is involved in detecting and repairing DNA stand breaks (Masutani et al, 2005). Mice with null mutations in *Parp* have exhibited higher rates of carcinogenesis. Mutations and single-nucleotide polymorphisms in the human *PARP* gene have been implicated with the development of prostate cancer in association with the age-related accumulation of mutation (Masutani et al, 2005).

One cannot underestimate the contribution of epigenetic regulation in the progression to CRPC when considering prostate tumor heterogeneity and the complex paracrine signaling of the tumor microenvironment. Genetic studies have identified mutations that are necessary and presumably influential in creating an engaging tumor microenvironment (Zhu et al, 2002; Hill et al, 2005). The androgen receptor, along with other genes involved in prostate gland development or homeostasis discussed in this review, could prove a viable target for epigenetic silencing or activation. Mice engineered with global and conditional mutations in the *AR* have contributed to the current understanding of the complex development, maturity, and disease state of the prostate gland (reviewed in Zhou, 2010).

Advancements in genetic recombinant technology offer myriad new directions and attractive opportunities for exploitation. Perhaps the most reproducible and efficient of the animal models involves the overexpression of an oncogene as the prime mechanism driving tumorigenesis. Conventional theory has attributed overexpression of these genes to the manifestation of a proliferative advantage in mutant subsets of cells (Gonzalzo and Isaacs, 2003; Levy, 2008). The recently published, elegant review account of the last decade's molecular events by Hannahan and Weinberg (2011) lucidly illustrates that increased intracellular activity of such oncoproteins as RAS, MYC, and RAF causes induction of cellular senescence and even apoptosis. This contradiction to the fundamental understanding of oncogenesis creates a profound opportunity to reinvestigate at the molecular level the roles that cell cycle regulatory proteins and proto-oncogenes play in homeostatic and pathological states of the cell, and how these pathways can be targeted in the treatment of cancer.

For a period of rigorous dissection of cancer-signaling pathways scanning 2 decades, the ultimate recommendation has been that a global database be instituted to catalogue each model and the corresponding preclinical significance (Green et al, 1998; Pienta et al, 2008) for the benefit of investigative efforts around the world. Gene expression profiles, mechanistic patterning of tumorigenesis, and therapeutic response of each model should be gathered and stored in a public domain. It is critical to fully understand temporal and spatial expression of genes driven by currently utilized promoters so that synthetic promoters may be developed or novel promoters can be implemented to target oncogene expression more specifically. None of the currently available models can reproduce skeletal metastasis at a consistent frequency. Current models could be treated with intraosseous injection of

oncogenes or tumorigenic precursors to facilitate this phenotype. Efforts should be dedicated to understanding the epigenetic changes involved in human cancer initiation and progression to insure the continued development of preclinical models to establish the efficacy and safety profile of novel therapeutic approaches.

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Figure.

Signaling axis exploited in the phosphatase and tensin homologue deleted on chromosome 10 (*PTEN*) mutant mouse model of prostate tumorigenesis. Receptor-ligand complex formation initiates an intracellular signaling cascade ultimately controlling phosphorylation (activation) of the AKT survival pathway. *PTEN* is a phosphatase that acts by cleaving a phosphate group from PIP_{3,4,5} and effectively down-regulating AKT activation. Mutations in *PTEN* designed to mimic those detected in human disease enhance the AKT-mediated phosphorylation of target proteins, functionally contributing to a tumorigenic phenotype. Phosphorylation of the androgen receptor (AR) induces nuclear localization, binding to the androgen response region (ARR), and transcriptional regulation of target gene expression.

Table

Current well-characterized mouse models for prostate cancer

Model	Genetic Manipulation	Pathology	Translational Significance	References
Pten	Germline knockout	Homozygous deletion results in embryonic lethality (E9.5). 50% of heterozygotes develop multifocal PIN, dysplasia, and carcinogenesis.	Progression from early stage PIN to metastatic adenocarcinoma (conditional knockout).	Di Cristofano et al, 2001; Podsypanina et al, 1999; Di Cristofano and Pandolfi, 2000; Shen and Abate-Shen, 2007
	Conditional knockout	Homozygous deletion results in high-grade PIN with advancement to invasive metastasis.		Wang et al, 2003; Backman et al, 2004
Nkx3.1	Germline knockout	Homozygous deletion results in prostatic hyperplasia and neoplasia. Heterozygotes develop similar but less severe phenotypes.	PIN development parallels that seen in early human disease.	Bhatia-Gaur et al, 1999; Abdulkadir et al, 2002; Kim et al, 2002a; Asatiani et al, 2005; Abate-Shen et al, 2008
	Conditional knockout	Homozygotes develop PIN.		Abdulkadir et al, 2002
Pten/Nkx3.1	Compound deletion (Nkx3.1 ^{-/-} , Pten ^{+/-})	High-grade PIN by 6 mo with progression to adenocarcinogenesis and invasive metastasis.	Progression to poorly differentiated, multifocal adenocarcinoma. Development of CRPC following androgen ablation.	Kim et al, 2002b; Park et al, 2002; Abate-Shen et al, 2003, 2008
TRAMP	SV40 transgene expression	Progressive PIN by 6 wk. Invasive adenocarcinoma and metastasis by 6 mo, with 100% penetrance by 24 mo.	Progression to invasive metastasis. Skeletal metastasis is unique to this model. 80% of castrated animals develop CRPC.	Gingrich et al, 1996; Green et al, 1998; Kaplan-Lefko et al, 2003; Winter et al, 2003; Klein, 2005; Jeet et al, 2010
LADY	SV40 transgene expression	Neoplasia PIN by 10 wk. Undifferentiated adenocarcinoma with neuroendocrine differentiation by 20 wk.	Progression from initial androgen-dependent regression to androgen-independent relapse following castration.	Kasper et al, 1998; Masumori et al, 2001; Klein, 2005

Abbreviations: CRPC, castration-resistant prostate cancer; E9.5, embryonic day 9.5; PIN, prostatic intraepithelial neoplasia.