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Review

Genetically engineered mouse models of prostate cancer

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ABSTRACT

Despite major improvement in treatment of early stage localised prostate cancer, the distinction between indolent tumors and those that will become aggressive, as well as the lack of efficient therapies of advanced prostate cancer, remain major health problems. Genetically engineered mice (GEM) have been extensively used to investigate the molecular and cellular mechanisms underlying prostate tumor initiation and progression, and to evaluate new therapies. Moreover, the recent development of conditional somatic mutagenesis in the mouse prostate offers the possibility to generate new models that more faithfully reproduce the human disease, and thus should contribute to improve diagnosis and treatments. The strengths and weaknesses of various models will be discussed, as well as future opportunities.

1. Introduction

Prostate cancer is the most common malignant visceral neoplasm in males in Western societies and the second leading cause of cancer-related deaths in these populations (Bray et al., 2010; Ferlay et al., 2010; Jemal et al., 2010; Siegel et al., 2012). It is a multistage disease that develops during decades; even though men in their 20s can display preneoplastic lesions called prostatic intraepithelial neoplasia (PIN), clinically detectable prostate cancer usually appears after the fifth decade. Its etiology remains largely unknown and its clinical course unpredictable. In western world 1 man in 6 will be diagnosed with this disease during his lifetime. If detected at early stages when locally confined, prostate cancer is eradicated in 70–80% of the patients by radical prostatectomy, radiation therapy or cryotherapy (Siegel et al., 2012). Unfortunately, around 5 years after primary treatment, the remaining 20–30% develop metastasis, mainly in bone, as well as in liver, lung and adrenal glands. Relapses can be managed efficiently only for a limited time period by targeting the androgen–androgen receptor (AR) axis through androgen ablation therapy and/or androgen receptor (AR) antagonists (castration) (Bubendorf et al., 2000; Denmeade and Isaacs, 2002). These treatments are not curative, and ultimately lead to the recurrence of highly aggressive tumors. Following the emergence of castrate-resistant prostate cancer, docetaxel chemotherapy has been shown to be therapeutically efficacious, but the median increase in survival was only 4 months (Petrylak et al., 2004; Singh et al., 2010). Serum prostate specific antigen (PSA) is widely used as a biomarker for prostate cancer detection, but does not predict whether tumors will remain indolent or become clinically aggressive. Large scale clinical

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Table 1 – Common transcriptional regulatory elements used to drive transgene expression in the mouse prostate.

Promoter	Expression	Ectopic expression	Reference
Rat C3(1)	VP > DP	Seminal vesicles, testis, thyroid and salivary gland, cartilage.	(Allison et al., 1989; Maroulakou et al., 1994; Zhang et al., 1997, 2000b)
−426 bp to +28 bp rat probasin	VP > DLP, AP (starts before puberty)	Seminal vesicles	(Greenberg et al., 1995, 1994)
−11.5 kb to +28 bp rat probasin	VP > LP > DP > AP (starts before puberty)	Seminal vesicles	(Yan et al., 1997)
ARR_2PB	VP, DLP > AP (starts in newborn mice)	Prostate stroma, seminal vesicles, testis	(Wu et al., 2001; Zhang et al., 2000a)
6 kb PSA	LP > DP > VP, AP (starts at puberty)	None reported	(Cleutjens et al., 1997)

studies indicate that systematic use of this marker leads to many unnecessary transrectal prostatic needle biopsies, over diagnosis and over treatment with severe side effects on patients and increased costs for healthcare systems (Schroder et al., 2012; Whitson and Carroll, 2010). Thus, there is an urgent need for improvements in both diagnosis and therapy for prostate cancer.

The human prostate is a glandular organ composed of central, peripheral and transitional zones that are not clearly demarcated. The three zones contain acini located within a fibromuscular stroma. Acini are formed by columnar epithelial cells which secrete prostatic proteins and fluids from their apical surfaces into a lumen, and are surrounded by basal cells attached to the basement membrane and scattered neuroendocrine cells. PIN lesions are formed by cells that proliferate within the prostatic epithelium and disrupt its well-defined architecture (Bostwick and Qian, 2004; McNeal and Bostwick, 1986; McNeal et al., 1986; Timms, 2008). They are graded according by their degree of atypia. Low grade PINs (LGPINs) define areas of proliferative glandular epithelial cells that present enlarged nuclei, variable in size, non-prominent nucleoli and an intact basal cells layer (Bostwick, 1989). High grade PINs (HGPINs) mainly differ from LGPINs by the presence of prominent nucleoli, a nuclear hyperchromasia and a fragmented or lack of basal cells layer (Montironi et al., 2011), and are broadly accepted to be precursors of prostate carcinoma (Chrisofos et al., 2007; Montironi et al., 2011). Most of the human prostate cancers correspond to acinar adenocarcinoma, while neuroendocrine prostate cancers represent less than 2% of the cases (Grignon, 2004). However, focal regions of neuroendocrine differentiation are more commonly observed following recurrence after prostatectomy and androgen deprivation therapy (Komiya et al., 2009; Yuan et al., 2007).

A large number of studies have been performed with immortalized human prostate cancer cell lines, such as LNCaP, VCap, PC-3 or MDA-PCa, to gain insight into the biology of tumor progression, androgen-independent diseases and metastatic prostate cancer, and test putative chemopreventive compounds (Peehl, 2005; Wang et al., 2005). These lines are derived from advanced/metastatic tumors, and thus do not allow to recapitulate the various stages of the human disease. Moreover, studies of clonal cell lines in culture do not reproduce their interaction with the various cellular compartments of the prostate, such as the basal and neuroendocrine epithelial cells, stromal cells, or of the metastatic site (e.g. osteoblasts, osteoclasts), with vascular and lymphatic circulation and immune cells. In addition, xenograft transplantation models based on transformed human cell lines do not mimic the heterogeneity of human tumors and their microenvironment, and require immunodeficient host animals, that lack crucial modulators of tumorigenesis.

These major limitations prompted the development of animal models of prostate cancer to investigate tumor genetics and gene–environment interactions. Even though the gross anatomy of the mouse prostate differs from that of the human prostate, as it is composed of four distinct lobes [i.e. anterior (AP), ventral (VP), dorsal (DP) and lateral (LP)], the prostate of both species are composed of glands and ducts of similar organization (Cunha et al., 1987). Mouse prostatic glands contain however fewer basal and neuroendocrine cells, and looser and less fibromuscular stroma, with only few smooth muscle cells (Marker et al., 2003). Mice are not prone to develop spontaneous benign or malignant prostate pathologies, and rodent models of prostatic hyperplasia and malignancy using chemical carcinogenic agents are not sufficiently reliable (Bosland and Prinsen, 1990; Pollard et al., 1989). Importantly, the possibility to genetically manipulate the mouse genome with various technologies developed over the last 3 decades allowed the generation of a number of models of prostate cancer. This review presents an historical synopsis of various genetically engineered mouse (GEMs) lines of prostate cancer, highlighting their strengths and weaknesses, and future directions for research in the field, with an emphasis on the opportunities offered by temporally-controlled targeted somatic mutagenesis in the prostate to generate mouse models faithfully mimicking prostate carcinogenesis in humans.

2. Prostate cancer mouse models generated by targeted protein overexpression

The mouse genetic tools developed during the late 70s led to the establishment of a number of transgenic mice that express oncoproteins. Transgene expression in the mouse prostate became possible with the characterization of

Table 2 – Examples of prostate cancer GEM.

Mouse line	HGPIN latency	Invasive tumors (latency)	Incidence of metastasis	Site of metastasis	Latency of metastasis	References
TRAMP	3 months	Neuroendocrine carcinoma (4–7 months)	About 100%	Lymph node, lung, adrenal gland, bone (low penetrance)	4–9 months	(Gingrich et al., 1996; Greenberg et al., 1995)
ARR_2PB-c-Myc	<3 months	Adenocarcinoma (3–6 months)	0%	NA	NA	(Ellwood-Yen et al., 2003)
$PTEN^{+/-}$	8–10 months	None	0%	NA	NA	(Di Cristofano et al., 1998)
$PTEN^{+/-}$/ $Nkx3.1^{-/-}$	>6 months	(> 6 months)	25%	Lymph node	>1 year	(Abate-Shen et al., 2003; Kim et al., 2002b)
PB-Cre4/ $PTEN^{L2/L2}$	6–9 weeks	Adenocarcinoma (3 months)	50%	Lymph node, lung	3 months	(Wang et al., 2003)
PB-Cre4/ $Trp53^{L2/L2}/Rb^{L2/L2}$	Not reported	Adenocarcinoma; neuroendocrine differentiation. (< 6 months)	70%	Lymph node, liver, lung, adrenal gland	<7 months	(Zhou et al., 2006)
PB-Cre4/$PTEN^{L2/L2}$/ $SMAD4^{L2/L2}$	>2 months	Adenocarcinoma (< 3 months)	100%	Lymph node and lung	<8 months	(Ding et al., 2011)
PB-Cre4/$PTEN^{L2/L2}$/ $Trp53^{L2/L2}/SMAD4^{L2/L2}$	<4 months	(< 4 months)	> 10%	Bone metastasis	<4 months	(Ding et al., 2012)
PSA-Cre-$ER^{T2}/PTEN^{L2/L2}$	8–10 months	Adenocarcinoma (14–16 months)	0%	NA	NA	(Ratnacaram et al., 2008)

transcriptional regulatory elements of genes expressed in the prostate.

Promoter/enhancer regions of the rat C3(1) gene, that encodes a subunit of the prostatic steroid-binding protein, a major secretory protein of the rat ventral prostate, direct transgene expression in prostatic epithelial cells of transgenic mice. Transgene expression is however also found in seminal vesicle and testis, as well as in thyroid, salivary gland and cartilage of some lines (Allison et al., 1989; Maroulakou et al., 1994; Zhang et al., 1997, 2000b) (Table 1). Various promoter/enhancer regions of the androgen sensitive rat probasin gene also enabled the development of transgenic lines that express proteins in the prostatic epithelium (Greenberg et al., 1995, 1994; Kasper et al., 1994; Yan et al., 1997) (Table 1). A small (−426 bp to +28 bp) rat probasin DNA segment (also called Pb), encompassing two androgen receptor response elements (ARE), is sufficient to direct transgene expression to the prostate epithelium (Greenberg et al., 1995, 1994). However, the levels are low and not strictly restricted to the prostate epithelium, with some expression occurring in the seminal vesicles. By using a larger (−11.5 kb to +28 bp) DNA segment of this gene (also called LPB), expression level is often higher in the prostate epithelium, but also occurs in the seminal vesicles, and starts before puberty (Yan et al., 1997). To obtain high levels of expression in the prostate and circumvent the use of a large transgenic construct, a modified minimal probasin promoter was engineered by fusing the small rat probasin promoter (−426 bp to +28 bp) with a segment of its enhancer region encompassing the two AREs. The resulting chimeric ARR_2PB promoter ensures high transgene expression level in the mouse prostatic epithelium and is strongly regulated by androgens (Wu et al., 2001; Zhang et al., 2000a). Expression starts in the prostate bud of newborn mice, reaches high levels in the developing prostate before puberty, and is also detected in the prostate stroma, the seminal vesicles and the testes (Song et al., 2002; Wu et al., 2001). Finally, a 6 kb promoter/enhancer segment of the human PSA gene allows the expression of high level of transgenes in the mouse prostatic luminal epithelium after puberty (Cleutjens et al., 1997) (Table 1).

The first reported transgenic mouse line for prostate cancer [C3(1)-Tag] has been established by expressing, under the control of the aforementioned C3(1) promoter, the simian virus (SV) 40 large T antigen (Tag), a viral oncogene encoded by the SV 40 early sequence, known to inactivate critical cellular tumor-suppressor proteins (e.g. p53 and Rb) (Maroulakou et al., 1994). Male C3(1)-Tag transgenic mice develop prostatic hyperplasia in the DP and VP by 2–3 months of age, PINs by 6 months and adenocarcinoma by 7–11 months. Rare prostate cancer metastases to lung have been reported. T antigen expression is also observed in other tissues, including the thyroid, salivary gland and cartilage, and induces lethal lesions in these tissues by 1 year (Shibata et al., 1998), thereby limiting the use of these transgenic mice.

To restrict SV40 T antigen expression to the prostate, various versions of the probasin transcriptional regulatory elements have been used. The TRAMP (transgenic adenocarcinoma mouse prostate) model was developed by linking the (−426 bp to +28 bp) rat probasin promoter region to the SV40 early region (Greenberg et al., 1995). TRAMP mice express the T antigen oncoprotein in the dorso-lateral lobe (DLP) and VP. When maintained on a C57BL/6 genetic background, all male TRAMP mice develop PINs between 2 and 3 months of age

and progression to poorly differentiated neuroendocrine carcinoma occurs by 4–7 months (Gingrich et al., 1996; Kaplan-Lefko et al., 2003). Distant metastases are primarily present in lymph nodes and lung, and occasionally in the liver, kidney and adrenal glands by 4 to 9 months (Table 2). TRAMP mice on a mixed C57BL/6/FVBn genetic background exhibit increased tumor incidence compared to the parental strain, and develop bone metastasis at low frequency. Importantly, and in contrast to C3(1)-Tag transgenic mice, TRAMP mice do not develop other primary pathologies. Moreover, several LPB-Tag mouse lines were generated by expressing the SV40 large T antigen under the control of the large (−11.5 kb to +28 bp) rat probasin promoter DNA segment (Kasper et al., 1998). The rate of prostate tumor growth varies among individual lines, likely due to different levels of transgene expression, but all transgenic males develop PINs by 10 weeks of age. LPB-*Tag* lines that display a rapid prostate enlargement (e.g. 12T-7f) develop HGPINs and stromal hypercellularity. The large size of the prostate precluding the maintenance of most mice to later ages, local microinvasion and metastases are rarely seen. In contrast the LPB-*Tag* line 12T-10, that shows the slowest prostate tumor growth, develops HGPINs without associated prominent stromal hypercellularity and develops invasive adenocarcinoma, as well as neuroendocrine prostate carcinoma, that commonly metastasize to regional lymph nodes, liver and lung (Masumori et al., 2001). Thus, tumor development in these mice is similar to that in TRAMP mice, even though progression is slower.

TRAMP and LPB-Tag mice have been used by many investigators for pre-clinical testing of chemoprevention strategies and to identify pathways involved in prostate cancer initiation and progression (Ahmad et al., 2008; Kasper and Smith, 2004; Klein, 2005), even though they present the following drawbacks: (i) prostate tumor formation is driven by viral proteins that are not involved in the generation of human prostate cancers. (ii) they develop neuroendocrine carcinoma that rarely occur in human prostate cancer. Thus these models are relevant only to a small subpopulation of prostate cancer patient. (iii) T antigen is expressed in the prostate at the first week(s) of life at a low level, and thus might affect the development of this organ. (iv) the activity of the promoter driving T antigen expression is regulated by androgen at adulthood, thus effects observed in hormone ablation experiments might result from decreased transgene expression.

To circumvent some of these problems, transgenic mice overexpressing in the prostate wild-type or modified prostatic proteins rather than viral oncoproteins were generated. To determine whether chronic overexpression of the anti-apoptotic protein bcl-2 might affect the development and/or progression of prostate cancer, C(3)1-bcl-2 and Pb-bcl-2 transgenic mice, that express the human bcl-2 protein under C(3)1 regulatory elements and the (−426 bp to +28 bp) probasin promoter/enhancer region, respectively, were established (Bruckheimer et al., 2000; Zhang et al., 1997). C(3)1-bcl-2 transgenic mice develop an unusual form of neoplasia in the VP characterized by increased number of both epithelial and stromal cells, that resembles human benign prostatic hyperplasia (BPH). They do however not develop anaplastic transformation of prostatic cells even at advanced age (Zhang et al., 1997). In contrast, Pb-bcl-2 transgenic mice do not exhibit hyperplastic and neoplastic growth (Bruckheimer et al., 2000). Thus, bcl-2 overexpression in prostatic epithelial cells has little impact on tumor initiation. The basis for the phenotypic difference between C3(1)-bcl-2 and Pb-bcl-2 transgenic mice might reflect differences in timing and/or level of bcl-2 expression, or of genetic background.

The transcription factor c-Myc, known to regulate cell proliferation and apoptosis, is frequently overexpressed or amplified in prostate cancer (Fleming et al., 1986; Jenkins et al., 1997; Nesbit et al., 1999; Qian et al., 1997; Sato et al., 1999). To determine the consequence of c-Myc overexpression in the prostate, C3(1)-c-Myc, ARR_2PB-Myc and probasin-Myc transgenic mouse lines, that express c-myc under the control of the rat C3(1), the rat probasin ARR_2PB and the (−426 bp to +28 bp) rat probasin promoter/enhancer elements, respectively, were generated (Ellwood-Yen et al., 2003; Zhang et al., 2000b). Overexpression of c-Myc in the ventral prostate epithelial cells of C3(1)-c-Myc transgenic mice induces LGPINs that do not progress to adenocarcinoma over the lifetime of the mice. The use of these mice is limited by loss of reproductive capacity within five generations, which most likely results from transgene expression in male and female reproductive tissues (Zhang et al., 2000b). ARR_2PB-Myc and Pb-Myc express c-Myc in the prostate as early as at 2 weeks of age, but at higher levels than in the former (Ellwood-Yen et al., 2003). PIN lesions appear from 2 weeks of age in ARR_2PB-Myc mice, and these lesions progress to invasive adenocarcinomas by 3–6 months of age with a reliable kinetics. Lesions occur at full penetrance in the VP and DLP, and at a lower extent in the AP. These transgenic mice do however not present metastases (Table 2). Pb-Myc mice develop similar pathological changes, but with a slower kinetics. As ARR_2PB-Myc and Pb-Myc mice express a non-viral oncogene and develop invasive adenocarcinoma and not neuroendocrine carcinoma, they offer advantages over those expressing SV40 T antigen. However, they do not develop metastasis.

Transgenic mouse lines that overexpress growth factors (e.g. IGF-1, FGF-7, FGF-8b) (Foster et al., 2002; Kaplan-Lefko et al., 2008; Song et al., 2002), growth factor receptors [e.g. chimeric FGFR [iFGFR-1 (JOCK1 line) and iFGFR-2] that bind a lipid-permeable chemical inducer of dimerization instead of its cognate FGF ligand (Freeman et al., 2003), or a dominant negative truncated FGFR2 splice variant (Foster et al., 2002)], kinases [e.g. expressing a constitutively activated form of AKT (myr-Akt) (MAKT line) (Majumder et al., 2003), transcription factors encoded by genes that are rearranged in human prostate cancer (e.g. ETV1 and ERG) (King et al., 2009; Tomlins et al., 2007) or subunits of ubiquitin E3 ligase complexes (e.g. SKP2) (Shim et al., 2003) that might be involved in prostate cancer initiation or progression, were generated using either the ARR_2PB or the (−426 bp to +28 bp) rat probasin promoter enhancer elements. These mouse lines develop epithelial hyperplasia and PINs, except for ARR_2PB-ERG and iFGFR-2 mice, thus providing evidence that overexpression of many single proteins can initiate prostatic neoplasia. Progression to adenocarcinoma was only reported for the JOCK1 line (Acevedo et al., 2007). Note however, that in numerous studies, only a limited number of aged mice was analyzed [e.g. (Tomlins et al., 2007)], and that full development of prostate cancer could not always be determined, as bladder

obstruction required euthanasia (e.g. MAKT line) (Majumder et al., 2003).

To identify cooperating genetic events that might modulate pathogenesis of prostate cancer, transgenic mouse lines have been intercrossed to generate mice overexpressing several proteins in the prostate. For instance, prostate tumor formation is accelerated in TRAMP mice bearing the Pb-bcl-2 transgene, providing evidence that enhanced bcl-2 expression contributes to prostate carcinogenesis (Bruckheimer et al., 2000). The incidence of metastases is however not affected. Moreover, even though Pb-Des transgenic mice which express Insulin-like growth factor-1 (IGF-1) under the control of the (−426 bp to +28 bp) rat probasin regulatory elements develop PINs (Kaplan-Lefko et al., 2008), cancer progression is reduced in TRAMP/Pb-Des mice, indicating that the IGF signaling exerts differential effects on epithelial cell and tumor growth. In addition, whereas chemical inhibition of mTOR efficiently reverses PIN formation in MAKT mice, it only induces a partial regression in MAKT/Pb-bcl-2 double transgenic mice, as bcl-2 blocks the induction of apoptosis triggered by mTOR inhibition (Majumder et al., 2004). In contrast, MAKT/ARR$_2$PB-Myc bitransgenic mice exhibit accelerated progression of PINs to microinvasive adenocarcinoma, and are resistant to mTOR inhibitors (Clegg et al., 2011).

Taken together, transgenic mice overexpressing proteins in prostatic epithelium have provided important insights into prostate cancer initiation and progression, and results gained from these studies have important implications for clinical studies. Their major limitations remain expression of active transgenic protein before prostate maturation, except for the lines expressing chimeric FGFR, as their activity is selectively induced by an exogenous ligand, as well as androgen-regulated expression, which might confound interpretation of tumor regression and androgen-independent growth after castration.

3. Prostate cancer mouse models generated by loss of function mutations

The generation of knock-out mice, in which a given gene is ablated in all their cells (Capecchi, 1994), opened new avenues to study the impact of genetic alterations found in human cancer, by introducing the corresponding modification in the mouse genome.

Deletion or mutation of PTEN (phosphatase and tensin homolog deleted on chromosome 10) gene, that encodes a lipid phosphatase, the major function of which is the dephosphorylation of phosphatidylinositol-3-phosphate leading to down-regulation of the Akt/PKB pathway, is one of the most frequent genetic alterations in human prostate cancer. Bi-allelic ablation of PTEN in mice results in embryonic lethality, thus precluding the analysis of PTEN function in prostate cancer using null mutants. However, heterozygous mutant (PTEN$^{+/-}$) mice develop neoplasia in multiple tissues, including intestines, lymphoid cells, mammary gland, thyroid, endometrial and adrenal glands (Di Cristofano et al., 1998; Podsypanina et al., 1999; Stambolic et al., 2000; Suzuki et al., 1998), and most of them die within 8 months from massive lymphosplenomegaly. PTEN$^{+/-}$ survivor exhibit PINs by 8–10 months of age, but no invasive adenocarcinoma (Table 2). As the gene encoding the homeobox transcription factor *NKX3.1* maps to a region of chromosome 8p21 which undergoes allelic deletion in ~ 80% of prostatic neoplasia, mice with Nkx3.1 null-alleles were generated. *Both Nkx3.1$^{+/-}$ and Nkx3.1$^{-/-}$* mice develop PINs by one year of age (Bhatia-Gaur et al., 1999; Kim et al., 2002a), and the occurrence of PINs in *Nkx3.1$^{+/-}$* mice is concomitant with loss of Nkx3.1 protein expression (Kim et al., 2002b), as seen in human cancer (Bowen et al., 2000).

To investigate the contribution of genetic alterations that modulate PINs formation and/or promote cancer progression in PTEN$^{+/-}$ mice, compound GEM models have been generated. In agreement with AKT activation in PTEN-deficient prostate epithelial cells, AKT deficiency strongly reduces tumor development in PTEN$^{+/-}$ mice, and even AKT haplodeficiency attenuates HGPIN formation (Chen et al., 2006).

In contast, ablation of various tumor suppressors in PTEN$^{+/-}$mice enhances prostate tumorigenesis. The Ink4a/Arf locus encodes two distinct tumor suppressors, p16INKa and p19ARF, which are regulators of the pRB and p53 pathways, respectively. Inactivation of INK4a/ARF is known to be associated with a variety of human cancers (Burri et al., 2001; Sharpless and DePinho, 1999). PINs develop at younger age in Pten$^{+/-}$/Ink4a/Arf$^{+/-}$ mice than in Pten$^{+/-}$ mice and the onset is further accelerated in Pten$^{+/-}$/Ink4a/Arf$^{-/-}$ compound mice (You et al., 2002). However, progression to invasive adenocarcinoma is not observed in these mouse models. Even though p27^{Kip1} expression is frequently downregulated in human prostate cancer, the prostate of mice p27-null mice exhibits no pathological changes (Kiyokawa et al., 1996; Nakayama et al., 1996). However, Pten$^{+/-}$/p27$^{-/-}$ mice develop prostate carcinoma within 3 months with complete penetrance, which become invasive thereafter, but no metastases (Di Cristofano et al., 2001). The latency of PIN development is also reduced in Pten$^{+/-}$/Nkx3.1$^{+/-}$ mice (Kim et al., 2002b), and the incidence of HGPINs, invasive adenocarcinoma and lymph node metastasis is increased (Abate-Shen et al., 2003). Interestingly, NKX3.1 and PTEN proteins are not expressed in focal areas of tumors, but whereas the second allele of PTEN is lost, the coding region of that of Nkx3.1 is not mutated (Kim et al., 2002b).

Taken together, these results indicate a cooperative role of PTEN, p16INKa and p19ARF, p27 and Nkx3.1 in prostate tumor initiation and progression. Interestingly however, even though the incidence of HGPINs and/or adenocarcinoma is higher in *Pten$^{+/-}$/Nkx3.1$^{+/-}$/p27$^{+/-}$* and *Pten$^{+/-}$/Nkx3.1$^{-/-}$/p27$^{+/-}$* than in *Pten$^{+/-}$/Nkx3.1$^{+/-}$* and *Pten$^{+/-}$/Nkx3.1$^{-/-}$* mice, *Pten$^{+/-}$/Nkx3.1$^{+/-}$/p27$^{-/-}$* and *Pten$^{+/-}$/Nkx3.1$^{-/-}$/p27$^{-/-}$* develop less HGPINs and/or adenocarcinoma (Gao et al., 2004), indicating that *p27^{kip1} positively and negatively* controls prostate cancer progression in a dosage-dependent manner.

4. Compound transgenic and knock-out mouse models of prostate cancer

By intercrossing mice bearing null alleles with TRAMP mice, several factors and/or pathways that modulate prostate tumorigenesis have been identified.

Table 3 – Mouse lines expressing Cre recombinase in prostatic epithelial cells.

Mouse line	Transcriptional regulatory elements	Cre recombinase activity	Ectopic Cre expression	References
Pb-Cre	Rat probasin (−426 bp to +28 bp)	VP > DP, AP, LP	Bladder, seminal vesicles	(Maddison et al., 2000)
PB-Cre4	ARR_2PB	LP > VP, DP, AP, starting before puberty	Prostatic basal and stromal cells; seminal vesicles, seminiferous tubules	(Wang et al., 2006; Wu et al., 2001)
ARR_2PBi-Cre	ARR_2PB	LP, VP, DP, AP	Seminal vesicles, ductus deferens	(Jin et al., 2003)
PSA-Cre	PSA (6 kb)	All lobes of mature prostate	None reported	(Abdulkadir et al., 2002)
PSA-Cre	PSA (6 kb)	All lobes of mature prostate	None reported	(Ma et al., 2005)

The early growth response protein 1 (EGR1) transcription factor, overexpressed in a majority of human prostate carcinoma, regulates the expression of genes important for prostate tumor progression. Ablation of Egr1 in TRAMP mice delays progression from PINs to invasive carcinoma (Abdulkadir et al., 2001). The steroid receptor co-activator-3 (SRC-3), a co-regulator of a number of transcription factors including the androgen receptor, is overexpressed in prostatic luminal cells of TRAMP mice during cancer progression (Tien et al., 2009). TRAMP mice bearing SRC-3 null alleles develop PINs and early-stage carcinoma, but rarely poorly differentiated carcinoma (Chung et al., 2007). By ablating the two alleles encoding the ubiquitin ligase Siah2 in TRAMP mice, the formation of neuroendocrine prostate tumors and metastases is strongly reduced (Qi et al., 2010). Caveolin-1, a major structural component of flask-shaped plasma membrane invaginations called caveolae, is implicated in many cellular processes, and has tumor suppressor properties in various tissues (Glenney, 1989). Invalidation of one or both Cav-1 alleles in TRAMP mice attenuates the progression of prostate carcinoma and decreases metastasis to distant organs (Williams et al., 2005). Thus, various signaling pathways promote tumorigenesis in TRAMP mice.

TRAMP mice bearing a germ-line mutation that inactivates the growth hormone (GH) releasing hormone receptor (GHRH-R) have low GH and IGF serum levels. Even though they develop a higher number of PINs, progression toward prostate cancer is reduced (Majeed et al., 2005). These results contrast with those of Kaplan-Lefko et al. showing that transgenic overexpression of IGF in luminal prostatic cells (Pb-Des mice) induces PINs, and that progression of organ-confined tumors and emergence of metastatic lesions is delayed in young TRAMP/Pb-Des mice (Kaplan-Lefko et al., 2008), and with those of DiGiovanni et al. showing that overexpression of IGF-1 in various epithelia, including basal epithelial cells of the prostate using a bovine K5 promoter region, induces PINs and adenocarcinoma (DiGiovanni et al., 2000). Thus, complex GH and/or IGF-1 signaling pathways influence prostate carcinogenesis.

The aryl hydrocarbon receptor (AhR) is a transcription factor that binds 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), a compound that might promote prostate cancer (Akhtar et al., 2004; Fritz et al., 2005). However, prostate tumor incidence is increased in TRAMP mice in which one AhR allele is ablated, and the frequency of tumor formation is further enhanced by bi-allelic AhR ablation (Fritz et al., 2007). Moreover, the rate of prostate cancer progression is increased in TRAMP mice in which one PTEN allele is ablated (Kwabi-Addo et al., 2001). In addition, overexpression of ERG in the prostate of $PTEN^{+/-}$ mice accelerates PIN formation and induces progression to invasive carcinoma at 6 months of age (Carver et al., 2009; King et al., 2009).

Taken together, the analysis of transgenic, KO and compound mutant mice indicate that many signaling pathways positively and negatively control prostate cancer initiation and/or progression. However, results gained from KO mice in which gene(s) are ablated in all cells and from mice that overexpress transgenic proteins in a large number of prostatic cells, and/or before complete prostate development, might induce complex alteration of the prostate tissue and physiology, and thus do not faithfully mimic focal mutations in given cell types of the prostate that might occur in human prostate cancer.

5. Generation of prostate cancer mouse models through targeted somatic mutations

The development of techniques to introduce targeted somatic mutations in the mouse allowed to overcome some of the limitations of conventional gene knockout. They rely on site-specific recombinases that efficiently catalyze recombination of DNA segments flanked by cognate recognition sites. The most widely used conditional system is based on the bacteriophage P1 Cre recombinase which recognizes DNA elements called LoxP sites, and induces recombination between two of them, resulting in deletion or inversion of the intervening sequence (Sternberg et al., 1981). Conditional inactivation of a gene is generally achieved by flanking, via homologous recombination in ES cells, one or several exons of interest with LoxP sites (floxed allele; L2). By expressing Cre recombinase in a cell/tissue specific manner, using appropriate

promoters, the targeted exon(s) are selectively excised in a spatially-controlled manner (Gu et al., 1994).

To express Cre recombinase in the prostatic epithelium, its coding sequence was first inserted downstream of the (−426 bp to +28 bp) rat probasin promoter/enhancer segment (Maddison et al., 2000). Even though Cre expression is higher in the VP of the resulting Pb-Cre line, Cre-mediated excision occurs in all lobes (Table 3). However, as Cre is also expressed at low levels in the bladder and seminal vesicles (Maddison et al., 2000), recombination might also occur in these tissues. Two additional transgenic mouse lines, PB-Cre4 and ARR_2PBi-Cre, expressing Cre under the control of the ARR_2PB promoter/enhancer regions were established (Jin et al., 2003; Wu et al., 2001). Recombination efficiency is higher in luminal epithelial cells of LP of young adult PB-Cre4 mice than in the VP, DP and AP. As Cre is expressed at birth in the developing prostate, and throughout the life of these mice, the number of cells undergoing recombination increases with time (Wu et al., 2001). In addition, Cre activity is present in basal and stromal cells of the prostate, in seminal vesicles, as well as in seminiferous tubules (Wang et al., 2006; Wu et al., 2001). The ARR_2PBi-Cre line exhibits efficient recombination in epithelial cells of all prostatic lobes, as well as in seminal vesicle and ductus deferens (Jin et al., 2003). Moreover, two PSA-Cre transgenic mouse lines expressing Cre under the control of the 6 kb promoter/enhancer region of the human PSA gene were independently established (Abdulkadir et al., 2002; Ma et al., 2005). Cre-mediated recombination in these lines is restricted to luminal epithelial cells of mature prostate, in contrast to Probasin-based transgenic Cre lines (Table 3).

MMTV-Cre transgenic mice, that express Cre recombinase under the control of the mouse mammary tumor virus (MMTV) long terminal repeat (Wagner et al., 2001), and Nkx3.1-Cre mice generated by inserting Cre into the Nkx3.1 locus via homologous recombination in ES cells (Stanfel et al., 2006), also induce Cre-mediated recombination in prostatic epithelium. However, as these lines express Cre in a number of cell types during development and exhibit strong Cre activity in organs such as seminal vesicles, vas deferens, epididyme, skin, salivary gland and kidney, lung, intestine, respectively (Bierie et al., 2003; Gounari et al., 2002; Stanfel et al., 2006; Wagner et al., 2001), and as one Nkx3.1 allele is ablated in Nkx3.1-Cre mice, their use to generate prostate cancer models is limited.

Transgenic Cre lines were extensively used to determine the impact of loss of function of various genes in the prostate, and allowed for the generation of new cancer-prone GEMs. Mice in which PTEN is ablated in the prostate were generated in various laboratories by intercrossing mice bearing floxed (L2) PTEN alleles with Pb-Cre, PB-Cre4, PSA-Cre and MMTV-Cre mice (Backman et al., 2004; Ma et al., 2005; Trotman et al., 2003; Wang et al., 2003, 2006). All lines develop HGPINs and invasive carcinoma, but tumorigenesis is markedly accelerated and its penetrance increased in PB-Cre4/$PTEN^{L2/L2}$ (also called $PTEN^{pc-/-}$) (Table 2) and MMTV-Cre/$PTEN^{L2/L2}$ mice in which PTEN is ablated before full development of the gland. Moreover, metastasis to lymph nodes occurs in $PTEN^{pc-/-}$ and PSA-Cre/$PTEN^{L2/L2}$ mice, but only the former develop lung metastasis (Ma et al., 2005; Wang et al., 2003). Differences in genetic background, variable efficiencies of PTEN ablation in luminal epithelial cells and/or PTEN ablation in prostatic basal and stromal cells in some lines might account for variation in tumor promotion and aggressiveness amongst the lines.

PB-Cre4/$PTEN^{L2/L2}$ mice were crossbred with many GEMs to characterize signaling pathways that modulate prostate tumor formation. Even though PB-Cre4/$mTOR^{L2/L2}$ mice ($mTOR^{pc-/-}$), in which both alleles of the AKT target mTOR are ablated in the prostate, do not exhibit any pathological defects in the prostate, PTEN/$mTOR^{pc-/-}$ mice in which PTEN and mTOR are ablated in mouse prostate, develop less PINs than $PTEN^{pc-/-}$ mice, and no invasive carcinoma (Nardella et al., 2009). Similarly, concomitant ablation in the prostate of PTEN and Rictor, a component of the mTOR complex 2, protects cells from transformation by PTEN (Guertin et al., 2009). Thus, mTORC2 inhibitors might have clinical applications to counteract AKT activation. Moreover, the incidence of HGPINs is decreased in $PTEN^{pc-/-}$ mice bearing p19Arf null alleles (Chen et al., 2009), in contrast to $PTEN^{+/-}$/Ink4a/$Arf^{-/-}$ mice (You et al., 2002).

Mice expressing Cre selectively in the prostate are also valuable tools for conditional transgene expression in this tissue. For instance, to overexpress Bmi, a core component of the polycomb repressive complex 1 (PRC1), in the prostate of mice bearing a hemizygous PTEN mutation in prostatic epithelial cells, PB-Cre4/$PTEN^{L2/+}$ mice were bred with BmiLSL transgenic mice in which Bmi is expressed after Cre-mediated deletion of a floxed stop cassette (Nacerddine et al., 2012). PB-Cre4/$PTEN^{L2/+}$/BmiLSL offsprings develop PINs with a shorter latency than PB-Cre4/$PTEN^{L2/+}$ and PB-Cre4/BmiLSL mice, and invasive adenocarcinoma, in contrast to mice bearing a monoallelic PTEN ablation or only Bmi overexpression, thus indicating that Bmi cooperates with PTEN haploinsufficiency in invasive postate cancer.

$PTEN^{pc-/-}$ mice bearing floxed alleles of c-jun NH2-terminal kinases (JNK) 1 and 2 or of MKK4 and MKK7, that encode two kinases that activate JNK, develop HGPINs and invasive adenocarcinoma earlier than $PTEN^{pc-/-}$ mice. In addition, lymph node metastasis occur in PTEN/JNK1/$JNK2^{pc-/-}$ mice, but not in $PTEN^{pc-/-}$ mice at a similar age (Hubner et al., 2012), thus demonstrating that JNK and PTEN signaling pathways negatively control tumorigenesis in a cooperative manner.

Chen et al. have shown that PB-Cre4/$Trp53^{L2/L2}$ ($Trp53^{pc-/-}$) mice, in which the tumor suppressor gene p53 is ablated in the prostate, do not exhibit any pathological defects in the prostate, while combined inactivation of PTEN and Trp53 in the prostate results in HGPINs and invasive carcinoma formation in 10 week-old PTEN/$Trp53^{pc-/-}$ mice, indicating that p53 loss accelerates the progression of tumors initiated by the loss of PTEN (Chen et al., 2005). $PTEN^{pc-/-}$/$Trp53^{pc-/-}$ mice do not develop distant metastases and die at around 7 months of age, due to bladder obstruction and renal failure. In contast, Zhou et al. reported that aged PB-Cre4/$Trp53^{L2/L2}$ and PB-Cre4/$Rb^{L2/L2}$ develop PINs, and that PB-Cre4/$Trp53^{L2/L2}$/$Rb^{L2/L2}$ mice exhibit invasive carcinoma and metastasis in regional lymph nodes, liver, lung and adrenal gland (Zhou et al., 2006). Concomitant ablation of PTEN and SMAD4 in the prostate also induces invasive carcinoma before 3 month of age,

and death by 8 month due to bladder obstruction, whereas PB-Cre4/SMAD4$^{L2/L2}$ (SMAD4$^{pc-/-}$) mice do not exhibit prostate neoplasia beyond 2 years of age (Ding et al., 2011). The incidence of lymph node and lung metastasis is markedly increased in PTEN/SMAD4$^{pc-/-}$ mice compared to PTEN$^{pc-/-}$ mice. Remarkably, PTEN/Trp53/SMAD4$^{pc-/-}$ mice develop even more aggressive prostate tumor, as well as bone metastasis in about 12% of mice at 4–5 months of age (Table 2) (Ding et al., 2012). Telomerase reactivation, by Cre-mediated deletion of LoxP-flanked stop cassette located in the telomerase reverse transcriptase allele, in the prostate of PTEN/Trp53$^{pc-/-}$ mice that had telomere dysfunction, also generates aggressive tumors and skeletal metastases in 25% of them before 6 months of age. Thus, ablation of PTEN, Trp53 and SMAD4 in prostate or reactivation of telomerase activity in prostatic cells deficient for PTEN and Trp53 is sufficient to induce prostate tumors with bone metastases, and mimics prostate cancer progression in men.

The PB-*Cre4* mouse line was also crossbred with mice bearing floxed alleles of the nuclear receptor RXRα and its heterodimeric partner PPARγ, to generate RXR$\alpha^{pc-/-}$ and PPAR$\gamma^{pc-/-}$ mice (Huang et al., 2002; Jiang et al., 2010). Both lines develop LGPINs between 3 and 5 months and HGPINs at later stage, but no invasive carcinoma. Oxidative stress induced by PPARγ loss-of-function induces lysosomal autophagy which might contribute to malignant progression. Note however that tumor incidence, latency and disease progression of TRAMP mice was not affected by PPARγ haploinsufficiency (Saez et al., 2003).

Deletion of the β-catenin regulator APC, mediated by *PB-Cre4*, results in elevated levels of β-catenin protein in the prostate, and induces hyperplasia as early as 4.5 weeks of age (Bruxvoort et al., 2007). Adenocarcinoma are formed by 7 months, and the large size of the tumors requires euthanasia of the mice between 12 and 15 months of age. Moreover, to express a dominant stable form of β-catenin in prostate, mice bearing a floxed exon 3 Catnb allele were bred with PB-Cre4 mice. The resulting *PB-Cre4/Catnb*$^{+/\Delta ex3}$ mice display HGPIN lesions after 3 months of age and adenocarcinoma by 7 months, which become locally invasive with time. MMTV-Cre mice bearing such Catnb floxed alleles develop HGPINs in all prostatic lobes after 10 weeks (Gounari et al., 2002), indicating that β-catenin stabilization might be a crucial event for initiation of PIN lesions in the prostate. Similar mice generated by another group developed however only squamous metaplasia at 8–10 weeks, and older mice could not be analyzed, as activation of β-catenin in other cell types affected their viability (Bierie et al., 2003). This discrepancy might result from subtle genetic background differences. To determine whether the Wnt/β-catenin and K-ras pathways synergize during prostatic tumorigenesis, PB-Cre4 mice were bred with mice harboring a conditional allele from which oncogenic K-rasV12 is expressed after Cre-mediated deletion of floxed stop sequences (Pearson et al., 2009). After 6 months of age, about 60% PB-Cre4/K-rasV12 mice display LGPINs, but only few have adenocarcinoma. *PB-Cre4/K-rasV12/Catnb*$^{+/\Delta ex3}$ mutants exhibit however invasive carcinoma at earlier time and higher incidence than *PB-Cre4/Catnb*$^{+/\Delta ex3}$ *mice*, indicating that Ras and Wnt signaling cooperates to promote prostate tumorigenesis.

ARR$_2$PBi-Cre mice were successfully used to conditionally ablate IGF-IR in the prostate (Sutherland et al., 2008). Such mice develop focal hyperplasia in all lobes, but no carcinoma. Moreover, when bred with TRAMP mice, less differentiated aggressive tumors appear earlier. PSA-*Cre* transgenic mice were bred with mice bearing floxed *Nkx3.1* alleles. PSA-Cre/Nkx3.1$^{L2/+}$ mice develop PINs, in which the expression of the wild-type Nkx3.1 allele is lost (Abdulkadir et al., 2002). Thus, these results support a role of Nkx3.1 in tumor initiation, and confirm results observed in mice bearing germline *Nkx3.1*-null alleles.

Although most of the above mouse models of prostate cancer show that specific gene alterations can lead to prostate cancer in mice, pathological lesions develop in young animals and disease progression is often very fast, whereas in men, disease occurs in late adulthood and can extend over a period of many years. The introduction of genetic modifications in a large number of cells of the mouse prostate before its full development, and sometimes in various cell types could account for these differences. Thus, to accurately model sporadic prostate cancer, mutations should arise in a limited number of cells of the prostate after puberty.

Ligand-controlled site-specific recombinases, developed over the recent years allow to circumvent most of these limitations. They are based on fusion proteins between Cre or Flp recombinases and mutated ligand binding domains of nuclear receptors (Metzger and Feil, 1999). The most largely used chimeric recombinase to introduce spatio-temporally controlled targeted somatic mutations in the mouse is the Cre-ER^{T2} recombinase, a fusion protein between Cre and a mutated human estrogen receptor, the activity of which is selectively induced by the synthetic ligand Tamoxifen (Metzger and Chambon, 2001).

Four transgenic lines expressing ligand-dependent Cre recombinases in the prostate have been recently developed (Birbach et al., 2009; Luchman et al., 2008b; Ratnacaram et al., 2008; Wang et al., 2009) (Table 4). The PSA-Cre-ER^{T2} and ARR$_2$PB-CreER^{T2} transgenic lines express the

Table 4 – Mouse lines expressing tamoxifen-dependent Cre recombinases in prostatic epithelial cells.

Mouse line	Chimeric Cre recombinase	Transcriptional regulatory elements	Cre recombinase activity	References
PSA-Cre-ER^{T2}	Cre-ER^{T2}	PSA (6 kb)	Luminal cells: DLP, VP > AP	(Ratnacaram et al., 2008)
ARR$_2$PB-Cre-ER^{T2}	Cre-ER^{T2}	Probasin ARR$_2$PB	Luminal cells: AP, DLP, VP	(Luchman et al., 2008b)
Probasin-MerCreMer	MerCreMer	Probasin genomic region (Bac)	Luminal cells: AP, DLP > VP	(Birbach et al., 2009)
Nkx3.1$^{Cre-ERT2}$	Cre-ER^{T2}	Nkx3.1 (KI)	Luminal cells and 10% basal cells	(Wang et al., 2009)

Tamoxifen-dependent Cre-ER^{T2} recombinase from the 6 kb human PSA promoter, and the rat probasin ARR$_2$PB promoter, respectively (Luchman et al., 2008b; Ratnacaram et al., 2008). In addition, a transgenic line expressing the Tamoxifen-dependent MerCreMer chimeric recombinase under the control of the regulatory elements of the probasin gene located on a bacterial artificial chromosome was established (Birbach et al., 2009). These transgenic lines express the chimeric recombinases selectively in the prostatic luminal epithelial cells, and their recombinase activity is strictly tamoxifen-dependent. The level of recombination in the various lobes varies however amongst the lines. Whereas ARR$_2$PB-CreER^{T2} mice induce recombination at high efficiency in all lobes, PSA-Cre-ER^{T2} mice exhibit a high recombination efficiency in the DLP and VP (>up to 80%) and a lower efficiency in the AP (10–60%), and MerCreMer mice induce recombination in around 50% in AP and DLP, and 20% in VP. Interestingly, by lowering the Tamoxifen doses, the number of targeted epithelial cells is reduced, thus offering the possibility to modulate the number of focal genetic lesions and generate sporadic mutations in mature prostate at various ages (Birbach et al., 2009; Ratnacaram et al., 2008).

NKX3-1$^{Cre\text{-}ERT2}$ KI mice, in which the Cre-ER^{T2} recombinase is under the transcriptional control of the endogenous *Nkx3.1* promoter, were generated via homologous recombination in ES cells (Wang et al., 2009). Tamoxifen administration to these mice induces Cre-mediated recombination in most of luminal cells and in about 10% of basal cells (Table 4).

To evaluate the consequences of PTEN loss in luminal epithelial cells of adult mice, PTEN ablation was induced by Tamoxifen administration to adult PSA-Cre-ER^{T2} mice bearing PTEN L2 alleles. All such mutant mice develop PINs in all lobes within 2–3 months after Tamoxifen-induced PTEN ablation and adenocarcinoma within 8–10 months, but no distant metastases up to 20 months after PTEN ablation (Ratnacaram et al., 2008) (Table 2). Moreover, Luchman et al. have shown, using ARR$_2$PB-Cre-ER^{T2}/PTEN$^{L2/L2}$ mice, that PTEN inactivation before puberty results in much faster apparition of prostate carcinoma compared to PTEN inactivation at adulthood (Luchman et al., 2008a), showing that tumor development depends on the timing of PTEN ablation. Interestingly, monoallelic Cre-ER^{T2}-mediated PTEN ablation in epithelial cells of adult prostate also generates PINs, but in much lower number and after a longer latency, but no adenocarcinoma (Ratnacaram et al., 2008). The lack of PTEN protein expression in PINs of these mice demonstrates that loss of PTEN function is a permissive event for uncontrolled cell proliferation. Thus, Cre-ER^{T2} –mediated PTEN ablation in luminal cells of adult prostate mimics well early stages of prostate cancer formation in humans. These mice are valuable both for exploring the molecular mechanisms underlying prostate cancer and its progression and for the development and validation of preventive and therapeutic approaches in preclinical settings.

Another genetic system based on the tetracycline operator and reverse tretracycline transactivator (rtTA) has recently been developed to temporally control trangene expression in the prostate. Indeed, transgenic mice in which rtTA is placed under the control of the ARR$_2$PB promoter or Hoxb13 transcriptional regulatory elements located on a large genomic segment were generated, and doxycycline administration to such mice induces expression of trangenes located under the control of the tetracyclin operator in prostate epithelial cells (Rao et al., 2012; Wang et al., 2012). These genetic systems are useful to conditionally overexpress transgenes in the normal prostate or during diseases progression to determine their role, or site-specific recombinases to introduce genetic modifications. Combined with the Cre-ER^{T2} system, they will allow to conditionally express or mutate combinations of genes in a sequential manner.

6. Characterization of androgen signaling in the mouse prostate

AR signaling is essential for prostate development, and the conventional concept of the AR role in prostate cancer is to promote cancer progression (Shen and Abate-Shen, 2010). After androgen deprivation, AR activity might be affected by AR mutations or amplification, post-translational modifications, changes in AR and AR coregulator interactions, or growth factor/kinase signal pathways (Mostaghel et al., 2007; Visakorpi et al., 1995). Studies in cells in culture and xenografts indicate that AR might have distinct roles in various prostatic cells and/or at given prostate cancer stages. To investigate *in vivo* androgen signaling in prostate, GEM have been extensively studied. Even though castration induces a decrease of tumor burden in various mouse prostate cancer models, androgen-independent tumors often grow at later time (Abate-Shen et al., 2003; Ellwood-Yen et al., 2003; Gingrich et al., 1997). Epithelial cell proliferation and apoptosis is increased in the prostate of transgenic mice overexpressing AR under the control of the (−426 bp to +28 bp) probasin regulatory elements. Focal PIN lesions develop with time, and HGPINs are observed in aged mice only, but no adenocarcinoma (Stanbrough et al., 2001). Early expression of AR in the urogenital sinus epithelium during development and in prostatic basal and luminal epithelial cells of the prostate of Ors1-Cre mice bearing a transgene in which AR is expressed after excision of a floxed stop cassette, also induces PIN lesions, and adenocarcinoma at low frequency (Zhu et al., 2011). Thus, AR can function as a positive regulator of proliferation in normal prostate epithelium, and stimulate the development of neoplasia. The low penetrance of adenocarcinoma indicates that secondary genetic or epigenetic events are required to enhance AR-mediated oncogenic transformation of the prostate. Interestingly, overexpression of an AR mutant (E231G) with increased ligand-independent basal activity and increased responsiveness to coregulators, under the control of the (−426 bp to +28 bp) probasin promoter, causes rapid development of PINs, invasive carcinoma and lung metastases by 1 year of age (Han et al., 2005).

Specific ablation of AR in mouse prostatic epithelium in PB-Cre4 mice bearing floxed AR alleles (pes-ARKO mice) induces apoptosis of epithelial luminal cells and increased proliferation of epithelial basal cells (Wu et al., 2007). In contrast, ablation of AR in stromal smooth muscle cells using transgelin-Cre mice or in stromal fibroblasts, using FSP1-Cre mice, reduces the expression of various growth factors, including IGF-1, and decreases proliferation of epithelial cells in the prostate (Yu et al., 2012, 2011). These studies indicate that androgens

control prostate homeostasis through complex signaling pathways mediated by AR in both epithelial and stromal cells.

To inverstigate the role of AR in prostatic epithelial cells during cancer formation, AR was ablated in the prostatic epithelium of PB-Cre4/PTEN$^{L2/L2}$ mice. Proliferation of PTEN/AR-null cancer cells is stimulated by preventing AKT inhibition (Mulholland et al., 2011), indicating a cross-talk between AR and PI3K signaling pathways. Moreover, pes-ARKO mice were bred with TRAMP mice to generate PB-Cre4/AR$^{L2/y}$/TRAMP mice (pes-ARKO-TRAMP mice). Even though this Cre line has been shown to induce recombination of floxed alleles in epithelial basal cells, in pes-ARKO-TRAMP mice recombination was reported to be restricted to luminal cells (Niu et al., 2008a). Loss of AR in these cells increases apoptosis in epithelial luminal cells and proliferation of basal cells, leading to an expansion of CK5/CK8-positive basal intermediate cells, and increased size of primary and metastatic tumors in lymph nodes and more metastases in liver. Moreover, TRAMP mice in which AR is ablated in a temporally-controlled manner in the prostatic epithelium and stroma, as well as in many other cell types, were generated by breeding TRAMP/AR$^{L2/y}$ mice with Mx-Cre mice, resulting in ind-ARKO-TRAMP mice after induction of Cre expression by pIpC administration (Niu et al., 2008b). Ablation of AR in both stromal and epithelial cells after puberty reduces tumor size, but cells are less differentiated than those of TRAMP mice. The proliferation rate of basal-intermediate cells is lower in primary prostate tumors from ind-ARKO-TRAMP mice than from pes-ARKO-TRAMP mice. Moreover, ablation of AR in both epithelial and stromal cells of TRAMP mice leads to smaller and less aggressive metastatic tumors in lymph nodes than in TRAMP mice. Thus, these results indicate that prostate stromal AR may play a major stimulating role in primary prostate tumor growth and migration to distant tissues, and that selective targeting stromal AR at early stages of prostate cancer might improve the treatment of this cancer.

Replacement of threonine 877 to alanine (T877A) in human AR is a common mutation in prostate cancer. The transcriptional activity of this mutant receptor is induced by non-androgenic steroid hormones and AR antagonists. To determine the effect of this mutation on prostate cancer growth, the corresponding mutation was selectively introduced in epithelial luminal cells of adult mice (Takahashi et al., 2011). To this end, a mouse line was engineered in which the exons encoding the AR ligand binding domain encompassing T877 was replaced with the corresponding human coding sequence flanked by LoxP sites, and followed by a similar DNA segment in which the codon 877 was mutated to encode an Alanine. Thus, Tamoxifen administration of adult PSA-Cre-ER^{T2} mice bearing this AR allele induces excision of the DNA segment encoding T877 and fuses the duplicated segment encoding A877, thereby generating ARpe-T877A/Y mice, expressing the ART877A mutant selectively in prostatic luminal epithelial cells. Even though epithelial cell proliferation is increased in these mutant mice, no prostate tumors are formed. However, by introducing this mutation in TRAMP mice, the onset of tumor growth is accelerated, indicating that prostatic factors potentiate the effect of AR hyperfunction. As haploinsufficiency of Wnt-5a abrogated this effect, and Wnt-5a expression is increased in malignant human prostate tumors, a non canonical Wnt signaling might stimulate the development of prostatic tumors expressing hyperfunctional AR.

7. Use of GEM to identify stem cells in the prostate

The location of prostate stem cells and origin of prostate cancer stem cells has been controversial for many years. Several lines of evidence support the existence of a stem cell population in the basal epithelium of the prostate (Kasper, 2008; Lang et al., 2009; Leong et al., 2008; Visvader, 2011). The characterization of PB-Cre4/PTEN$^{L2/L2}$ mice indicates that basal like cells of PTEN-null prostate cancer contain the majority of tumor initiating activity (Mulholland et al., 2009). However, explants from Trp63-null mice can form prostate tissue and undergo several rounds of regression/regeneration in the absence of basal cells (Kurita et al., 2004), indicating that luminal cells might self-renew. Analyzes of PSA-Cre/PTEN$^{L2/L2}$ mice identified luminal epithelial progenitor cell that might initiate tumor formation after PTEN ablation (Korsten et al., 2009). The discrepancy between the results obtained with PB-Cre4/PTEN$^{L2/L2}$ and PSA-Cre/PTEN$^{L2/L2}$ mice might result from differences in the promoter activities driving Cre expression, the probasin promoter beeing active in multipotent progenitor cells, whereas PSA promoter/enhancer might be active in lineage-specific luminal progenitor cells. Moreover, elegant genetic lineage tracing based on PSA-Cre-ER^{T2}–induced prostatic luminal epithelial cell labeling in the adult prostate has shown that luminal epithelial cell can survive androgen deprivation and proliferate upon androgen replacement (Liu et al., 2011). In addition, rare castrate resistant luminal epithelial stem cells that express Nkx3.1 (CARN) were identified by cell labeling through Nkx3.1$^{Cre\text{-}ERT2}$ –mediated recombination (Wang et al., 2009). As androgen-induced regeneration of the prostate of castrated Nkx3.1$^{Cre\text{-}ERT2}$/PTEN$^{L2/L2}$ mice in which PTEN in selectively ablated in CARNs at adulthood induces a rapid formation of HGPINs and carcinoma with local invasion, CARN cells might be at the origin of prostate cancer. Interestingly, human CARN-like cells, that may represent the cell-of-origin of tumor reinitiation in castration-resistant prostate cancer, have been recently identified (Germann et al., 2012).

8. Conclusion and future directions

The genetic tools allowing to manipulate the mouse genome led within the past 20 years to the establishment of a number of mouse lines that provided tremendous insights in the signaling pathways involved in prostate cancer initiation and progression. Even though the first genetic models based on viral oncogene overexpression only partially mimicked the human disease, recent models, in which 3 endogenous genes (PTEN, Trp53 and Smad4) are concomitantly conditionally ablated in a the prostate epithelium, develop spontaneously aggressive prostate tumors and bone metastasis. Spatio-temporally controlled targeted somatic mutagenesis should further improve these models by generating more focal mutations at adult stage. Moreover, combinatorial use of various recombinases (Cre, Flp, Cre-ER^{T2}, Flp-ER^{T2}) (Branda and Dymecki, 2004; Metzger and Chambon, 2001)

and the tetracycline regulatory system (Rao et al., 2012; Wang et al., 2012) will allow to express or ablate various genes in different cell types in a temporally-controlled manner, and thus provide exciting opportunities to faithfully mimic focal gene disruptions during the multistep tumorigenesis process. The availability of such mouse models will be highly valuable to identify new biomarker of disease evolution and prognostic signatures, from prostate biopsies or in fluids (blood, urine), using massive sequencing, transcriptomic and/or proteomic and metabolomic approaches. An extensive characterization of the cellular and molecular mechanisms underlying castrate resistant prostate cancer and bone metastases in such GEMs, should provide new drug targets. Combined with powerful non-invasive imaging techniques (utrasound, magnetic resonance imaging, positron emission tomography and micro-CT scans) (Nardella et al., 2009; Nastiuk et al., 2007; Schoder and Larson, 2004; Zhang et al., 2009) or bioluminescent/fluorescent transgenic reporter mice (Ellwood-Yen et al., 2006; Hsieh et al., 2005; Iyer et al., 2005; Jiang et al., 2011; Liao et al., 2007; Lyons et al., 2006; Xie et al., 2004) GEMs will be very useful to perform longitudinal studies to test chemopreventive or selective therapeutic strategies that delay the onset or progression of prostate cancer. Results gained from such studies should allow to develop new diagnostic tools and effective treatments that would enhance survival and life quality of prostate cancer patients.

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