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Animal models relevant to human prostate carcinogenesis underlining the critical implication of prostatic stem/progenitor cells

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Abstract

Recent development of animal models relevant to human prostate cancer (PC) etiopathogenesis has provided important information on the specific functions provided by key gene products altered during disease initiation and progression to locally invasive, metastatic and hormone-refractory stages. Especially, the characterization of transgenic mouse models has indicated that the inactivation of distinct tumor suppressor proteins such as phosphatase tensin homolog deleted on chromosome 10 (PTEN), Nkx3.1, p27^{KIP1} and p53 and retinoblastoma (pRb) may cooperate for the malignant transformation of prostatic stem/progenitor cells into PC stem/progenitor cells and tumor development and metastases. Moreover, the sustained activation of diverse oncogenic signaling elements, including epidermal growth factor receptor (EGFR), sonic hedgehog, Wnt/ β -catenin, c-Myc, Akt and nuclear factor-kappaB (NF- κ B) also may contribute to the acquisition of more aggressive and hormone-refractory phenotypes by PC stem/progenitor cells and their progenies during disease progression. Importantly, it has also been shown that an enrichment of PC stem/progenitor cells expressing stem cell-like markers may occur after androgen deprivation therapy and docetaxel treatment in the transgenic mouse models of PC suggesting the critical implication of these immature PC cells in treatment resistance, tumor re-growth and disease recurrence. Of clinical interest, the molecular targeting of distinct gene products altered in PC cells by using different dietary compounds has also been shown to counteract PC initiation and progression in animal models supporting their potential use as chemopreventive or chemotherapeutic agents for eradicating the total tumor cell mass, improving current anti-hormonal and chemotherapies and preventing disease relapse.

Keywords

Prostate cancer; Metastases; Transgenic mouse models; Prostatic cancer stem/progenitor cells; PTEN; Akt; Dietary compounds; Chemopreventive treatments; combination therapy

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1. Introduction

Human prostate cancer (PC) is a heterogeneous and multifactorial disease that proceeds through multisteps of defined pathological and cytological stages. In general, the PC etiopathogenesis occurs through the formation of pre-cancerous lesions designated as prostatic intraepithelial neoplasms (PINs), tumor development follows by the progression to locally invasive and metastatic disease stages that are often accompanied by the acquisition of a hormone-refractory phenotype by PC cells [1–7]. Although the etiopathological causes of the human PC development are not well established, the risk factors often associated with PC initiation include intense oxidative stress, chronic inflammation and hormonal changes, and more particularly with advancing age [3–5,8–10]. Numerous investigations have also revealed that the alterations in a defined subset of key gene products and molecular pathways typically occur along PC etiopathogenesis and progression. The genetic disruption, epigenetic silencing and/or decreased expression through different mechanisms of diverse tumor suppressor genes that control cell cycle progression such as tensin homolog deleted on chromosome 10 (*PTEN*), homeodomain-containing transcription factor *Nkx3.1*, cyclin-dependent kinase inhibitor *p27^{KIP1}*, *p53* and retinoblastoma (*pRb*) may provide a critical role for PC development (Fig. 1) [11–17]. Moreover, the sustained activation of diverse oncogenic signaling cascades such as epidermal growth factor receptor (EGFR), sonic hedgehog, Wnt/ β -catenin, stromal cell-derived factor-1 (SDF-1)/CXC chemokine receptor 4 (CXCR4) and downstream effectors such as Akt and nuclear factor-kappaB (NF- κ B) frequently occurs during PC progression to locally invasive, metastatic and hormone-refractory PCs (HRPCs) (Fig. 1) [3,4,18–32]. These tumorigenic cascades can cooperate for the sustained growth, survival, invasion, metastases and treatment resistance of PC cells [33–36]. In addition, the release of different oncogenic factors by activated myofibroblasts and intense remodeling in extracellular matrix (ECM) components in the tumor reactive stroma at the primary and secondary neoplasms is generally involved in the malignant transformation of PC cells and disease progression and metastases (Fig. 1) [3,4,7,9,37–39].

Importantly, a growing body of experimental evidence has also revealed that the accumulation of genetic and/or epigenetic alterations in prostatic stem/progenitor cells or their early progenies may lead to their malignant transformation into highly tumorigenic and migrating PC stem/progenitor cells, also designated as PC- and metastasis-initiating cells (Figs. 1 and 2) [4,40–49]. It has been shown that the highly tumorigenic PC stem/progenitor cells expressing specific markers such as telomerase, CD133, CD44^{hi}, $\alpha_2\beta_1$ -integrin^{hi}, multidrug transporter ABCG2^{hi} and aldehyde dehydrogenase (ALDH^{hi}) but low or undetectable androgen-receptor (AR) level and endowed with a high self-renewal ability and an aberrant differentiation potential can give rise to the total PC cell mass [40,43,47–51]. Therefore, these immature PC cells can provide important functions in PC initiation, progression, metastases at distant tissues and resistance to current therapies [40–49,52–55]. In support with this, several prostate regenerative system and transplantable primary xenograft models as well as diverse transgenic mouse models of PC have indicated that the PC stem/progenitor cells can provide critical roles for prostate carcinogenesis, metastases at distant sites and resistance to androgen ablation therapy and chemotherapy [56–62]. In this matter, we review recent gain-of and loss-of function studies using different prostatic tissue regenerative systems and animal models of PC suggesting the critical implication of specific gene products deregulated in PC stem/progenitor cells for PC development and metastases. The emphasis is on the data obtained from transgenic mouse models of PC, including *PTEN* knockdown transgenic mice that are representative of the genetic and epigenetic alterations often observed in PC patients during disease etiopathogenesis and progression. In addition, recent advances on the validation of different dietary compounds as potential chemopreventive and chemotherapeutic agents for treating the PC patients at early and late stages of disease are also reviewed.

2. Implications of the malignant transformation of prostatic stem/progenitor cells into highly tumorigenic and migrating PC stem/progenitor cells during prostate carcinogenesis and metastases

Several investigations have revealed the presence of a small subpopulation of prostatic stem/progenitor cells expressing specific stem cell-like markers such as telomerase, CD133, CD44^{hi}, $\alpha_2\beta_1$ -integrin^{hi}, stem cell factor receptor KIT (CD117), tumor-associated calcium signal transducer (Trop-2), ALDH^{hi}, ABCG2^{hi}, Bcl-2 and/or stem cell antigen-1 (Sca-1) in mouse but low or undetectable AR level in human and rodent prostate glands [4,50,63–65]. These multipotent prostatic stem/progenitor cells endowed with a high self-renewal ability localized within the basal cell layer of the prostatic epithelium were able to drive prostate regeneration by giving rise to basal cells, neuroendocrine (NE) cells and the total epithelial cell mass including secretory luminal epithelial cells expressing AR and cytokeratin 8 (CK8)/CK18 [4,64,66]. More specifically, it has been reported that the prostate involution in the mouse model following androgen withdrawal may be restored due at the persistence of a self-renewing stem cell-like subpopulation in basal epithelial compartment enriched in the proximal region of the prostate gland that can regenerate the prostate gland upon androgen restoration [4,66]. For instance, a single subpopulation of KIT^{+/−} prostatic stem cells from C57BL/6 mouse donors, which was implanted with rat embryonic urogenital sinus mesenchymal (UGSM) cells under the renal capsule of host athymic nude mouse, was able to generate a functional and secretion-producing prostate *in vivo* [66].

Accumulating lines of experimental evidence also suggest that the occurrence of genetic and/or epigenetic alterations occurring in adult prostatic stem/progenitor cells during the lifespan may result in their malignant transformation into highly tumorigenic and migrating PC stem/progenitor cells, also designated as PC- and metastasis-initiating cells (Figs. 1 and 2) [3,4,40,41,43,47,49,67,68]. In fact, the progressive accumulation of specific genetic aberrations in prostatic stem/progenitor cells and their progenies during chronological aging leading to the inactivating mutations in distinct tumor suppressor genes such as *PTEN* and *p53* and sustained activation of diverse oncogenic products is frequently associated with PC initiation and disease development (Figs. 1 and 2) [3,4,11,12,14–17,45,69]. The alterations in different tumor suppressor proteins are necessary to prevent the irreversible growth arrest designated as cellular senescence and apoptotic cell death induced in response to particular oncogenic events, thereby promote their malignant transformation into tumorigenic PC stem/progenitor cells (Figs. 1 and 2) [3,4,45,69,70].

In addition, this model of prostate carcinogenesis also implicates that the acquisition of a more malignant behavior by highly tumorigenic PC stem/progenitor cells, including a migratory phenotype and changes in their local tumor microenvironment during the epithelial-mesenchymal transition (EMT) program, may result in their malignant transformation into migrating PC stem/progenitor cells (Figs. 1 and 2) [3,4,9]. Hence, the highly tumorigenic and migrating PC stem/progenitor cells endowed with a migratory ability and survival advantages can undergo invasion and dissemination through the peripheral circulation and provide critical functions for the formation of metastases at near lymph nodes and distant sites including bones (Figs. 1 and 2) [3,4,9]. In support with this model of prostate carcinogenesis and metastases, recent studies have led to the identification of a subpopulation of highly tumorigenic PC stem/progenitor cells expressing the stem cell-like markers in malignant prostatic adenocarcinomas and metastatic neoplasms and PC cell lines that were able to give rise to the total tumor cell mass (Fig. 1) [40,43,44,47–50,50,53–55,68,71–83]. It has also been shown that the up-regulation of stemness gene expression and the acquisition of a migratory ability by tumorigenic PC stem/progenitor cells induced during the EMT program and under tumor hypoxic conditions may contribute to their

aggressive behavior and treatment resistance (Figs. 1 and 2) [3,4,9,53–55,72–77,84,85]. For instance, it has been reported that the interleukin-6 (IL-6) secreted by PC cells can activate the cancer-associated fibroblasts, which in turn may secrete MMPs that induce the EMT program in a paracrine manner and an enhanced expression of stem cell-like markers in PC cells as well as their ability to form prostaspheres and to self-renew, and thereby promote their metastatic spread through the induction of cyclooxygenase-2/NF- κ B/hypoxia inducible factor-1 α (HIF-1 α) signaling elements [84,85]. Importantly, we are reporting recent data from animal models that have also indicated that the occurrence of specific genetic alterations in prostatic stem/progenitor cells in the prostate gland may lead to PC initiation and progression to invasive and metastatic disease stages and treatment resistance.

3. Animal models of PC carcinogenesis and metastases

3.1. Tissue regeneration system and primary xenograft models of PC

Some studies performing by using tissue regeneration systems consisting to subcutaneously transplant the transformed prostate epithelial cells with UGSM cells in immunocompromised mice and primary PC xenograft models have indicated that the occurrence of the specific oncogenic events in normal basal prostatic stem/progenitor cells or their local microenvironment can drive their malignant transformation into PC stem/progenitor cells and prostate tumorigenesis [67,86–89]. For instance, it has been shown by a prostate regeneration procedure that the Sca-1⁺ regenerative cell fraction enriched from mouse prostate engineered for expressing the constitutively active form of Akt1 formed the PIN lesions when infected at a low-input multiplicity of infection (MOI) and fully developed carcinoma *in situ* when infected at high MOI [86]. Moreover, the overexpression of the Ets family transcription factor Erg-1 or a constitutive activated form of Akt1 in Sca-1⁺/CD49^{fhi} basal stem cells from mouse prostate also resulted in the formation of dysplastic and PIN lesions in the prostate regeneration assay while same alterations in luminal prostatic epithelial cells did not induce tumor formation [87]. The Sca-1⁺/CD49^{fhi} basal stem cells combined with UGSM cells engineered for overexpressing fibroblast growth factor-10 (FGF-10) also formed PCs suggesting a critical role of stromal environment in PC development [87]. In the same way, a single prostasphere generated from the CD133^{hi} cell subpopulation isolated from the immortalized intermediate basal cell strains that have been established from peripheral zone of normal and Gleason score 6 prostate glands of a patient also formed dysplastic lesions in nonobese diabetic/severe combined immunodeficient (NOD/SCID) mice *in vivo* but not the CD133^{lo} cell fraction [88]. The treatment of CD133^{hi} prostaspheres with c-Myc antisense oligonucleotide also abrogated their survival and prevented the formation of the pre-malignant lesions derived from the CD133^{hi} prostaspheres suggesting a potential role of c-Myc oncogene in the malignant transformation of these immature cells at the early stages of prostate carcinogenesis [88]. Moreover, the introduction of sonic hedgehog (SHH) ligand-expressing vector in the normal prostate of mice also caused PIN lesions within 30 days, invasive PCs with a high incidence and metastatic lesions that were detected in supra-epididymal lymph nodes along with an up-regulated expression of the transcription factor twist [67,89]. It has also been noted that the SHH-induced prostate tumorigenesis in this mouse model was accompanied by the morphological changes in epithelial and stromal compartments of the prostate, and the hedgehog signaling elements were co-localized with p63 and CD44 in hyperplastic basal epithelial cells [67,89]. Together these data suggest that the basal and intermediate cells with stem cell-like properties found in the prostate epithelial compartment in mice can be involved in PC etiopathogenesis.

3.2. Transgenic mouse models of PC

The establishment of transgenic mouse models derived from targeted germline or conditional prostate-specific gene alterations that spontaneously develop PIN lesions, which progress to locally invasive, hormone-refractory and metastatic PCs, has also shed more light on the molecular transforming events that might occur in the PC stem/progenitor cells and their progenies during early and late stages of prostate carcinogenesis. Based on the fact that the alterations of genes having critical functions such as *PTEN* homozygous loss may lead to the early embryonic lethality and aberrant developmental programs or postnatal lethality, some studies have consisted to perform prostate-specific conditional gene mutation, deletion or overexpression. Especially, several gene-targeted strategies and conditional transgenic mouse models of PC have been generated by using the gene promoters that are specifically expressed in the epithelium of mouse prostate to induce prostate-specific gene alterations [57,58,60,61,90–94]. Among the most used approaches, there is the combined use of prostate-specific probasin (*PB*) promoter constructs, which are specifically expressed in prostate epithelial cells of mice at 2–3 weeks of the age and at a higher level at the puberty and Cre recombinase/*loxP* site-specific recombination system [60,61,92–96]. More specifically, the *PB-Cre4* transgenic mice, in which the expression of the Cre recombinase from bacteriophage P1 is under the control of a composite promoter containing two androgen-responsive regions (*ARR2*) linked with the endogenous *PB* promoter fragment, has been largely used to generate diverse conditional transgenic mouse models of PC [60,61,95,96]. These techniques have led to the establishment of diverse gene-targeted and transgenic mouse models harboring different genetic alterations. Among the most investigated transgenic mouse models of PC, there are diverse animal models engineered for expressing simian virus 40 (SV40) oncoproteins such as transgenic adenocarcinoma of the mouse prostate (TRAMP) model as well as different gene-targeted transgenic mouse models harboring one or compound genetic alterations such as *PTEN* deletion and/or overexpressing specific oncogenic products such as *erbB2*, *FGF8b*, *c-Myc*, β -catenin and *Vax3* in the prostate (Tables 1 and 2) [57–61,90,91,97–114]. In this regard, we reviewed the transgenic mouse models of PC that have been shown to develop PIN lesions and/or invasive and metastatic PCs reflecting the frequent molecular events seen in the PC patients.

3.2.1. SV40/T-antigen-derived transgenic mouse models of PC

3.2.1.1. TRAMP mouse model of PC—The TRAMP mice were generated by using a region of prostate-specific rat PB promoter to drive the specific expression of the SV40/large tumor antigen-coding region (Tag) in the prostatic epithelial cells [57,58,90,91]. The TRAMP mice expressing a high level of the SV40/Tag transgene spontaneously develop intraepithelial hyperplasias and high-grade PIN lesions that rapidly progress to invasive PCs after about 4–6 months and metastases that are detected with a high incidence at the periaortic lymph nodes and lungs but rarely at bones, kidney and adrenal gland [58]. The analyses of different biomarkers detected in premalignant tissues in the TRAMP mouse model at 12 weeks of the age, prior to the development of solid tumors, have revealed that AR, estrogen receptor- α , EGFR, *erbB2*, EGF, transforming growth factor- α (TGF- α), insulin-like growth factor-1 (IGF-1), IGF-1R receptor, vascular endothelial growth factor receptor-2 (VEGFR-2) and extracellular signal-regulated kinase-1 (erk-1) and erk-2 mRNA were significantly increased in the prostates of these transgenic mice as compared to wild-type C57BL/6 mice [115]. Moreover, it has been reported that the expression levels of downstream signaling elements such as PI3K, phosphorylated-Akt (Ser473), phosphorylated I κ B α , NF- κ B p65 and p50 subunits, Bcl-2, cyclin D1, matrix metalloproteinase-9 (MMP-9) and vascular endothelial growth factor (VEGF) were increased during PC progression in TRAMP mice between 24 and 32 weeks of the age as compared to non-transgenic littermates [116]. The results from a recent study consisting to cross congenic ABCA2

transporter knockout (KO) mice and wild-type TRAMP mice have also revealed that KO/TRAMP mice did not form metastases compared to wildtype TRAMP mice [117]. In fact, the tumor cells in KO/TRAMP mice displayed reduced chemotactic potential that may be due to an altered sphingolipid metabolism leading to an increase of galactosylceramide synthase mRNA levels combined with a decreased lactosylceramide synthase and CTP:choline cytidyltransferase [117]. These data indicate that the ABCA2 multidrug transporter can provide critical functions for metastases of PC cells in the TRAMP mouse model.

Of particular therapeutic interest, it has also been observed that an early castration in TRAMP mice performed at 4 weeks of the age reduced the prostate tumor progression while a castration at 12, 15 and 20 weeks of the age inhibited only the tumor progression in certain castrated mice and the growth of microscopic androgen-independent (AI) lesions and metastases was not delayed as compared to the non-castrated mice used as controls [90,118,119]. It has been noted that the average lifespan of TRAMP mice was 54.1 weeks after an early castration performed at 4 weeks of the age, 59.9 weeks after a delayed castration at 28 weeks of the age or later, and 39.1 weeks in the sham-castrated mouse group indicating that an early or delayed surgical castration provides comparable survival advantages in the TRAMP mouse model [118]. Importantly, the androgen deprivation therapy (ADT) performed on TRAMP mice resulted in a temporary cessation of PC growth, which was followed by a rapid tumor re-growth accompanied with the acquisition of a more malignant behavior and increased distant metastases that were observed in 78% castrated mice relative to 30% intact animals by the end of the experiment at 36 weeks of the age [120]. An overexpression of diverse stem cell-like markers such as Sca-1, CD133 and KIT as well as the anti-apoptotic factor Bcl-2 and binding immunoglobulin protein GRP78/BIP was also observed in prostatic tumors in castrated TRAMP mice at an early stage of 10 weeks post-castration as compared to non-castrated controls while the expression of the stem cell-like markers was decreased and returned at the basal level after 20 weeks post-castration [119,120]. Moreover, it has also been observed that the number of stem cell-like cells expressing Sca-1 detected in the total PC cell mass isolated from the dorsolateral lobes of prostatic tumors using anti-Sca-1 antibody conjugated to the magnetic beads was significantly higher in mice at 10 weeks post-castration relative to 20 weeks post-castration or wild-type TRAMP mice used as controls [119]. Together these observations suggest that the persistence of the AI PC stem/progenitor cells expressing Sca-1, CD133 and KIT after ADT in the TRAMP mouse model can contribute to the tumor re-growth and metastases after anti-hormonal therapies. Additional studies are however required to establish the tumorigenic and metastatic properties of PC stem/progenitor cells expressing stem cell-like markers such as Sca-1 and CD133 *versus* their differentiated progenies isolated from the primary and secondary tumors of TRAMP mice before and after ADT to ascertain their potential contribution to tumor development, metastases, treatment resistance and disease relapse.

In addition, another histopathological feature observed in the TRAMP mouse model is the detection of tumor cells with a NE phenotype as observed in certain cases of human aggressive and metastatic PCs and which may be induced under tumor hypoxia conditions during disease progression (Fig. 1). In this matter, it has been shown that the formation of NE prostate tumors or NE lesions in PCs in the TRAMP mouse model was suppressed in mice lacking the seven in absentia homolog 2 (Siah2) [121,122]. In fact, E3 ubiquitin-protein ligase Siah2 is involved in the regulation of the availability of HIF-1 α that can cooperate with a forkhead transcription factor box A2 (FoxA2) for promoting the expression of specific genes such as Hes6, Sox9, and histone demethylases Jmjd1a that provide critical functions in the formation of NE prostate tumors or NE lesions in PCs in TRAMP mice [121,122]. Importantly, it has also been observed that the stress-induced by castration or a

treatment with the current clinical chemotherapeutic drug, docetaxel of TRAMP mice led in over 30% of mice to the formation of NE prostate tumors as compared to untreated transgenic mice [123]. The NE prostate tumors exhibited particular features, including high expression levels of proliferating cell nuclear antigen (PCNA), metastasis-associated protein (S100A4) and protein gene product (Pgp) but undetectable expression levels of AR and E-cadherin [123]. In considering the fact that the PB-SV40/Tag is selectively expressed in prostate epithelial cells at early stages of precancerous lesions while a high level of SV40-tumor antigen is detected in prostatic NE carcinomas and adenocarcinomas, it has been suggested that these tumors may arise from the malignant transformation of common prostatic progenitor cells with mixed NE and epithelial phenotypes during cancer progression in TRAMP mice [123]. In this regard, it has also been noted that the genetic background of the mouse strains used may influence the incidence of the formation of prostatic tumors with a NE phenotype in the TRAMP mouse model of PC. More specifically, it has been observed that FVB-TRAMP mice rapidly developed NE prostate tumors with an increased frequency as compared to B6-TRAMP mice [124]. It has also been proposed that NE tumors formed in FVB-TRAMP mice may originate from the malignant transformation of multipotential or bipotential prostatic stem/progenitor cells that co-express the epithelial (E-cadherin) and neuroendocrine (synaptosin) markers as well as FoxA1 and FoxA2 at an early stage of prostate carcinogenesis [124]. Hence, these observations suggest that the malignant transformation of common progenitor cells in the prostate epithelium that are committed to both the luminal epithelial and NE cell lineages, may result in the development of prostatic adenocarcinomas and NE tumors or NE lesions in PCs in the TRAMP mouse model which may be promoted by the anti-hormonal or docetaxel treatment.

3.2.1.2. Fetal Globin-SV40/T-antigen transgenic mouse model of PC—The SV40 transgenic mouse model of PC designated as fetal γ -globin-SV40/T-antigen (γ -globin-SV40 Tag), which has been derived from SV40/Tag expression driven by the human fetal γ -globin promoter that is expressed in $p63^+$ cells of basal cell layer of prostatic epithelium, also develop the PIN lesions that progress to invasive carcinomas with epithelial and NE features accompanied by the metastases at the near lymph nodes, kidneys, lungs, bones, adrenal gland and thymus (Table 1) [125–128]. It has been shown that the tumors formed in fetal γ -globin-SV40/Tag mouse model may originate from a subset of non-neoplastic Tag expressing- $p63^+$ basal epithelial cells, possibly adult prostatic stem cells, detected before the formation of preneoplastic lesions such as high-grade PIN and dysplasia in these transgenic mice [99,127,128]. Moreover, the data from immunohistochemical analyses have indicated that the primary tumor tissues and PC cell lines established from primary tumors of these fetal γ -globin-SV40/Tag transgenic mice expressed the stem cell-like markers such as CD133 and CD44 and mixed NE and epithelial phenotypes, including high expression levels of synaptophysin and cytokeratin 8 (CK8) but a low expression of CK18 [128]. Also, the results from a comparative high-throughput genomic analysis of primary tumors formed in fetal γ -globin-SV40/Tag mice *versus* normal prostatic mouse tissues have revealed that several deregulated genes associated with human PC metastases were altered in this transgenic mouse model of hormone-refractory and highly aggressive and metastatic PCs [99]. The deregulated gene products include diverse signaling elements involved in the DNA replication and repair (pRb-1, p53, c-Myc, proliferating cell nuclear antigen, DNA (cytosine-5)-methyltransferase 3A), growth factor signaling pathways (TGF- β 2, Erk1/2, N-Ras, Notch 1 as well as stathmin-1 which can contribute to the destabilization of microtubules and taxane resistance) and transcription factors (Sp-1, NF-Y, cAMP response element-binding (CREB), member of Ets oncogene family Elk-1 and E2F) [99]. Importantly, it has also been observed that the castration performed at 4–6 weeks of the age in these fetal γ -globin-SV40/Tag mice did not prevent the prostate carcinogenesis.

All together, these observations suggest that the NE tumors and PCs formed in the fetal γ -globin-SV40/Tag mice may arise from the malignant transformation of a subpopulation of $p63^+$ basal and/or intermediate epithelial cells endowed with an AI phenotype [126]. Additional studies of the co-localization of the specific stem cell-like and cell lineage markers in Tag-expressing prostatic epithelial cells during tumor initiation and progression combined with the *ex vivo* characterization of the tumorigenic potential of malignant Tag⁺ cells is however required to further ascertain the specific functions of prostatic stem/progenitor cells and their malignant counterparts in tumor development, metastases and treatment resistance in this fetal γ -globin-SV40/Tag mouse model of PC.

3.2.1.3. Potential applications and limitations of SV40/T-antigen-derived transgenic mouse models of PC—The SV40/Tag-driven transgenic mouse models may be useful to establish the genetic alterations that frequently occur during the PC initiation and development of aggressive, AI and metastatic PCs and the implication of the acquisition of NE phenotype by PC- and metastasis-initiating cells and their progenies as well as to test potential chemotherapeutic agents. The important limitations of these SV40/Tag-derived transgenic mouse models of PCs, however, are that they are driven by viral oncogenic products and a high rate of NE tumor formation is also observed in certain transgenic mice models that are not representative of the most human PCs. Consequently, the use of another approach consisting to perform gain-of or loss-of function studies of specific or combined gene products by gene-targeting in the normal prostate may represent a better strategy to establish the transgenic animal models exhibiting genetic changes that frequently occur during human prostate carcinogenesis and metastases. We are discussing different gene-targeted and transgenic mouse models that have provided important insight into the specific functions provided by distinct key tumor suppressor genes such as *PTEN* and oncogenes in the malignant transformation of prostatic epithelial cells, including prostatic stem/progenitor cells, into PC stem/progenitor cells.

3.2.2. *PTEN* knockdown transgenic mouse models of PC

The *PTEN* transgenic mouse models are among the better animal models mimicking the continuum of genetic and histopathological changes that are frequently associated with human PC initiation, progression and metastases [60,61,95,129]. Numerous studies have been performed by using germline *PTEN*^{+/-} heterozygous mutants or conditional mutants with a prostate-specific deletion of one or two alleles of *PTEN* suppressor gene [60,61,95,130–133]. The results have revealed that the *PTEN*^{+/-} monoallelic partial inactivation in the prostate is sufficient for inducing the formation of PIN lesions and PCs after a long latency of 10 to 16 months in certain mouse strains while the conditional *PTEN*^{-/-} biallelic complete inactivation in *PB-Cre4* mice resulted in the invasive PC formation in 100% of transgenic mice at about 9–12 weeks (Table 1) [60,61,95,130,132,133]. The metastases or micrometastases have also detected in *PB-Cre4 PTEN* null transgenic mice at lymph nodes and lungs [60]. Nevertheless, no bone metastasis was detected in all of these *PTEN* knockdown transgenic mouse models suggesting that additional genetic alterations are essential for promoting bone metastases.

Importantly, the data from a study performed by using specific cell lineage markers have also revealed that the prostate-specific *PTEN*^{-/-} biallelic inactivation in the *PB-Cre4* transgenic mice led to the proliferation and expansion of a Sca-1⁺/p63⁺/Bcl-2⁺ prostatic stem/progenitor cell subpopulation in basal and suprabasal cell compartments of the prostate gland without blocking their differentiation [61]. These transforming events culminated to the formation of hyperplasias at 4 weeks, PIN lesions at 6 weeks, invasive PCs at 9 weeks and metastases at 12 weeks [61]. More recently, it has been shown that a Lin⁻/Sca-1⁺/CD49f^{hi} (LSC) prostatic stem/progenitor subpopulation comprising the CK5⁺/p63⁺ cell

fraction was increased during PC initiation, progression and after castration in *PB-Cre4* *PTEN* homozygous mice, and the sorted LSC cells were able to regenerate a prostate epithelial structure with a cancerous morphology mimicking the primary PCs [134].

Collectively, these observations suggest that the immature Sca-1⁺ PC stem/progenitor cells or their more cell lineage-committed progenies with an AI phenotype can act as the PC-initiating cells in these *PTEN*^{-/-} transgenic mouse models and their persistence after hormone withdrawal may lead to tumor re-growth. These data support then the critical functions of the *PTEN* loss-induced sustained PI3K/Akt activation in PC stem/progenitor cells in prostate carcinogenesis and treatment resistance. In regard with this, it has also been shown that the *PTEN* loss may cooperate with other transforming events during PC progression in compound transgenic mouse models of PC.

3.2.2.1. Compound PTEN knockdown transgenic mouse models of PC—Several studies carried out with compound transgenic mouse models have indicated that the monoallelic or biallelic deletion of distinct tumor suppressor genes such as *Nkx3.1*, *p27^{KIP1}* (*Cdkn1b*) and *p53* can cooperate with *PTEN* loss for promoting PC development (Figs. 1 and 2; Table 1) [60,62,135–137]. More specifically, it has been observed that the loss of heterozygous (LOH) of *PTEN* in TRAMP mice significantly shortened the average lifespan of TRAMP mice from 245 days to 159 days [57]. Moreover, it has been reported that *Nkx3.1* and *p27^{KIP1}* are haploinsufficient tumor suppressor genes and only loss of a single allele is sufficient to lead to the development of prostatic intraepithelial hyperplasias and PIN-like lesions [131,135–140]. In contrast, the loss of one or two alleles of *Nkx3.1* or *p27^{KIP1}* combined with a loss of *PTEN* synergistically promoted the PI3K/Akt activation, high-grade PIN formation and prostate tumorigenesis [131,135–137,141]. Interestingly, it has also been shown that the exposure of *Nkx3.1*;*PTEN* transgenic mice to low levels of androgen accelerated the disease progression and formation of PC tumors exhibiting a gene expression pattern like AI PCs including an up-regulated expression of Vav 5 and runt-related transcription factor 1 (Runx1) [142]. These data indicate that a supplementation with low androgen levels in this double transgenic mice model of PC may promote rather reduce the transition to aggressive HRPCs, at least in part, by favoring the enrichment of an AI PC stem/progenitor cell subpopulation.

In the same way, the studies consisting to a conditional ablation of one or two alleles of *p53* and/or *PTEN* in the prostate have revealed that the *p53*^{+/-} or *p53*^{-/-} knockdown transgenic mice did not develop it to PIN lesions or PCs while 50% of *PTEN*^{-/-};*p53*^{-/-} double mutants exhibited invasive PCs at 10 weeks of the age that were invariably lethal by 7 months of age [69,143–145]. It is noteworthy that despite the prostate-specific homozygous inactivation either *p53* or *pRb* genes only results in hyperplasia or PIN formation, it has been observed that the compound transgenic mice with the *p53*^{-/-}; *Rb*^{-/-} developed rapidly growing tumors that metastasized to the regional lymph nodes, lungs, liver and adrenal glands [146]. The malignant neoplasms observed in this *p53*^{-/-};*Rb*^{-/-} mouse model appear to originate from AI Sca-1/CK8 PC progenitor cells found in the proximal region of the prostatic ducts that can give rise to the tumor cells expressing AR and/or NE markers such as synaptophysin and chromogranin A [146].

Together these data suggest that the progressive compound loss of the one allele of *PTEN*, *Nkx3.1* and/or *p27^{KIP1}* in early stages and deletion of two alleles of *PTEN*, *p53* and *pRb* in late stages of prostate carcinogenesis may cooperate for PIN formation and progression to locally invasive and metastatic PCs by overcoming the cellular senescence and apoptotic death of PC stem/progenitor cells and their progenies as frequently observed in human PCs (Figs. 1 and 2). In addition to these genetic alterations, it has also been shown that the

activation of multiples oncogenic products, alone or in combination therapy, in transgenic mouse models may contribute to PC progression.

3.2.3. Other targeted oncogene activation in transgenic mouse models of PC

The specific genetic alterations of different oncogenic growth factor signaling elements including erbB2, FGF8b, prolactin (PRL) and downstream effectors such as PI3K/Akt, K-Ras, Myc, β -catenin, protein kinase LKB1, disintegrin and metalloproteinase domain-containing protein 9 (ADAM12) and Vax3 in the mouse prostate, alone or combined with the *PTEN* deletion, have also led to the establishment of different transgenic mice developing PIN lesions and/or invasive PCs (Table 2) [100–114]. For instance, it has been observed that the *PB-erbB-2* transgenic mice developed PIN lesions and invasive PCs at about 18 months of the age that were associated with a progressive elevation of EGFR expression levels in tumor-adjacent normal tissues, PIN lesions and PCs while erbB3 expression was only increased in PIN lesions and PCs [104]. Moreover, the results from another study have indicated that the monoallelic loss of *PTEN* in the *PB-erbB2* model resulted in the formation of prostatic adenocarcinomas with 15% incidence at about 8 months of the age through the activation of PI3K/Akt/mammalian target of rapamycin (mTOR)/p70S6K signaling pathway and enhanced expression of nuclear cyclin D1 and proliferating cell nuclear antigen (PCNA) [100–102]. Importantly, the characterization of prostate-specific PRL transgenic mice has revealed that the prostate carcinogenesis in this mouse model progresses through the formation of pre-malignant lesions and invasive PCs that was accompanied by a dramatic accumulation and abnormal spreading of a Sca-1⁺ prostatic stem/progenitor-like subpopulation and p63-positive basal epithelial cells and activation of STAT5 [103]. Of therapeutic interest, it has been observed that the expression of a competitive PRL-receptor antagonist in a double-transgenic mice model Delta1-9-G129R-hPRL prevented early stages of prostate tumorigenesis by reducing or inhibiting STAT5 activation, cell proliferation, formation of abnormal basal cell pattern, and frequency or grade of PIN lesions [103]. In the same way, the *PB-Myc^{lo}* and *PB-Cre4 Myc^{hi}* transgenic mice engineered for expressing low or high levels of human c-Myc oncogenic product in the prostate also developed PIN lesions and invasive prostatic adenocarcinomas that were accompanied by an activation of the Pim-1 kinase, a gene known to cooperate with c-Myc in human PC tumorigenesis, and the loss of *Nxk3.1* transcriptional activator at later stages as compared to the *PTEN* null mouse model [62,106,109]. The *PB-Cre4 Myc^{hi}* transgenic mice crossed with the *PB-hepsin* mice overexpressing hepsin, which is a cell surface protease that is overexpressed in more than 90% of human PCs, also cooperated for promoting PC progression [110]. In contrast, the *PB-Cre4 Z-Myc* transgenic mice focally expressing Myc in the prostate, alone or in combination with a deletion of one or two alleles of the *PTEN*, only formed low- or high-grade PIN lesions that did not progress to PCs due, in part, at inducing a p53-dependent apoptotic response [113]. These data are consistent with the fact that the *PTEN* loss-induced p53 apoptotic pathway may limit the PC progression in this double mutant, focal *Myc;PTEN^{-/-}* transgenic mouse model.

The characterization of other transgenic mouse models of PC has also indicated that the intense inflammatory conditions and a sustained activation of Wnt/ β -catenin and growth factor-induced K-Ras/MAPKs pathways may cooperate for PC development [147,148]. For instance, it has been reported that a down-regulation of adenomatosis polyposis coli (APC), which acts as an important inhibitory regulator of Wnt/ β -catenin pathway, in a heterozygous *APC^{Min/+}* mouse model resulted with increasing age to the formation of PIN lesions and microinvasive prostatic carcinomas after 3 to 6 months in about 40% of mice that was accompanied by the enhanced levels of several proinflammatory cytokines mimicking the molecular events seen in human PCs [149]. Moreover, the transgenic mice engineered for expressing a dominant stabilized β -catenin (*Catnb^{+lox9ex3}*) or activated *K-Ras^{+V12}* only

developed the PCs after a long latency while 100% of compound *PB-Cre Catnb^{+/lox(ex3)};K-Ras^{+V12}* transgenic mice formed invasive PCs through a synergistic elevation of c-Myc, cyclooxygenase-2 (COX-2) and AR (Table 2) [107]. In the same way, the transgenic mice engineered for overexpressing a constitutive active form of Vav3 oncogenic product in the prostatic epithelium also developed non-bacterial chronic prostatitis in the prostate gland accompanied with an infiltration of lymphomononuclear cells such as monocytes, lymphocytes, and plasma cells [108]. The mice overexpressing Vav3 also formed PIN lesions and invasive PCs at about 3 months of the age while no metastasis at distant tissues was detected [108]. The oncogenic effects of Vav3 appear to be mediated, at least in part, through the activation of the PI3K/Akt signaling elements that in turn can stimulate the pro-inflammatory mediator NF- κ B-induced gene expression as well as AR signaling axis in AR⁺ PC epithelial cells [108].

Future investigations of prostate epithelial cell lineage markers in these transgenic mouse models are however necessary to more precisely establish the specific implication of these oncogenic alterations in the malignant transformation of prostatic stem/progenitor cells into PC stem/progenitor cells *versus* their differentiated progenies and treatment resistance as well as for their use as animal models of PC to test novel chemopreventive and chemotherapeutic agents. In this regard, we are reporting potential dietary compounds that represent promising chemopreventive and chemotherapeutic agents to eliminate the total PC cell mass, reverse drug resistance and thereby prevent PC progression and disease relapse.

4. Novel chemopreventive and chemotherapeutic strategies using natural dietary compounds for preventing PC progression and overcoming treatment resistance

Numerous studies aiming to develop novel therapies for preventing or treating PCs have led to the identification of a variety of potential chemopreventive and anti-tumoral agents, including natural and dietary compounds and synthetic agents or gene therapies, to prevent PC development or counteract its progression to aggressive and metastatic HRPCs. Among the chemopreventive anti-tumoral agents showing potential therapeutic interest for treating PCs, there are diverse anti-androgens (finasteride), anti-estrogens (tamoxifen), phytoestrogens, antioxidants (selenium and vitamin E), non-steroidal antiinflammatory (pro-apoptotic) drugs “NSAIDs”, differentiation agents and anti-angiogenic agents (COX-2 inhibitors) [115,150–162]. Some of these agents have been observed to prevent PC initiation and progression by triggering anti-proliferative, apoptotic, anti-invasive, anti-metastatic and/or antiangiogenic effects in pre-malignant and malignant prostatic epithelial cells *in vitro* and in PC xenograft and transgenic mouse models *in vivo* [115,150–161]. In general, it has been shown that these chemopreventive and chemotherapeutic agents act through multiple molecular mechanisms by blocking diverse tumorigenic cascades such as EGFR, erbB2, hedgehog, CD44 and/or AR axes and their downstream oncogenic signaling elements including PI3K/Akt, MAPKs and NF- κ B as well as by down-regulating diverse drug resistance-associated molecules including ATP-binding cassette (ABC) multidrug transporters (Fig. 1) [115,150–161,163,164]. For instance, a natural polyphenolic flavonolignan isolated from milk thistle, silibium has been shown to induce anti-proliferative, antiinvasive and apoptotic effects and potentiated the cytotoxic effects induced by mitoxantrone on PC cells *via* the inhibition of numerous mitogenic and survival signaling elements, including EGFR, CD44, PI3K/Akt and MAPKs, MMPs and urokinase-type plasminogen activator (uPA) [150,151,163,165]. Importantly, the soy isoflavone genistein has also been shown to improve the growth inhibitory, anti-invasive and anti-metastatic effects induced by current chemotherapeutic drug docetaxel on PC3 xenograft model in mice *via* the inhibition of the expression and secretion of receptor activator of NF- κ B

(RANK)/RANKL ligand/MMP-9 signaling and drug resistance-associated molecules such as NF- κ B [152,153].

Some pre-clinical investigations have also consisted to establish the chemopreventive and anti-carcinogenic effects induced by diverse compounds such as dietary curcumin, lycopene, silibinin, feeding of dibenzoylmethane, green tea polyphenols, genistein, alpha-difluoromethylornithine, toremifene, R-flurbiprofen, celecoxib and sulindax and their chemical derivatives using the TRAMP and *PTEN* knockdown transgenic mouse models of PC. The results have revealed that these chemopreventive agents significantly decreased the incidence of PIN lesions and PC formation and/or delayed the disease progression in the TRAMP model [154–161]. More specifically, the inclusion of non-toxic flavanoid, silibinin as supplement diet has been shown to suppress the tumor growth, angiogenesis and metastases at distant sites in the TRAMP mouse model by decreasing the expression of platelet endothelial cell adhesion molecule-1/CD-31, VEGF/VEGFR-2, HIF-1 α , inducible nitric oxide synthase, MMPs, mesenchymal marker snail-1 and fibronectin in the prostatic tissue [158,166]. The treatment with a bark extract from *Phellodendron amurense*, nextrutine has also been observed to prevent prostate tumor formation when administer prior to the high-grade PIN development as well as the progression of established tumors, invasion and metastases of AI PC cells in the TRAMP model [157]. The anti-carcinogenic effects of nextrutine was associated with an inhibition of transcriptional activities of NF- κ B and CREB [157]. Interestingly, several botanic compounds such as genistein, apigenin, baicalein, curcumin, epigallocatechin 3-gallate (EGCG), quercetin, and resveratrol have also been reported to act as the specific hedgehog inhibitor like cyclopamine by inhibiting the GLI expression and suppressing the *in vitro* growth of PC cell lines and disease progression in the TRAMP model [159].

In addition, the administration of a combination of liposomal forms of curcumin and resveratrol in prostate-specific *PTEN* knockout mice at 4 weeks of the age also inhibited the PC growth and decreased the incidence of PCs detected after 7 weeks of drug treatment and induced the apoptosis in *PTEN*-CaP8 cancer cells *in vivo* by down-regulating phosphorylated Akt/mTOR and cyclin D1 [160]. It has also been reported that the small leucine-rich proteoglycan, decorin inhibited the growth and induced the apoptosis in cultured *in vitro* PC cells, and the systemic intraperitoneal delivery of decorin delayed tumor growth and prevented the metastases in immunocompetent *PTEN*^{-/-} transgenic mice at 4 weeks of the age as compared to *PTEN*^{+/+} mice used as control [161]. The anti-carcinogenic effects of decorin were mediated, at least in part, through an inhibition of the expression and activation of EGFR, PI3K/Akt and AR signaling pathways in tumor cells [161].

Several clinical trials are also ongoing with some of these chemopreventive and chemotherapeutic agents to ascertain their anti-carcinogenic efficacy *versus* systemic toxicity. Although several previous trials with a single agent, have generally given no significant improvement of overall survival of PC patients, the validation of new combinations of these dietary agents with the current anti-hormonal and chemotherapeutic treatments offers great promise for a more effective management of the patients diagnosed at early and late stages of disease progression. Future studies are however necessary to more precisely establish the molecular mechanisms of actions of these chemotherapeutic agents and their cytotoxic effects on PC stem/progenitor cells and potential secondary effects. In this regard, recent studies have also indicated that the natural compounds such as parthenolide, gossypol and sulforaphane induced the apoptotic death of PC stem/progenitor cells and their progenies *in vitro* and *in vivo* [76,167–169].

5. Conclusions and future directions

Significant progress in the development of novel animal models of PC has led to a better understanding of the molecular mechanisms and specific functions of gene products altered in PC stem/progenitor cells *versus* their progenies that may cooperate to prostate etiopathogenesis, carcinogenesis, metastases and resistance to current therapies. Several potential biomarkers and molecular targets, including the PTEN loss-induced Akt and NF- κ B activation that have been identified to be implicated in the prostate carcinogenesis offer great promising to develop novel multiplex biomarkers and multitargeted strategies for improving the efficacy of diagnosis and therapeutic treatment of PC patients at early and late disease stages.

Although this important advance, few preclinical xenograft and animal models of PC are available that accurately recapitulates together frequent molecular genetic and histopathologic events occurring in PC- and metastasis-initiating cells and their progenies and the tumor microenvironment during human PC initiation and progression as well as the high incidence of bone metastasis as observed in human. Consequently, it will be important to further establish new xenograft and compound transgenic mouse models reflecting the different genetic changes that frequently occur in PC stem/progenitor cells and their progenies during human prostate carcinogenesis and metastases at distant tissues including bones. These studies will shed more light on the molecular transforming events and mechanisms that can cooperate to drive PC initiation and progression to invasive, hormone-refractory and metastatic disease stages and thereby help to design novel animal models of PC relevant human carcinogenesis and metastases.

Of great clinical interest, it will be important to further establish the cytotoxic effects induced by combinations of different chemotherapeutic agents, including diverse dietary compounds with current anti-hormonal treatments and chemotherapies on the immature PC stem/progenitor cells and their progenies detected in different animal models relevant to human prostate carcinogenesis and metastases. Hence, these additional investigations should lead to the therapeutic validation of novel chemopreventive and chemotherapeutic agents that could be used in combination to prevent or counteract the disease progression to locally invasive, metastatic and lethal HRPCs.

Abbreviations

ADT	androgen deprivation therapy
AI	androgen-independent
ALDH	aldehyde dehydrogenase
AR	androgen receptor
ARR₂	two androgen-responsive regions
BM	bone marrow
CXCR4	CXC chemokine receptor 4
CK	cytokeratin
ECM	extracellular matrix
EGFR	epidermal growth factor receptor
EMT	epithelial-mesenchymal transition
FGF	fibroblast growth factor

HIF-1α	hypoxia-inducible factor-1 α
HRPCs	hormone-refractory prostate cancers
IGF	insulin-like growth factor
IL-6	interleukin-6
MAPKs	mitogen-activated protein kinases
MMPs	matrix metalloproteinases
NOD/SCID	nonobese diabetic/severe combined immunodeficient
NE	neuroendocrine
NF-κB	nuclear factor-kappaB
Nrf2	nuclear factor (erythroid-derived 2)-like 2
PB	probasin
PC	prostate cancer
PI3K	phosphatidylinositol 3'-kinase
PINs	prostatic intraepithelial neoplasms
PIP3	phosphatidylinositol 3,4,5-trisphosphate
PRL	prolactin
PTEN	phosphatase tensin homolog deleted on chromosome 10
pRb	retinoblastoma protein
Sca-1	stem cell antigen-1
SDF-1	stromal cell-derived factor-1
SHH	sonic hedgehog
STAT	signal transducer and activator of transcription
SV40	simian virus 40
Tag	antigen-coding region
TGF-β	transforming growth factor- β
TRAMP	transgenic adenocarcinoma of the mouse prostate
UGSM	urogenital sinus mesenchymal
uPA	urokinase-type plasminogen activator
VEGF	vascular endothelial growth factor

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Fig. 1.

Scheme showing the molecular transforming events occurring in PC stem/progenitor cells and their local microenvironment involved in the prostate carcinogenesis and metastases. The accumulation of different genetic and epigenetic alterations leading to a down-regulation of diverse tumor suppressor proteins such as phosphatase tensin homolog deleted on chromosome 10 (PTEN), Nkx3.1, p21^{WAP1} and p27^{KIP1} cyclin-dependent kinase inhibitors, p53 and/or retinoblastoma (pRb) combined with a persistent activation of diverse tumorigenic cascades initiated by different growth factors in PC stem/progenitor cells is illustrated. These molecular transforming events can contribute to the sustained growth, invasion, metastases at distant sites and treatment resistance of PC stem/progenitor cells. Moreover, the changes in the local microenvironment of PC stem/progenitor cells during the epithelial-mesenchymal transition (EMT) process and under hypoxic conditions, including the release of diverse growth factors and cytokines by the adjacent myofibroblasts, which may induce in a paracrine manner an increase of stemness gene expression and oncogenic effects in these immature PC cells, is also indicated. In addition, the scheme also shows the potential anticarcinogenic effects induced by the dietary compounds through an inhibition of the expression levels and/or activities of diverse receptor tyrosine kinases (RTKs) such as EGFR and erbB2 and their downstream signaling elements and sonic hedgehog (SHH/SMO/GLIs) in PC stem/progenitor cells. Moreover, the potential inhibitory effect of the dietary agents on the activity of ATP-binding cassette (ABC) multidrug transporters such as

ABCG2, which may result in an intracellular accumulation of chemotherapeutic drugs and increase of their cytotoxic effects, is also illustrated.

Fig. 2.

Model of the prostate carcinogenesis and metastases mediated through the malignant transformation of prostatic stem/progenitor cells into tumorigenic and migrating PC stem/progenitor cells. The scheme shows the malignant transformation of prostatic stem/progenitor cells into tumorigenic and migrating PC stem/progenitor cells that can give rise to the total PC cell mass, and which may be induced through the inactivation of different tumor suppressor genes and sustained activation of distinct growth factor signaling pathways during the epithelial-mesenchymal transition (EMT) program. The possible dissemination of tumorigenic and migrating PC stem/progenitor cells through the peripheral circulation and secondary tumor formation driven by metastasis-initiating cells at distant tissues is also indicated.

Table 1
Summary of SV40/Tag- and *PTEN* knockdown-derived transgenic mouse models of prostate carcinogenesis and metastases

Mouse model	Preneoplastic lesions	Prostatic adenocarcinoma latency/incidence	Phenotypic features of tumors	Metastatic sites	Ref.
<i>PB-SV40/Tag</i> (TRAMP)	Hyperplastic and low- and high-grade PIN lesions	12–20 weeks/100%	Well differentiated tumors exhibiting the poorly differentiated and AI features after castration	Periaortic lymph nodes and lungs but rarely at bones, kidneys and adrenal glands	[57,58]
<i>SV40/Tag-Fetal Gγ globin</i>	PIN-like foci	16–20 weeks/100%	Tumors with the epithelial and neuroendocrine features exhibiting an AI phenotype after castration	Renal lymph nodes, kidneys and adrenal glands as well as micrometastases at lungs and bones	[125,126]
<i>PB-Cre PTEN^{+/-}</i>	Hyperplastic and PIN lesions	12–16 months/ 100%	Tumors showing an activation of PI3K/Akt pathway	ND	[60]
<i>PB-Cre4;PTEN^{-/-}</i>	Hyperplastic and PIN lesions	9–12 weeks/100%	Tumors showing an activation of PI3K/Akt pathway and an AI phenotype after castration	Lymph nodes and lungs	[60,61]
Germline deletion or <i>PB-Cre Nkx3.1^{+/-} or Nkx3.1^{-/-}</i>	Hyperplastic and low-grade PIN lesions	ND	ND	ND	[136,138,139]
<i>Nkx3.1^{+/-};PTEN^{+/-}</i>	Hyperplastic and low- and high-grade PIN lesions	12–15 months/100%	Invasive tumors exhibiting activated Akt and AR and an AI phenotype after castration	Iliac lymph nodes	[136,141]
<i>p27^{KIP1}^{+/-} or p27^{KIP1}^{-/-}</i>	Hyperplastic and dysplastic lesions	ND	ND	ND	[131,139]
<i>p27^{KIP1}^{+/-};PTEN^{-/-}</i>	PIN lesions	3–5 months/100%	Invasive tumors	ND	[131]
<i>PB-Cre4 p53^{-/-}</i>	PIN lesions with reduced apoptotic potential	ND	ND	ND	[144]
<i>PB-Cre4 p53^{-/-}; PTEN^{-/-}</i>	High-grade PIN lesions	10 weeks/50%	Invasive tumors	ND	[69]
<i>p53^{-/-};Rb^{-/-}</i>	Hyperplastic and PIN lesions	160 days/100%	Poorly differentiated and highly aggressive tumors showing a rapid growth and both luminal and neuroendocrine differentiation as well as an AI phenotype after castration	Regional lymph nodes, lungs, liver and adrenal glands	[170]

Abbreviations: AI, androgen independent; AR, androgen receptor; PI3K, phosphatidylinositol 3'-kinase; ND, no detected; PIN, intraepithelial prostatic neoplasm; PB, probasin promoter fragment; PTEN, phosphatase tensin homolog deleted on chromosome 10; Rb, retinoblastoma; SV40/Tag, simian virus 40 large tumor antigen region and TRAMP, transgenic adenocarcinoma of the mouse prostate.

Table 2
Summary of targeted genes in transgenic mouse models of prostate carcinogenesis and metastases

Mouse model	Preneoplastic lesions	Prostatic adenocarcinoma latency/incidence	Phenotypic features of tumors	Metastatic sites	Ref.
<i>PB-erbB2</i>	PIN lesions	18–20 months/11.7%	Tumors showing enhanced expression levels of EGFR, erbB2 and ErbB3 and phosphorylated AR during disease progression	ND	[100]
<i>PB-erbB2; PTEN^{+/-}</i>	Hyperplastic and PIN lesions	8 months/15%	Tumors showing an activation of PI3K/Akt/p70S6K pathway and enhanced expression of nuclear cyclin D1 and proliferating cell nuclear antigen	ND	[101,102]
<i>PB-Cre4 FGF8b</i>	Hyperplastic and PIN lesions	ND	ND	ND	[105]
<i>PB-Cre4 FGF8b; PTEN^{+/-}</i>	Hyperplastic and PIN lesions	18–19 months/90%	Poorly differentiated tumors that progress through the loss of heterozygosity of PTEN and Akt activation and showing intense inflammation. A rare form of mucinous adenocarcinoma was detected in 2 of the 31 animal analyzed.	Pelvic lymph nodes	[105]
<i>PB-Cre4 Myc^{hi}</i>	PIN lesions	6–12 months/100%	Invasive tumors exhibiting a loss of Nkx3.1, enhanced expression of serine/threonine kinase Pim-1 but no evidence of a neuroendocrine phenotype	ND	[106]
<i>PB-Cre Catmb^{+/-lox(ex3)}</i>	PIN-like keratinized squamous metaplasias	End-point (longevity average of 428 days)/100%	Invasive tumors exhibiting a high level of nuclear β -catenin and an up-regulated expression of its target CD44	Bulbourethral, preputial and urethral glands keratinized squamous metaplasias	[107]
<i>PB-Cre Catmb^{+/-lox(ex3)}; K-Ras^{+V12}</i>	PIN-like keratinized squamous metaplasias	End-point (longevity average of 189 days)/100%	Invasive tumors exhibiting a high level of nuclear β -catenin and an up-regulated expression of its target CD44 as well as an activation of Ras/MAPK cascade and enhanced expression and activation of AR	Bulbourethral, preputial and urethral glands keratinized squamous metaplasias	[107]
<i>PB-Cre4 APC^{-/-}</i>	Intraepithelial PIN lesions	7–15 months/---	Highly proliferative and invasive tumors exhibiting a high level of β -catenin	ND	[149]
<i>PB-Cre4 Vax3- IRES-hrGFP</i>	Intraepithelial PIN lesions	3–10 months/48%	Tumors exhibiting a high activation of PI3K/Akt, NF- κ B and AR signaling pathways and a significant inflammatory response	ND	[108]

Abbreviations: APC, adenomatosis polyposis coli; AR, androgen receptor; Catmb, β -catenin; FGF8b, fibroblast growth factor 8b; hrGFP, *Renilla* green fluorescence protein; MAPK, mitogen-activated protein kinase; ND, not detected; NF- κ B, nuclear factor-kappaB; PB, probasin promoter fragment; PI3K, phosphatidylinositol 3'-kinase; PIN, prostatic intraepithelial neoplasm and PTEN, phosphatase tensin homolog deleted on chromosome 10.