Journal of Cellular Biochemistry 94:279–297 (2005)

Survey of Genetically Engineered Mouse Models for Prostate Cancer: Analyzing the Molecular Basis of Prostate Cancer Development, Progression, and Metastasis

Susan Kasper*

Department of Urologic Surgery, Cancer Biology, Cell and Developmental Biology, the Vanderbilt Prostate Cancer Center and the Vanderbilt Ingram Cancer Center, Nashville, Tennessee

Abstract Genetically engineered mouse models have been generated to study the molecular basis of prostate cancer (PCa) development, progression, and metastasis. Selection of a prostate-specific promoter, such as the probasin (PB) and prostate specific antigen (PSA) promoters, is critical for generating sufficient levels of transgene expression to elicit a phenotypic response. To date, target genes have included growth factors, cell cycle regulators, pro- and anti-apoptotic proteins, steroid hormone and growth factor receptors, oncogenes, tumor suppressors, and homeobox genes. The experimental approaches used to generate these mouse models include overexpression of the transgene, knock-out/knock-in of transgene expression and conditional regulation of expression using Cre/lox technology. This review summarizes the promoters, which have been utilized to create genetically engineered mouse models for PCa. Furthermore, the effects of gene disruption on promoting low- and high-grade intraepithelial neoplasia (LGPIN and HGPIN, respectively), locally invasive carcinoma and metastatic lesions will be discussed. To date, the PB-Cre4 × $PTEN^{loxp/loxp}$ model appears to be the only model that represents the entire continuum of prostate adenocarcinoma development, tumor progression, and metastasis, although models that develop prostatic neuroendocrine (NE) cancer can be generated by disrupting one genetic event. Indeed, analysis of bigenic mouse models indicates that two genetic events are generally required for progression from HGPIN to locally invasive adenocarcinoma and that two to five genetic events can promote metastasis to distant sites. Studying the effects of genetic perturbation on PCa biology will increase our understanding of the disease process and potentially provide targets for developing novel therapeutic approaches. J. Cell. Biochem. 94: 279–297, 2005.

Key words: prostate cancer; probasin; PSA; epithelial cell-specific; PIN; adenocarcinoma metastasis; growth factors; SV40; cell cycle; homeobox; androgen

The development of prostate cancer (PCa) in humans is a multistep process, advancing through a number of stages to eventually metastasize to distant sites such as bone, lung, and liver. Developing mouse models for elucidating the mechanisms, which control early genetic changes, tumor progression, and metastasis presents a considerable challenge, since no individual model appears to reflect all of the changes observed in human PCa. The development of prostate disease in genetically engineered mice appears to fall into several categories. In some mouse models, the transgene induces prostate epithelial hyperplasia and stromal proliferation, thereby mimicking processes that are more analogous to the development of benign prostatic hyperplasia (BPH). In other models, the transgene induces marked epithelial cell proliferation, resulting in one or more layers of atypical cells also observed in human LGPIN. In models representing human HGPIN, epithelial cell growth progresses further and cells acquire large pleomorphic nuclei with prominent nucleoli, thereby filling the glandular lumen. In a limited number of mouse models, epithelial cells invade the fibro-

*Correspondence to: Susan Kasper, Vanderbilt University Medical Center, A-1302 MCN, 1161 21st Avenue South, Nashville, TN 37232-2765.
E-mail: susan.kasper@vanderbilt.edu

Received 17 September 2004; Accepted 20 September 2004
DOI 10.1002/jcb.20339

muscular sheath locally, suggesting that they have acquired malignant potential. Metastasis to distant sites has only been documented in a few models such as those,which express both SV40 Large-T/small-t antigen genes and where two transgenic lines have been crossbred to generate a bigenic mouse model. Thus, the metastatic model appears to be the most difficult to create. Of critical importance are the choices of promoter to target the prostate and transgene to induce or inhibit PCa development and progression. The following review compares the outcomes of promoter and gene selection on PCa development and progression (Fig. 1) and summarizes the current mouse models for PCa (see Tables I–IV). The pathological classifications presented in this review were taken from the pertinent references and do not necessarily reflect the Bar Harbor Classification System, which was implemented in 2004 [Shappell et al., 2004].

PROMOTER SELECTION

Promoter selection is critical for targeting the transgene to the tissue-of-interest. Several generations of the rat probasin (PB) promoter have been characterized and will be briefly summarized. The initial −426/+28 bp PB promoter fragment was extensively characterized in PC-3, DU-145, and LNCaP cell lines utilizing either the chloramphenicol acetyl transferase (CAT) or the luciferase reporter systems. Two distinct androgen receptor (AR) binding sites, ARBS-1 (located at position −236 to −223) and ARBS-2 (at position −140 to −117), were found to be required for maximal induction of CAT gene expression in response to androgen treatment [Rennie et al., 1993]. Since both binding sites were essential for transactivating the AR signaling pathway, the region encompassing both elements (−244 to −96) was named the androgen responsive region (ARR) [Kasper et al., 1994]. This promoter was also tested in numerous non-prostatic cell lines and was found to activate reporter gene expression in a prostate cell-specific manner [Brookes et al., 1998]. Furthermore, the −426/+28 bp PB promoter targeted CAT transgene expression to the prostate in vivo [Greenberg et al., 1994] and was utilized to drive SV40 Large-T/small-t expression in the TRAMP mouse model for PCa [Gingrich et al., 1996]. SV40 Large-T/small-t expression was seen in all prostatic lobes with the dorsolateral lobes (DLP) demonstrating the highest levels of expression.

Subsequently, 12 kb of the endogenous PB promoter was isolated; and in transgenic mice, this long PB promoter (termed LPB) elicited significantly higher levels of CAT gene expression specifically in luminal epithelial cells of the prostatic gland than those seen with the smaller −426/+28 bp PB promoter fragment [Yan et al., 1997]. Furthermore, CAT gene expression rose in parallel with increasing serum androgen levels during prostate growth and maturation, indicating that PB was developmentally regulated [Yan et al., 1997]. CAT gene expression dropped to basal levels following castration, and expression could be restored to pre-castration levels with exogenous androgen treatment [Yan et al., 1997]. The LPB construct was selected to develop the LADY model where LPB was linked to the Large-T antigen of SV40. This gene contained the deletion mutation d1 2005, which removes expression of the small-t antigen [Kasper et al., 1998; Kasper et al., 2003]. Transgene expression occurred in all prostatic lobes with the highest levels of expression occurring either in the dorsal lobes (DP) or the ventral lobes (VP), followed by the lateral (LP) and anterior (AP or coagulating gland) lobes [Kasper et al., 1998].

The composite ARR_2PB promoter was developed by linking two ARRs to the endogenous PB promoter. The ARR_2PB promoter was as efficient in directing high levels of transgene expression as the LPB promoter and targeted prostate-specific expression in transgenic mice [Zhang et al., 2000a]. Subsequently, the PB-Cre4 model was developed by targeting ARR_2PB linked to the *Cre* recombinase gene to the prostatic epithelium [Wu et al., 2001]. When PB-Cre4 mice were crossbred with R26R mice carrying the ROSA26 reporter gene inserted between two directly repeated *lox*P sites, the ROSA26 reporter was excised only in the prostate, allowing the expression of lacZ in prostatic epithelium [Wu et al., 2001] (Table I). When PB-Cre4 males were crossbred with R26R females, high levels of LacZ transgene expression were detected in the prostate lobes, whereas all other tissue tested showed no recombination [Wu et al., 2001]. However, when PB-Cre4 females were used to generate the double transgenics, small foci of expression were seen in the testes, seminal vesicles, and ovaries [Wu et al., 2001]. Since this was not observed when PB-Cre4 males were bred, it is possible that the PB gene may undergo imprinting

TABLE I. Comparison of Characteristics in Transgenic Models of Prostate Cancer Generated With the Prostate-Specific Probasin Promoter

Model	Target tissue	LGPIN	HGPIN	Invasive carcinoma	Metastasis	Ref.
(−426)PB-mAR	Prostate (VP > DLP)	>12 months	>12 months	1 animal	No	[Stanbrough et al., 2001]
(−426)PB-*EcoR1*	Prostate (DLP)	10 months	24 months	24 months	No	[Voelkel-Johnson et al., 2000]
(−426)PB-FGF7 (PKS model)	Prostate (LP in line 3258, LP and DP in line 3293)	Hyperplasia after one year	No	No	No	[Foster et al., 2002]
Dominant negative (−426)PB-FGFR2iiib (KDNR model)	Prostate	Hyperplasia/dysplasia after 1 year; increased neuroendocrine cells in basal compartment	No	No	No	[Foster et al., 2002]
(−426)PB-*fos*	Prostate (DLP)	16 months	No	No	No	[Voelkel-Johnson et al., 2000]
(−426)PB-*RAS*	Prostate (DLP > VP > AP) low levels in seminal vesicle and intestine (low RT-PCR signal)	3 months, hyperplasia, PIN, multiple intestinal metaplasia; 12 months, stromal hyperplasia	No	No	No	[Scherl et al., 2004]
(−426) 53/His273 mutant	Prostate (DLP)	Earlier time points not analyzed	12 months	No	No	[Elgavish et al., 2004]
(−426)PB-Large-T/ small-t (TRAMP model)	Prostate (DLP > VP)	8–12 weeks	8–12 weeks	>12 weeks	Lung, liver, salivary gland, kidney, lymph node, bone; exhibits neuroendocrine features	[Greenberg et al., 1995; Gingrich et al., 1996; Kaplan-Lefko et al., 2003]
(−426)PB-Large-T/ small-t (transgenic rat model)	Prostate (VP, DLP, AP) tongue (neuroblastoma)	15 weeks	15 weeks	30–35 weeks	Neuroendocrine, into surrounding tissues	[Asamoto et al., 2002; Cho et al., 2003]
(−426)rPb-myr-HA-Akt1 (MPAKT model)	Prostate (VP ⋙ LP)	>38 weeks	No	No	No	[Majumder et al., 2003]
(−426)PB-bcl2	Prostate	No	No	No	No	[Bruckheimer et al., 2000]
(−426)PB-*Myc*-PAI (Lo-Myc model)	Prostate (DPL,VP > AP)	4 weeks	6–12 months	>12 months	No	[Ellwood-Yen et al., 2003]
ARR2PB-*myc*-PAI (Hi-Myc model)	Prostate (DPL,VP > AP)	2 weeks	3–6 months	>6 months	No	[Ellwood-Yen et al., 2003]
(−454)PB-IGF	Prostate (VP > DP, LP)	No	No	No	No	[Konno-Takahashi et al., 2003]
(−454)PB-T*ras*	Prostate (VP, DLP) epididymis	>6 months hyperplasia and stromal proliferation, no PIN	No	No	No	[Barrios et al., 1996]
(−458)PB-rPRL	Prostate (DLP ≥ VP > AP) seminal vesicles (weak RT-PCR signal)	12 weeks, hyperplasia and stromal proliferation	No	No	No	[Kindblom et al., 2003]
ARR_2PB-FGF8b	Prostate (VP, LP > DP > AP) epididymis, seminal vesicles, ductus deferens	>3 months	>15 months	No	No	[Song et al., 2002]
ARR_2PB-SKP2	Prostate (VP, LP > DP)	3–7 months	3–7 months	No	No	[Shim et al., 2003]
ARR_2PB-hepsin	Prostate (VP)	Disorganization and disruption of basement membrane	No	No	No	[Klezovitch et al., 2004]
LPB-SV40 Large-T antigen line 12T-7f (Lady model)	Prostate (VP > DLP, AP)	12–20 weeks	12–20 weeks	20 weeks	No	[Kasper et al., 1998; Masumori et al., 2001]
LPB-SV40 Large-T antigen line 12T-10 (Lady model)	Prostate (VP > DLP, AP)	2–5 months	2–12 months	6–12 months, adenocarcinoma, with neuroendocrine features	Lymph node, liver, lung, spleen, kidney, bone (primarily neuroendocrine)	[Masumori et al., 2001]

The length of 5′-probasin flanking sequence (base pairs) utilized to generate the different transgenic mouse models is provided in parentheses. The ARR_2PB promoter region is composed of (−244/−96)(−286/+28) probasin sequence and the LBP promoter region contains 10.8 kb of probasin 5′-flanking sequence.

TABLE II. Transgenic Models of Prostate Cancer Generated With Non-Probasin Promoter Regions

Model	Target tissue	LGPIN	HGPIN	Invasive carcinoma	Metastasis	Ref.
BK5-IGF1	Prostate (VP, DLP), male accessory glands	>6 months, primarily hyperplasia	>14 months, with neuroendocrine differentiation	Yes	Yes, locally into pelvic muscle mass, seminal vesicle walls, neuroendocrine	[DiGiovanni et al., 2000]
C3(1)-bcl-2	Prostate (VP), testes, uterus	>3 months, epithelial and stromal proliferation, no PIN	No	No	No	[Zhang et al., 1997]
C3(1)-c-myc	Prostate (VP), testes, uterus	>3 months, hyperplasia, dysplasia	No	No	No	[Zhang et al., 2000b]
C3(1)-Polyoma virus middle T	Prostate (VP, DLP, AP), epididymis, lung, testes, mammary and salivary glands, vas deferens, ampullary gland, urethra	Hyperplasia, dysplasia	Yes	Yes	No	[Tehranian et al., 1996]
C3(1)-SV40 Large-T/small t	Prostate (VP > DLP), mammary, sweat glands, urethral glands	8–12 weeks	20–32 weeks	>28 weeks with neuroendocrine differentiation	No	[Shibata et al., 1996b; Shappell et al., 2003]
Cryptdin 2-SV40 Large-T/small-t	Prostate	8-10 weeks, with neuroendocrine differentiation	10 weeks	12 weeks	16 weeks, lymph nodes, lung, liver, bone	[Garabedian et al., 1998]
Fetal gamma globin-SV40 Large-T/small-t	Prostate (VP, DLP), adrenal cortex, brown fat, also in embryonic non-erythroid tissues	Observed, derived from basal epithelial layer	Observed	16–20 weeks, epithelial with luminal epithelial and neuroendocrine features	3–4 months, adrenal, bone, lung, lymph nodes, thymus, intrascapular tissue of neck and shoulders	[Perez-Stable et al., 1996]
gp91-phox-SV40 Large-T/small-t	Prostate, lung, spleen, white blood cells	>8 weeks, neuroendocrine	>8 weeks, neuroendocrine	Neuroendocrine	Surrounding tissues	[Skalnik et al., 1991]
PSP94-SV40 Large-T/small-t	Prostate (VP, DLP), skin, liver, intestine, bone, lung,	>10 weeks	>12 weeks	>16 weeks	Renal lymph nodes, kidney	[Gabril et al., 2002]
MT-1-rPRL	Prostate (DP > VP), kidney, liver, pancreas, seminal vesicles, testis, thymus	>10–15 months, epithelial and stromal proliferation	No	No	No	[Wennbo et al., 1997]
MMTV-kgf	Prostate (VP, DLP), mammary and salivary glands, seminal vesicles, vas deferens	>9 months, papillary hyperplasia	No	No	No	[Kitsberg and Leder, 1996]
MMTV-wap	Prostate (AP), epididymis, seminal vesicles, lung, spleen, mammary gland, parotid, and submaxillary gland, skin	Hyperplasia	No	No	No	[Hennighausen et al., 1994]

TABLE III. Haplotype and Knock-Out Mouse Models of Prostate Cancer

Model	Tissues affected by loss of gene expression	LGPIN	HGPIN	Invasive carcinoma	Metastasis	Ref.
PB-Cre4 × RXRα$^{f/f}$	CRE activity in prostate, with low levels in epididymis and ductus deferens	>5 months (62% of mice)	>10 months (17% of mice)	No	No	[Huang et al., 2002]
PSA-CRE × Nkx3.1$^{f/f}$	CRE activity in prostate (VP, DLP > AP)	20 weeks hyperplasia, dysplasia	No	No	No	[Abdulkadir et al., 2002]
PB-Cre4 × PTEN$^{loxp/loxp}$	Cre activity in prostate (LP > VP > DL > AP) with trace amounts of recombination activity in seminal vesicles	4 weeks, hyperplasia 6 weeks, PIN	9 weeks	9 weeks	12 weeks, lymph nodes, lung	[Wang et al., 2003]
MMTV-Cre × PTEN$^{loxP/loxP}$	Prostate (VP, AP, DLP), skin, thymus, mammary gland	Not reported	2 weeks	3 weeks	No	[Backman et al., 2004]
Fsp1-Cre × Tgfbr2$^{f/f}$ (Tgfbr2^{fspKO} model)	All fibroblasts, prostate (DLP,AP), forestomach	5–7 weeks hyperplasia, dysplasias	No	No	No	[Bhowmick et al., 2004]
p53$^{-/-}$	Prostate	No	No	No	No	[Donehower et al., 1992]
Rb$^{-/-}$	Prostate	No	No	No	No	[Wang et al., 2000]
PTEN$^{+/-}$	Prostate (DLP > AP), adrenal medulla, thyroid, endometrium, small intestine, large intestine, lung	Dysplasia after 9 months (50% of mice)	No	No	No	[Podsypanina et al., 1999; Di Cristofano et al., 2001]
p27$^{Kip1-/-}$	Prostate	Mild hyperplasia	No	No	No	[Cordon-Cardo et al., 1998]
Nkx3.1$^{-/-}$	Prostate, bulbourethral gland	>2 months hyperplasia and dysplasia	No	No	No	[Schneider et al., 2000]
RARγ$^{-/-}$	Prostate, seminal vesicles	Squamous metaplasia	No	No	No	[Lohnes et al., 1993]
Stat5a$^{-/-}$	Prostate	Epithelial desquamation, in VP, no hyperplasia and no PIN	No	No	No	[Nevalainen et al., 2000]

TABLE IV. Comparison of Bigenic Mouse Models of Prostate Cancer

Model	Target tissue	LGPIN	HGPIN	Invasive carcinoma	Metastasis	Ref.
LPB-Tag (line12T-7) × ARR_2PB-hepsin	Prostate	Yes	Yes	Adenocarcinoma with neuroendocrine features	21 weeks, liver, lung, bone, neuroendocrine	[Klezovitch et al., 2004]
$PTEN^{+/-}$ × $Cdkn1b^{-/-}$	Prostate (DLP > AP) adrenal medulla, thyroid, endometrium, small intestine, large intestine,	>8 weeks	>12 weeks	>12 weeks (25% of mice)	No	[Di Cristofano et al., 2001]
$PTEN^{+/-}$ × $Nkx3.1^{-/-}$	Prostate, lymph nodes, and other tissues.	>6 months	>6 months	>6 months	No	[Kim et al., 2002b; Abate-Shen et al., 2003]
$PTEN^{+/-}$ × TRAMP	Prostate	Yes	Yes	Yes	Lung, lymph nodes, liver, kidney, neuroendocrine	[Kwabi-Addo et al., 2001]
TRAMP × $FGF2^{-/-}$	Prostate	Yes	Yes	Yes	Rate decreased by approximately 50%	[Polnaszek et al., 2003]
12T-7 × MT-DNIIR	Prostate (AP, DLP, VP)	12–23 weeks	12–23 weeks	>23 weeks	8 months lymph nodes, lung, liver, neuroendocrine	[Tu et al., 2003]
12T-10 × MT-DNIIR	Prostate (AP, DLP, VP)	<6 months	By 6 months	>6 months	8 months lymph nodes, lung, liver, neuroendocrine	[Tu et al., 2003]

during development. The PSA promoter has also been linked to the CRE recombinase gene to initiate recombination of the floxed alleles specifically in the prostate [Abdulkadir et al., 2002]. All other promoters utilized to date will target the transgene to multiple tissues (for summary, see Table II).

Promoter selection is also essential for obtaining levels of transcription that are sufficiently robust to promote a phenotype. The ARR_2PB promoter consistently results in high levels of transgene expression. The shorter −426/+28 PB promoter, although still prostate-specific, results in significantly lower levels of transcription. The effects of promoter selection on phenotype are clearly demonstrated in the C3(1)-c-myc, (−426)PB-Myc-PAI (Lo-Myc), and ARR_2PB-myc-PAI (Hi-Myc) models. Primarily hyperplasia with dysplasia are detected in the C3(1)-c-myc model [Zhang et al., 2000b]. In contrast, progression from LGPIN to locally invasive carcinoma occur in both the Lo-Myc and Hi-Myc models, albeit it that tumor progression in Hi-Myc mice occurs in half the time period than that required for Lo-Myc mice (Table I) [Ellwood-Yen et al., 2003]. Thus, rate of tumor progression can depend on promoter choice and to date, the PB promoter has provided many models to study the molecular basis of gene disruption on prostate tumorigenesis (Tables I and IV).

TARGET GENE SELECTION

Steroid Hormone Receptors

The AR appears to have the greatest impact on the development and progression of PCa. Androgen deprivation has been the "gold standard" in treating patients with advanced prostatic carcinoma [Brewster and Simons, 1994; Taplin et al., 1995]. Greater than 80% of the patients show biochemical evidence of a favorable response to androgen deprivation as measured by a decrease in serum PSA levels and tumor regression, but the duration of the response is usually 2–3 years [Taplin et al., 1995] before tumor growth resumes despite continuous treatment [Kelly and Scher, 1993]. Interestingly, immunohistochemical studies have demonstrated that AR expression is still present in >80% of metastatic lesions, suggesting that AR continues to be functional, even in the absence of androgen [van der Kwast et al., 1991; Hobisch et al., 1995]. Thus, it is possible to view

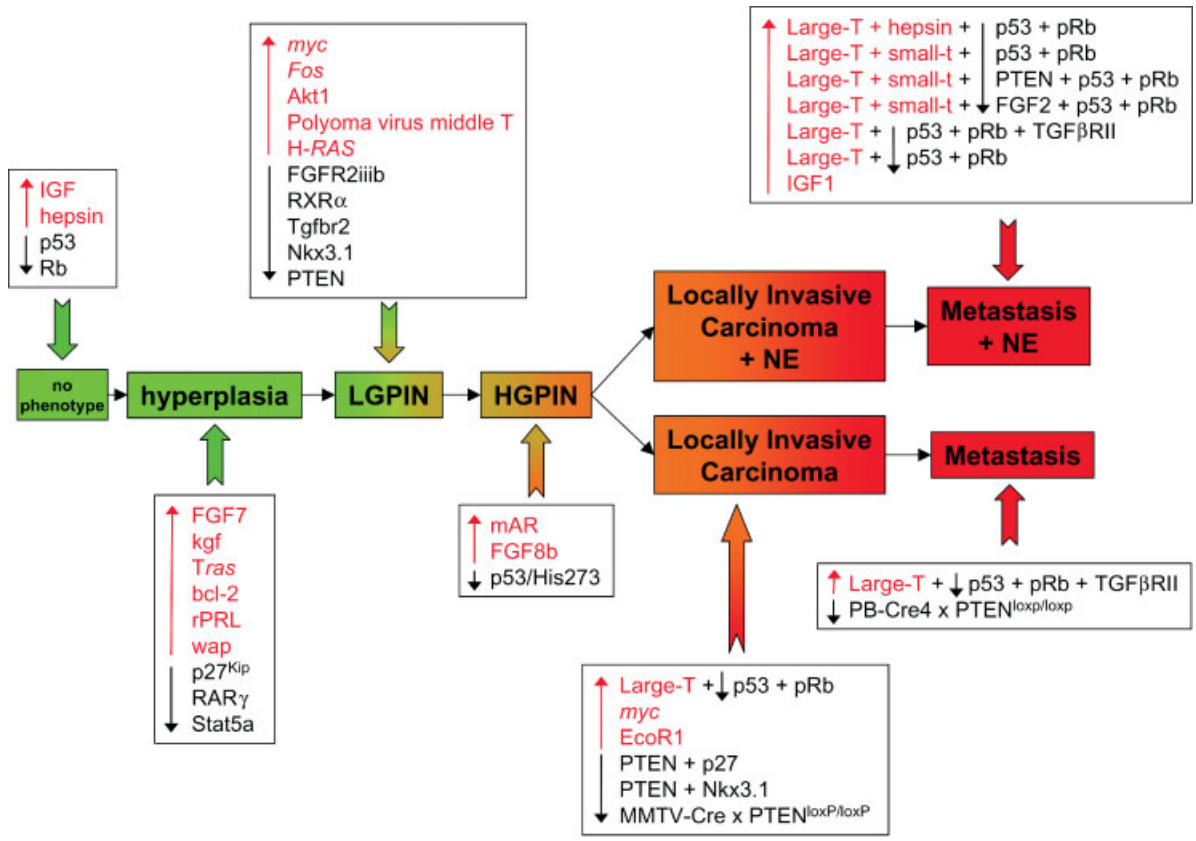

Fig. 1. Diagrammatic summary of the onset and progression of PCa resulting from one, two, or multiple genetic disruptions. The pathological classifications were reported in the cited references and do not necessarily reflect the Bar Harbor Classification System [Shappell et al., 2004]. Rather, they reflect the descriptions in the cited publications.

AR as a proto-oncogene, which when functioning normally, orchestrates both development and differentiation of the prostate under normal endocrine control. However, during tumorigenesis, AR undergoes gene amplification and/or acquires gain-of-function mutations to provide PCa cells with a selective growth advantage under conditions that previously precluded normal prostate epithelial cell proliferation [Feldman and Feldman, 2001].

Stanbrough and co-workers generated the PB-mAR transgenic model where murine AR under the regulation of the androgen-responsive PB promoter (−426/+28 bp) was targeted to the luminal epithelial cells of the prostate [Stanbrough et al., 2001]. No phenotypic changes were observed prior to 12 months of age. However, after 12 months, 1/11 mice demonstrated mild to moderate dysplasia, and 4/11 animals showed severe dysplasia with features similar to those seen in human PIN, including pronounced epithelial cell crowding, nuclear atypia and prominent nucleoli [Stanbrough et al., 2001]. A greater degree of dysplasia was observed in the VP and correlated with the higher levels of mAR expression observed in the VP as compared to the DLP lobes. Interestingly, only 45% of PB-mAR transgenic mice demonstrated a phenotype and no invasive carcinoma or metastatic lesions were observed [Stanbrough et al., 2001]. Thus, increased AR levels appear sufficient to promote moderate to severe dysplasia, but not metastasis.

Vitamin A is thought to play a role in normal prostate development and PCa. Exogenous retinoic acid (RA) inhibits ductal growth and branching and prostate tumor tissues contain decreased endogenous levels of retinoid [Huang et al., 2002]. Actions of retinoids are mediated through RA receptors which consist of RAR and RXR heterodimers. RXR-selective retinoids reduced clonal growth and tumorigenic potential in LNCaP, PC-3, and DU-145 human PCa cell lines [de Vos et al., 1997]. In RARγ$^{-/-}$ null mice, loss of RARγ expression resulted in prostatic squamous metaplasia [Lohnes et al.,

1993]. In contrast, normal prostate architecture was seen in transgenic mice lacking RXRβ or RXRγ [Huang et al., 2002]. Since RA receptor activity depends on the dimerization of RAR and RXR, these data suggest that RXRα may be the critical component in prostate tumorigenesis. Loss of RXRα expression is embryonically lethal and therefore, the PB-Cre4/lox system was utilized to disrupt RXRα specifically in prostate luminal epithelial cells. PB-Cre4 mice were crossbred with mice homozygous for the floxed RXRα allele ($RXR\alpha^{f/f}$). The resulting histopathological evaluation of $RXR\alpha^{f/f}$ prostates indicated that LGPIN lesions appeared after 5 months, and HGPIN lesions were detected after 10 months [Huang et al., 2002]. Incidence of LGPIN and HGPIN development was 62% and 17%, respectively [Huang et al., 2002]. A further observation was that LGPIN and HGPIN were also detected in heterozygous males at 11 and 14 months, respectively, demonstrating that RXR haploinsufficiency could promote PIN development [Huang et al., 2002]. Thus, loss of RXR expression appears to initiate proliferation and neoplastic transformation but is not sufficient to support the development of invasive carcinoma and metastasis.

Growth Factors

Growth factors are critical in many processes, including organogenesis, angiogenesis, wound repair, and tumorigenesis. For example, fibroblast growth factor 7 [FGF7, a.k.a. keratinocyte growth factor (KGF)] increases the expression of genes involved in epithelial cell proliferation. During normal growth and development, FGF7 functions as a paracrine factor since it is synthesized in stromal cells and secreted to promote proliferation of adjacent epithelial cells expressing the FGF2iiib receptor (FGFR2iiib) [Thomson, 2001]. In human prostate tumors, epithelial cells begin to express FGF7 and expression is maintained in carcinomas and metastatic lesions, suggesting that the FGF7 paracrine loop has been replaced by an autocrine mechanism that promotes prostate tumor progression and metastatic potential [McGarvey and Stearns, 1995]. Interestingly, only hyperplasia and/or dysplasia were seen in the PKS model ((−426)PB-FGF7) and KDNR model ((−426)PB-FGFR2iiib) where FGF7 or dominant negative FGF2iiib receptor expression, respectively, was overexpressed in prostatic epithelium [Foster et al., 2002]. Similarly, only hyperplasia was detected in MMTV-kgf transgenic prostates [Kitsberg and Leder, 1996]. Thus, although FGF7 expression appears to be elevated in advanced-stage human PCa, the mouse models suggest that the primary action of FGF7 is to induce proliferation and that other events are required to promote HGPIN, locally invasive adenocarcinoma, and metastasis.

Overexpression of FGF8b, another member of the FGF superfamily, is associated with decreased patient survival and FGF8b expression persists in androgen-independent disease [Dorkin et al., 1999]. FGF8b increases the growth rate of LNCaP cells and enhances their invasiveness in the soft agar assay in vitro and in the tumorigenesis/diaphragm invasion assay in nude mice [Song et al., 2000]. Therefore, the FGF8b isoform was linked to the ARR_2PB promoter to develop a mouse model for advanced PCa. In ARR_2PB-FGF8b mice, overexpression of FGF8b did promote HGPIN; however it was not sufficient to promote localized microinvasion or metastasis. Thus, this model could be utilized to investigate prostatic hyperplasia and PIN [Song et al., 2002].

Increased circulating levels of insulin-like growth factor-1 (IGF-1) have been associated with an increased risk of PCa [Chan et al., 1998]. IGF-1 acts as a mitogen, stimulating the proliferation of prostate epithelial cells [Chan et al., 1998]. The human PCa cell lines PC-3, DU-145, and LNCaP synthesize and secrete IGF-1 and contain constitutively autophosphorylated IGF-1 receptor (IGFR), suggesting that these cells can potentiate their own proliferation through an IGF-1/IGFR autocrine loop [Pietrzkowski et al., 1993]. IGF-1 also activates AR-mediated transcription to the same extent as the synthetic androgen methyltrienolone and this activity is inhibited by the anti-androgen Casodex, indicating that IGF-1-mediated transcription can occur through AR transactivation of the PSA promoter [Culig et al., 1994]. These studies suggest that IGF-I may play an important role in stimulating the growth and progression of PCa cells and that crosstalk between growth factor/receptor tyrosine kinase pathways and androgen-mediated signaling potentiate the development of PCa.

The (−454)PB-IGF and BK5 (bovine keratin 5)-IGF1 transgenic models were developed to determine the effects of IGF-1 overexpression on PCa progression. Epithelial cell abnormalities were not seen in prostates from (−454)PB-IGF

males [Konno-Takahashi et al., 2003]. In BK5-IGF1 mice where IGF1 was regulated by the bovine keratin 5 promoter, hyperplasia appeared after 6 months and HGPIN and metastasis into the surrounding tissue were detected after 14 months of age [Culig et al., 1994]. The basis for the pathological differences seen in these two models is not clear. In (−454)PB-IGF mice, transgenic IGF-1 levels were only two- to threefold greater than control levels, suggesting that transgene levels were inadequate to induce tumorigenesis [Konno-Takahashi et al., 2003]. A noteworthy observation was that neuroendocrine (NE) features were predominant in HGPIN and locally invasive lesions in the BK5-IGF1 model [Culig et al., 1994]. In the BON cell line derived from a human pancreatic carcinoid tumor, exogenously added IGF-I increased chromogranin A secretion and stimulated anchorage-dependent and anchorage-independent growth [von Wichert et al., 2000]. Furthermore, endogenously released IGF-I promoted the autonomous growth of BON cells in serum-free medium [von Wichert et al., 2000]. Thus, the predominance of NE tumor cells in HGPIN and locally invasive lesions in BK5-IGF1 mice may arise through the overexpression and autocrine activity of IGF-1.

Transforming growth factor-beta 1 (TGF-β1) is an important regulator of normal and malignant prostate cell growth. In normal prostate cells, TGF-β1 stimulates cell differentiation, inhibits epithelial cell proliferation, and induces apoptosis [Derynck, 1994; Glick et al., 1996]. In PCa, high levels of TGF-β are associated with tumor grade, angiogenesis, and metastasis [Wikstrom et al., 1998]. However, a concomitant loss of TGF-β receptor increases resistance to the anti-proliferative and pro-apoptotic effects of TGF-β1 [Wikstrom et al., 1998]. Loss of TGF-β receptors and resistance to the anti-proliferative and pro-apoptotic effects of TGF-β1 have been associated with poor prognosis [Kim et al., 1998].

TGF-β receptors are expressed both in prostatic epithelium (luminal and basal epithelial cells) and in stroma (fibroblasts, smooth muscle cells, and vascular endothelium) [Datta et al., 1998; Wikstrom et al., 1998]. To analyze the effects of TGF-β signaling in stromal fibroblasts, Bhowmick et al. [2004] selectively inactivated the TGF-β receptor type II (TβRII) in fibroblasts in $Tgfbr2^{fspKO}$ mice. Early development appeared normal, however, by 3 weeks of age, the number of prostatic stromal fibroblasts increased significantly and this was accompanied by the development of PIN predominantly in the DLP and AP lobes. These findings suggest that TGF-β-induced signaling in stromal fibroblasts modulates the growth and oncogenic potential of adjacent prostate epithelial cells.

Tumor Suppressor Genes

Tumor suppressor genes participate in the control of cell cycle and apoptosis to determine the fate of a cell. These genes can be inactivated through mutations or losses of chromosomal regions with moderate frequencies in advanced cancers. The p53 tumor suppressor gene is a transcription factor that binds to specific sequences in target genes to activate their transcription in response to cellular stress or genotoxic damage. Thus activation of $p21^{CIP1/WAF1}$ and 14-3-3σ, result in cell cycle arrest at G1 whereas GADD45 activity induces cell cycle arrest at the G2 checkpoint [Pietenpol and Stewart, 2002]. p53 also induces apoptosis through target genes which act through receptor-mediated signaling pathways or through increased expression of pro-apoptotic proteins, such as BAX, which activates the caspase cascade [Pietenpol and Stewart, 2002]. To investigate the role of p53 in tumorigenesis, Donehower et al. [1992] generated transgenic mice deficient for p53. Surprisingly, these mice developed normally; however, they were susceptible to spontaneous tumor formation. Loss of p53 expression did not affect prostate development and the onset of apoptosis was delayed for 5 days post castration, demonstrating that p53 was not essential for castration-induced involution of the prostate gland [Colombel et al., 1995].

The arginine to histidine mutation at codon 273 is one of the most frequently identified p53 mutations in breast, colorectal, and ovarian cancers [Lasky and Silbergeld, 1996]. The p53/His273 mutant was targeted to the prostate utilizing the −426/+28 bp PB promoter to test whether it decreased the rate of apoptosis in prostate epithelial cells. Hyperplasia/dysplasia with nuclear atypia was detected at 12 months of age, but further progression was not observed [Elgavish et al., 2004]. Interestingly, apoptosis was inhibited after castration, suggesting that despite the presence of endogenous p53, the p53/His273 mutant could render prostate epithelial cells resistant to factors, which induce apoptosis [Elgavish et al., 2004]. These observa-

tions suggest that inhibition of p53 through mutation is more effective in initiating cellular changes than loss of p53 gene expression itself. Hachiya et al. [1994] have postulated that mutant p53 acts as a "dominant negative" by interacting with oligomerization sequences in wild-type p53 to form chimeric complexes, which cannot transactivate target gene expression.

Loss of retinoblastoma gene (Rb) expression has been observed in human LGPIN [Phillips et al., 1994] and prostatic adenocarcinomas [Brooks et al., 1995], suggesting that inactivation of Rb may be an early event during prostate carcinogenesis. The $Rb^{-/-}$ null phenotype is embryonically lethal; however, prostate tissue can be rescued by grafting the pelvic organ rudiments from $Rb^{-/-}$ mouse embryos under the renal capsule of adult male nude mouse hosts [Wang et al., 2000]. These rescued prostates developed normally and continued to express AR and dorsolateral proteins [Wang et al., 2000]. In the tissue recombination model, testosterone plus estrogen treatment induced hyperplasia when $Rb^{+/+}$ prostatic epithelium was recombined with $Rb^{+/+}$ rat urogenital mesenchyme, whereas atypical hyperplasia and carcinoma were observed when $Rb^{-/-}$ prostatic epithelium was recombined with $Rb^{+/+}$ rat urogenital mesenchyme [Wang et al., 2000]. Thus, loss of Rb expression may predispose prostate epithelial cells to carcinogenesis.

SV40 Large-T and Small-t Antigens

It has become clear over the last few years that loss or mutation of a single tumor suppressor gene does not result in metastatic disease and suggests that multiple "hits" are required to potentiate this process. Although a direct relationship between Simian Virus 40 (SV40) and human PCa has not been proven, renewed interest in SV40 infections has been raised with the discovery that active SV40 particles were introduced into the human population by contaminated polio vaccines from 1955 to 1963 [Barbanti-Brodano et al., 2004]. Of the viral proteins that compose SV40, Large-T and small-t antigens have been studied most extensively. Both proteins have multiple functions, but a principle function of Large-T is to bind p53 [Pipas and Levine, 2001] and Rb [Decaprio et al., 1988] to promote viral replication and cell transformation [Asamoto et al., 2002]. In contrast, small-t itself does not cause transformation, but provides essential mitogenic signals for facilitating transformation [Asamoto et al., 2002]. For example, small-t binds the serine–threonine protein phosphatase PP2A, and inhibition of PP2A leads to constitutive activation of the Wnt pathway and stimulation of cell proliferation [Asamoto et al., 2002]. Furthermore, blocking PP2A activity also induces alterations in the actin cytoskeleton and tight junctions, which result in loss of cell polarity and promote tumor invasiveness [Asamoto et al., 2002].

A number of transgenic models have been generated with the SV40 Large-T and small-t antigens. The Lady model was created by targeting Large-T antigen (Tag) under regulation of the long PB promoter (LPB-Tag) to the transgenic mouse prostate [Kasper et al., 1998; Masumori et al., 2001]. Several LPB-Tag lines were obtained and have been described in detail elsewhere [Kasper et al., 1998; Shappell et al., 2004]. Line 12T-7 is one of the most extensively analyzed LPB-Tag transgenic lines and will be highlighted in this review. Briefly, Tag transformation was initially seen as multifocal lesions, containing clusters of cells with elongated hyperchromatic nuclei interspersed among normal epithelial cells exhibiting typically round, basally located nuclei. By 8–9 weeks of age, 100% of glandular epithelium expressed Tag. Tumor growth subsequently progressed to lesions containing stratified epithelia with mild nuclear atypia resembling human LGPIN and further on to marked nuclear atypia resembling HGPIN. Local microglandular patterns suggested that localized intraprostatic invasion was also detected [Kasper et al., 1998]. These tumors regressed upon castration and androgen treatment restored the pre-castration phenotype, demonstrating that tumor growth remained androgen dependent [Kasper et al., 1998]. Of the seven established LPB-Tag lines, only Line 12T-10 showed metastatic lesions. This line was unique in that the transgene was inserted into the X chromosome and that tumor growth clearly progressed from PIN with NE features to NE PCa [Masumori et al., 2001]. Invasive carcinoma was characterized by NE differentiation and metastasis occurred to lymph nodes, liver, lung, spleen, kidney, and occasionally bone [Masumori et al., 2001]. Interestingly, metastatic lesions were only detected after 6 months of age at one site and at multiple sites after 9–10 months of age. Bone metastases only occurred in 3/21 or 14% of mice [Masumori et al., 2001]. Thus, unlike in that observed in humans,

bone metastases are rare in the transgenic mouse model.

All other transgenic mouse models were generated with the SV40 early gene coding region expressing both Large-T and small-t antigen. These models include the TRAMP model [Greenberg et al., 1995; Gingrich et al., 1996; Kaplan-Lefko et al., 2003], C3(1)-SV40 Large-T/small-t [Shibata et al., 1996a,b], cryptdin 2-SV40 Large-T/small-t [Garabedian et al., 1998], fetal gamma globin-SV40 Large-T/small-t [Perez-Stable et al., 1996], gp91-phox-SV40 Large-T/small-t [Skalnik et al., 1991], PSP94-SV40 Large-T/small-t [Gabril et al., 2002], as well as the (−426)PB-Large-T/small-t transgenic rat model [Asamoto et al., 2002; Cho et al., 2003]. Prostate tumorigenesis and progression to HGPIN were observed in all of these models. Furthermore, metastasis, including NE differentiation and the development of metastatic NE carcinomas, were reported in all models carrying both Large-T and small-t antigen transgenes. Another common feature in the Large-T and small-t models was that tumors initially regressed upon castration but that after a period of time, tumor growth resumed in an androgen-independent manner. NE differentiation is seen focally in many if not most human prostate adenocarcinomas and is associated with disease progression [di Sant'Agnese and Cockett, 1996]. Recently, Jin et al. [2004] reported that the transplantable NE-10 tumor derived from the LADY Line 12T-10 model could maintain LNCaP tumor growth in castrated mice bearing both tumors. Furthermore, nuclear AR levels as well as PSA secretion increased in the absence of testicular androgens [Jin et al., 2004]. Thus NE cells can influence PCa progression by producing growth factors which stimulate proliferation in adjacent cells [di Sant'Agnese and Cockett, 1996; Jin et al., 2004]. In addition, NE cells may increase AR expression or activity, which could facilitate androgen-independent tumor growth [Jin et al., 2004].

Cell Cycle and Signaling Pathways

Oncogenesis and cancer progression are characterized by alterations in cell cycle-related events, including proliferation, differentiation, and apoptosis. These alterations can arise through changes in growth factor, tumor suppressor gene, and oncogene activity. As demonstrated by the Tgfbr2fspKO model, loss of TβRII signaling in stromal cells resulted in the development of PIN by 5–7 weeks [Bhowmick et al., 2004]. Another tumor suppressor gene whose inactivation has been associated with PCa is the phosphatase PTEN (phosphatase and tensin homolog deleted on chromosome-10). PTEN dephosphorylates PI3 kinase (phosphatidylinositol 3,4,5-triphosphate), which subsequently prevents phosphorylation/activation of AKT and results in apoptosis [Kwabi-Addo et al., 2001]. Although the $PTEN^{-/-}$ genotype is embryonically lethal, heterozygote $PTEN^{+/-}$ mice are viable [Podsypanina et al., 1999]. Increased epithelial proliferation, hyperplasia, and LGPIN were observed in this model, indicating that haploinsufficiency was sufficient to initiate carcinogenesis but not to promote metastasis [Podsypanina et al., 1999; Di Cristofano et al., 2001].

In the PB-Cre4 × $PTEN^{loxp/loxp}$ model, embryonic lethality was circumvented by crossbreeding PB-Cre4 mice with $PTEN^{loxp/loxp}$ mice to selectively flox out PTEN expression in the prostate. Homozygous loss of PTEN expression was detected only in prostate epithelial cells and resulted in increased AKT expression and cell proliferation [Wang et al., 2003]. Unlike the heterozygote $PTEN^{+/-}$ mice which only developed PIN, PB-Cre4 × $PTEN^{loxp/loxp}$ mice developed hyperplasia, PIN, invasive adenocarcinoma, and metastasis to lymph nodes and lung at 12 weeks of age [Wang et al., 2003]. Although apoptosis was induced when PTEN conditional knock-out mice were castrated, tumor regression was only partial, since residual invasive adenocarcinoma was still clearly evident [Wang et al., 2003]. Furthermore, $PTEN^{-/-}$ glands were larger and proliferation indexes were higher than those observed in wild-type controls, suggesting that loss of PTEN expression allowed PCa cells to proliferate in the absence of androgen [Wang et al., 2003].

Another PTEN conditional knock-out model was developed by crossbreeding MMTV-Cre mice with $PTEN^{loxP/loxP}$ mice [Backman et al., 2004]. In these mice, loss of PTEN expression resulted in HGPIN by 2 weeks of age with an increase in proliferation rate and cell number for both basal and luminal epithelial cells [Backman et al., 2004]. The difference in the time of onset of HGPIN between these two models could be the choice of promoter since unlike the PB promoter which is specifically regulated by androgen, transcription at the MMTV promoter can be induced by glucocorticoids and progestins as well as by androgens

[Cato et al., 1987]. Furthermore, promoter choice may also influence the development of metastasis since metastatic lesions to lymph nodes and lung were only detected in PB-Cre4 × $PTEN^{loxp/loxp}$ mice, where the promoter was specifically and developmentally regulated by androgens [Backman et al., 2004].

In non-tumor cells, the PTEN tumor suppressor gene antagonizes phosphoinositide 3-kinase (PI3K) signaling by dephosphorylating the lipid products generated by PI3K activity [Vazquez and Sellers, 2000]. However, in tumor cells, one of the effects of decreased PTEN activity is that PI3K signaling is deregulated and leads to the constitutive activation of downstream targets such as Akt [Vazquez and Sellers, 2000]. After phosphorylation on residues Thr-308 and Ser-473, activated Akt promotes cell survival and cell growth [Vazquez and Sellers, 2000]. Increased immunohistochemical Akt staining is seen in approximately 53% of human primary prostate carcinomas [Sun et al., 2001]. The MPAKT model [(−426)rPb-myr-HA-Akt1] was generated to determine the impact of constitutive Akt activity on prostate epithelial cell transformation and PCa development. HA-myr-Akt1 protein was primarily seen in the VP, with low levels of expression also detected in the LP [Majumder et al., 2003]. Although endogenous Akt and myr-HA-Akt1 levels were approximately equivalent, only myr-HA-Akt1 was phosphorylated and detected in focal lesions, demonstrating that activated Akt correlated with the development of PIN [Majumder et al., 2003]. The histological appearance of PIN lesions in MPAKT mice was very similar to that seen in $PTEN^{+/-}$ mice, confirming that Akt activation or PTEN haploinsufficiency results in a similar phenotype.

One of the downstream targets of PI3K is p27, a key regulator for progression from G1 to S phase. Activation of PI3K causes downregulation of $p27^{Kip1}$ gene expression [Alkarain and Slingerland, 2004]. The $p27^{Kip1}$ gene and p27 protein are both abundantly expressed in normal human prostate tissues [Cordon-Cardo et al., 1998]. During the development of PCa however, p27 protein levels are nearly undetectable, although $p27^{Kip1}$ gene expression is maintained [Cordon-Cardo et al., 1998]. These observations suggest that p27 activity is lost as a result of proteolysis [Cordon-Cardo et al., 1998]. The $p27^{Kip1-/-}$ mouse model was developed to mimic the clinical observations that loss of $p27^{Kip1}$ expression correlates with poor prognosis. Histopathological examination of the prostate determined that although cell proliferation increased, prostate tumor growth did not progress beyond mild hyperplasia [Cordon-Cardo et al., 1998]. Thus loss of p27 appears to initiate hyperplasia but is not sufficient to promote the development of advanced-stage disease.

p27 expression is regulated through ubiquitin-dependent degradation in late phase G_1 [Shim et al., 2003]. The ubiquitin complex SCF^{SKP2}, consisting of SKP1, CUL-1/CDC53, and SKP2, binds phosphorylated p27 and targets it for polyubiquitination and proteolysis [Shim et al., 2003]. In human PCa, SKP2 protein levels are elevated as p27 protein levels decline [Shim et al., 2003]. In the ARR_2PB-SPK2 mouse model, overexpression of SKP2 reduced p27 levels and promoted the development of HGPIN [Shim et al., 2003]. Thus, SKP2 overexpression appears more effective than the loss of p27 in promoting the early stages of tumorigenesis.

The observations that loss of pro-apoptotic regulators, such as p53 and pRb, do not result in overt PCa have been surprising. Thus, this raises the question whether a more aggressive tumor phenotype would be achieved by overexpressing anti-apoptotic molecules such as bcl-2 or Akt. In the C3(1)-bcl-2 model, overexpression of prostatic bcl-2 stimulated epithelial and stromal cell proliferation but was not sufficient to generate PIN lesions [Zhang et al., 1997]. In the (−426)PB-bcl2 mouse model, the histological appearance of the prostatic lobes was normal [Bruckheimer et al., 2000]. These models suggest that a primary function of bcl-2 is cell proliferation, not cell transformation.

As discussed earlier, constitutive Akt activity resulted in LGPIN lesions very similar to those seen in $PTEN^{+/-}$ prostates. Phosphorylation of Akt leads to the activation of mTOR and potentiation of cell survival, proliferation, and motility. Thus, derivatives of the mTOR inhibitor rapamycin are currently being tested in clinical trails in cancer patients [Majumder et al., 2004]. When rPb-myr-HA-Akt1 mice were treated with the mTOR inhibitor RAD001, cell size, polarization, and normal luminal cell architecture were restored [Majumder et al., 2004]. However, crossbreeding rPb-myr-HA-Akt1 with (−426) PB-bcl2 mice resulted in resistance to RAD001 treatment and partially rescued the prolifera-

tive phenotype [Majumder et al., 2004]. Thus, although overexpression of bcl-2 only promotes hyperplasia in (−426)PB-bcl2 mice, the increase in bcl-2 expression is sufficient to partially block apoptosis induced by RAD001. Thus, targeting pro-apoptotic regulators, such as bcl-2 with specific small molecule inhibitors may provide effective therapeutic treatments for human prostate carcinoma.

The signal transducers and activators of transcription (Stat) proteins are key mediators of cytokine signaling [Bromberg and Darnell, 2000]. During normal cell signaling, Stat activation is transient. However in many cancer cell lines, Stat proteins 1, 3, and 5 are often constitutively phosphorylated or activated [Bromberg and Darnell, 2000]. Although Stat5 does not contain intrinsic tyrosine kinase activity, it is phosphorylated and activated by the Janus kinase Jak2. The two isoforms Stat5a and Stat5b dimerize and translocate into the nucleus where they bind to specific DNA response elements to activate the transcription of target genes [Li et al., 2004]. One of the Stat target genes is prolactin, which inhibits apoptosis in androgen-deprived prostate epithelial cells [Li et al., 2004]. Prolactin also promotes proliferation in both normal and malignant prostate cells [Costello and Franklin, 1994]. Immunohistochemical analysis has shown that Stat5 activation strongly correlates with high Gleason score [Li et al., 2004]. Thus, increased Stat5 activation could contribute to the process of malignant transformation.

Prostates from Stat5a$^{-/-}$ null mice displayed desquamation of the epithelium and increased cyst formation without hyperplasia [Nevalainen et al., 2000]. Thus, loss of Stat5a expression resulted in a relatively benign phenotype. The expression of other Stat family members, such as Stat5b, was normal, and Stat1 and Stat3 levels did not change, suggesting that they could compensate for the loss of Stat5a expression. In contrast, overexpression of prolactin was presented with epithelial and stromal cell proliferation in MT-1-rPRL mice and hyperplasia and stromal proliferation in (−458)PB-rPRL mice. No advanced-stage disease or metastases were detected in these three models. In addition, no differences in serum prolactin levels were detected in men with PCa compared to their age-matched controls [Li et al., 2004]. Thus the role of prolactin in promoting PCa progression is uncertain.

Oncogenes

The c-Myc oncogene induces proliferation and has classically been considered to be critical for the process of oncogenesis [Nasi et al., 2001]. C-myc expression is typically elevated in human adenocarcinoma biopsy samples and has therefore been studied to determine its role in prostate tumor development and progression [Fleming et al., 1986]. Overexpression of c-myc levels in ARR$_2$PB-myc-PAI transgenic mice resulted in HGPIN with locally invasive adenocarcinoma within 3–6 months [Ellwood-Yen et al., 2003]. Complete tumor regression occurred when mice were castrated at 2 months of age [Ellwood-Yen et al., 2003]. However, residual tumor was still detected if castration was performed after the tumors were well established at 8 months [Ellwood-Yen et al., 2003]. The observation that they had not completely regressed implies the presence of hormone resistant cells, however, whether these residual tumors relapse remains to be determined. Thus, the time of hormone ablation appears to be critical both for eradicating tumor cells and for preventing the emergence of hormone resistant tumor cells. Microarray-based expression profiling indicated that Nkx3.1 expression was lost and that serine/threonine kinase PIM-1 was upregulated in these tumors [Ellwood-Yen et al., 2003]. Collectively, these data demonstrate that multiple events are required for developing metastasis.

Another oncogene, Ras, has also been implicated in PCa development [Matusik et al., 1987]. In the Dunning R3327 model, Harvey-*ras* (Ha-*ras*) expression increased as the androgen-dependent H lineage progressed to AT1 then MAT-Lu and finally to the metastatic MAT-Ly-Lu prostate tumor cell line [Cooke et al., 1988]. In the Dunning AT2.1 model, growth characteristics of Dunning AT2.1 cells stably transfected with Ha-*ras* did not change and s.c. inoculation into syngeneic rats resulted in tumor formation. However, unlike the controls, AT2.1 tumors carrying Ha-*ras* metastasized in over 80% of inoculated rats, suggesting that Ras overexpression promoted metastasis [Treiger and Isaacs, 1988]. In (−454)PB-T*ras* mice that overexpress Ras, epithelial hyperplasia with stromal proliferation were observed but no evidence of PIN was seen even out to 13 months of age [Barrios et al., 1996]. In (−426)PB-*RAS* mice, where the minimal −426 PB promoter was

linked to the activated Harvey-*RAS* gene, transgenic mice developed PIN at 3 months of age [Scherl et al., 2004]. In a similar manner, LGPIN was detected by 16 months but no further progression was observed in PB-*fos* mice [Voelkel-Johnson et al., 2000]. With the exception of Myc, the clinical implications of overexpression of proto-oncogenes is not clear since at best, they appear to initiate transformation but are not sufficient to promote a more aggressive PCa phenotype.

Homeobox Genes

Recent studies have indicated that homeobox genes required for prostate organogenesis also contribute to prostate carcinogenesis. The Nkx 3.1 gene, a possible homolog of the *Drosophila* gene bagpipe, is expressed in the somites, blood vessels, midgut, pituitary gland *anlagen*, and male urogenital tract during embryogenesis and continues to be expressed in the developing prostate and minor salivary glands postnatally [Schneider et al., 2000]. Overexpression of Nkx3.1 in PC3 and AT6 PCa cells resulted in decreased cell proliferation and anchorage-independent growth in vitro and decreased tumor growth in nude mice in vivo [Kim et al., 2002a]. Nkx3.1$^{-/-}$ null mice generated by homologous recombination developed into adults with no apparent external phenotypic abnormalities. Analysis of the prostate revealed that the number of prostatic ducts were reduced, suggesting that Nkx3.1 was involved in branching morphogenesis [Schneider et al., 2000]. Furthermore, marked hyperplasia appeared after 2 months followed by dysplasia after 10 months of age [Schneider et al., 2000]. Similar LGPIN lesions were observed in PSA-CRExNkx3.1$^{f/f}$ mice where prostatic Nkx3.1 expression was conditionally floxed out [Abdulkadir et al., 2002]. In both knock-out models, one Nkx3.1 allele was sufficient to generate the LGPIN phenotype [Schneider et al., 2000; Abdulkadir et al., 2002]. Thus, Nkx3.1 appears to function as a tumor suppressor and loss of expression initiates the development of early-stage disease.

Genomic Instability

Activity of the restriction enzyme *EcoRI* can induce genomic instability due to high levels of activity and nuclear localization [Voelkel-Johnson et al., 2000]. Voelkel-Johnson et al. [2000] utilized the −426 PB promoter to target the *EcoR1* gene to the prostate. High-grade PIN and localized invasion of epithelial cells into the adjacent stroma were observed in the DLP by 24 months, indicating that genomic instability was sufficient to promote localized advanced-stage disease [Voelkel-Johnson et al., 2000]. This model represents a latent PCa model where chromosomal abnormalities are already present but where another critical event is still required to promote a more malignant phenotype. A metastatic model, which represents the entire continuum for PCa development, progression, and metastasis appears elusive.

Bigenic Models

The mouse models demonstrate that disrupting the expression of a single gene leads, in most cases, to LGPIN and HGPIN and not to metastatic disease. Therefore, a number of bigenic models have been generated to determine whether at least two genetic events will promote metastasis. The Type II transmembrane serine protease hepsin is upregulated in 90% of prostate tumors and this increase is often greater than tenfold [Klezovitch et al., 2004]. No tumor formation was observed in the ARR_2PB-hepsin model [Klezovitch et al., 2004]. However, hepsin overexpression caused weakening in epithelial-stromal adhesion through the disorganization and disruption of the basement membrane in hepsin-expressing prostate glands [Klezovitch et al., 2004]. To determine the role of hepsin in PCa, ARR_2PB-hepsin mice were crossbred with LPB-Tag (Line12T-7) mice. In those bigenic prostates, overexpression of hepsin promoted adenocarcinoma with extensive glandular differentiation and cribriform lesions, local micrometastasis, and metastasis to liver, lung, and bone [Klezovitch et al., 2004]. Since no metastatic lesions were observed in the parent 12T-7 line, these observations imply that hepsin promotes PCa metastasis to bone [Klezovitch et al., 2004].

The bigenic models suggest that even if the phenotype of a given model is limited, crossbreeding it with another mouse model to generate a double mutant often results in much greater phenotypic changes. For example, the histopathology in p27Kip1$^{-/-}$ and PTEN$^{+/-}$ mice ranged from mild hyperplasia to dysplasia, respectively. However, when these two lines were crossbred (PTEN$^{+/-}$xCdkn1b$^{-/-}$ model), locally invasive tumors were identified in 25% of the mice examined. Since loss of PTEN

activity activates PI3K and Akt which subsequently downregulates $p27^{Kip1}$ gene expression [Alkarain and Slingerland, 2004], the $PTEN^{+/-} \times Cdkn1b^{-/-}$ model confirms that modulating the PI3K signaling pathway is one mechanism by which advanced-stage PCa develops. Similarly, although loss of Nkx3.1 expression causes hyperplasia and dysplasia, the loss Nkx3.1 in combination with PTEN haploinsufficiency in $PTEN^{+/-} \times Nk \times 3.1^{-/-}$prostates resulted in HGPIN and early carcinoma lesions [Kim et al., 2002b]. Furthermore, the percent of prostates with HGPIN/early carcinoma lesions increased in $PTEN^{+/-} \times Nk \times 3.1^{-/-}$prostates compared to $PTEN^{+/-} \times Nk \times 3.1^{+/-}$prostates, implying that rate of transformation was contingent on the number of alleles expressed or lost [Kim et al., 2002b]. $PTEN^{+/-}$ mice were also crossbred with TRAMP mice to determine the consequences of decreasing PTEN expression in combination with loss of p53 and pRb expression. The net result was that the animals died of local prostatic disease and not of metastatic disease [Kwabi-Addo et al., 2001]. Thus, PTEN appears to promote tumor growth rather than metastasis.

Increased FGF2 expression occurs specifically in stromal cells of human PCa specimens obtained by radical prostatectomy [Polnaszek et al., 2003]. Interestingly, high FGF2 levels have been determined in epithelial cells of locally advanced and metastatic disease as well as in the metastatic PCa cell lines PC-3 and DU-145 [Polnaszek et al., 2003]. In double $TRAMPxFGF2^{-/-}$ mutant prostates, loss of FGF2 expression resulted in a significant decrease in metastasis and inhibition of the development of poorly differentiated phenotype in primary prostate tumors [Polnaszek et al., 2003]. Thus, the evolution of FGF2 expression from stromal to epithelial cells suggests that in PCa, epithelial cells may potentiate their proliferation through an autocrine mechanism. Furthermore, these observations suggest that interference with FGF2 expression or function by specific drugs could represent a means for improving the efficacy of anticancer therapy.

Increased TGF-β expression and decreased TβRII expression are associated with tumor survival and metastasis [Kulkarni et al., 1993; Markowitz and Roberts, 1996]. To study the role of TβRII in PCa development, MT-DNIIR transgenic mice carrying the dominant negative mutant to TβRII were crossbred with LADY Line 12T-7f mice that develop HGPIN with local invasion. In 12T-7fxMT-DNIIR bigenic offspring, focal lesions of locally invasive carcinoma were observed more frequently [Tu et al., 2003]. In addition, the presence of two "hits" resulted in micrometastatic lesions to lymph nodes, lung, and liver and on rare occasions, to bone [Tu et al., 2003]. When MT-DNIIR transgenic mice were crossbred with LADY Line 12T-10 mice, which develop lung and liver metastases (12T-10xMT-DNIIR), metastatic rate was further accelerated as seen by the increased incidence of invasive carcinoma and the number of metastatic lesions compared to those identified in the parental 12T-10 line [Tu et al., 2003]. Systemic neutralization of TGF-β signaling is antagonistic to metastasis. However, blocking TGF-β receptor signaling in the epithelium, together with downregulating p53 and RB expression, appears to specifically enhance metastatic potential.

Bigenic models in which two or three genetic hits have occurred clearly demonstrate that progression to HGPIN and metastasis requires more than the perturbation of a single genetic event. Defining the mechanisms that confer oncogenic potential onto cancer cells is an important goal towards identifying critical events required for metastasis and targets for the prevention of metastasis.

CONCLUSIONS

Genetically engineered mouse models provide the means for addressing the mechanisms by which PCa develops and becomes a clinically significant disease. The PB and PSA promoters have been effectively utilized to target transgenes to the prostate and the new ARR_2PB promoter consistently induces high levels of transgene expression. The mouse models suggest that we should direct our attention towards several objectives. The first is that although numerous models of LGPIN and HGPIN have become available, few if any have been utilized to design protocols for the prevention of PCa. The TRAMP model for advanced PCa has been one of the few modes utilized in preventive trials [Kasper and Smith, 2004]. Another area which deserves further attention is the role of stromal factors on PCa development. The Fsp1 promoter is one of the first promoters utilized to disrupt gene expression in the prostatic stroma [Bhowmick et al., 2004]. The study of Bhowmick et al. [2004] demonstrated that loss of stromal

Tgfbr2 expression promoted the development of LGPIN. Lastly, in reviewing the available models, it has become clear that few models represent the entire continuum of PCa development, tumor progression, and metastasis (Fig. 1). Indeed, two genetic events are generally required for progression from HGPIN to locally invasive carcinoma, and two to five genetic events are proposed to promote metastasis (for summary, see Fig. 1). Identification and characterization of metastasis-promoting factors would facilitate the creation of genetically engineered mice to analyze the mechanisms by which PCa cells survive, proliferate, and colonize a non-prostatic environment.

REFERENCES

Abate-Shen C, Banach-Petrosky WA, Sun X, Economides KD, Desai N, Gregg JP, Borowsky AD, Cardiff RD, Shen MM. 2003. Nkx3.1; Pten mutant mice develop invasive prostate adenocarcinoma and lymph node metastases. Cancer Res 63:3886–3890.

Abdulkadir SA, Magee JA, Peters TJ, Kaleem Z, Naughton CK, Humphrey PA, Milbrandt J. 2002. Conditional loss of Nkx3.1 in adult mice induces prostatic intraepithelial neoplasia. Mol Cell Biol 22:1495–1503.

Alkarain A, Slingerland J. 2004. Deregulation of p27 by oncogenic signaling and its prognostic significance in breast cancer. Breast Cancer Res 6:13–21.

Asamoto M, Hokaiwado N, Cho YM, Shirai T. 2002. Effects of genetic background on prostate and taste bud carcinogenesis due to SV40 T antigen expression under probasin gene promoter control. Carcinogenesis 23:463–467.

Backman SA, Ghazarian D, So K, Sanchez O, Wagner KU, Hennighausen L, Suzuki A, Tsao MS, Chapman WB, Stambolic V, Mak TW. 2004. Early onset of neoplasia in the prostate and skin of mice with tissue-specific deletion of Pten. Proc Natl Acad Sci USA 101:1725–1730.

Barbanti-Brodano G, Sabbioni S, Martini F, Negrini M, Corallini A, Tognon M. 2004. Simian virus 40 infection in humans and association with human diseases: Results and hypotheses. Virology 318:1–9.

Barrios R, Lebovitz RM, Wiseman AL, Weisoly DL, Matusik RJ, DeMayo F, Lieberman MW. 1996. RasT24 driven by a probasin promoter induces prostatic hyperplasia in transgenic mice. Transgenics 2:23–28.

Bhowmick NA, Chytil A, Plieth D, Gorska AE, Dumont N, Shappell S, Washington MK, Neilson EG, Moses HL. 2004. TGF-beta signaling in fibroblasts modulates the oncogenic potential of adjacent epithelia. Science 303:848–851.

Brewster SF, Simons JW. 1994. Gene therapy in urological oncology: Principles, strategies and potential. Eur Urol 25:177–182.

Bromberg J, Darnell JE, Jr. 2000. The role of STATs in transcriptional control and their impact on cellular function. Oncogene 19:2468–2473.

Brookes DE, Zandvliet D, Watt F, Russell PJ, Molloy PL. 1998. Relative activity and specificity of promoters from prostate-expressed genes. Prostate 35:18–26.

Brooks JD, Bova GS, Isaacs WB. 1995. Allelic loss of the retinoblastoma gene in primary human prostatic adenocarcinomas. Prostate 26:35–39.

Bruckheimer EM, Cho S, Brisbay S, Johnson DJ, Gingrich JR, Greenberg N, McDonnell TJ. 2000. The impact of bcl-2 expression and bax deficiency on prostate homeostasis in vivo. Oncogene 19:2404–2412.

Cato AC, Henderson D, Ponta H. 1987. The hormone response element of the mouse mammary tumour virus DNA mediates the progestin and androgen induction of transcription in the proviral long terminal repeat region. EMBO J 6:363–368.

Chan JM, Stampfer MJ, Giovannucci E, Gann PH, Ma J, Wilkinson P, Hennekens CH, Pollak M. 1998. Plasma insulin-like growth factor-I and prostate cancer risk: A prospective study. Science 279:563–566.

Cho YM, Takahashi S, Asamoto M, Suzuki S, Inaguma S, Hokaiwado N, Shirai T. 2003. Age-dependent histopathological findings in the prostate of probasin/SV40 T antigen transgenic rats: Lack of influence of carcinogen or testosterone treatment. Cancer Sci 94:153–157.

Colombel M, Radvanyi F, Blanche M, Abbou C, Buttyan R, Donehower LA, Chopin D, Thiery JP. 1995. Androgen suppressed apoptosis is modified in p53 deficient mice. Oncogene 10:1269–1274.

Cooke DB, Quarmby VE, Petrusz P, Mickey DD, Der CJ, Isaacs JT, French FS. 1988. Expression of ras protooncogenes in the Dunning R3327 rat prostatic adenocarcinoma system. Prostate 13:273–287.

Cordon-Cardo C, Koff A, Drobnjak M, Capodieci P, Osman I, Millard SS, Gaudin PB, Fazzari M, Zhang ZF, Massague J, Scher HI. 1998. Distinct altered patterns of p27KIP1 gene expression in benign prostatic hyperplasia and prostatic carcinoma. J Natl Cancer Inst 90:1284–1291.

Costello LC, Franklin RB. 1994. Effect of prolactin on the prostate. Prostate 24:162–166.

Culig Z, Hobisch A, Cronauer MV, Radmayr C, Trapman J, Hittmair A, Bartsch G, Klocker H. 1994. Androgen receptor activation in prostatic tumor cell lines by insulin-like growth factor-I, keratinocyte growth factor, and epidermal growth factor. Cancer Res 54:5474–5478.

Datta PK, Chytil A, Gorska AE, Moses HL. 1998. Identification of STRAP, a novel WD domain protein in transforming growth factor-beta signaling. J Biol Chem 273:34671–34674.

de Vos S, Dawson MI, Holden S, Le T, Wang A, Cho SK, Chen DL, Koeffler HP. 1997. Effects of retinoid X receptor-selective ligands on proliferation of prostate cancer cells. Prostate 32:115–121.

Decaprio JA, Ludlow JW, Figge J, Shew JY, Huang CM, Lee WH, Marsilio E, Paucha E, Livingston DM. 1988. SV40 large tumor antigen forms a specific complex with the product of the retinoblastoma susceptibility gene. Cell 54:275–283.

Derynck R. 1994. TGF-beta-receptor-mediated signaling. Trends Biochem Sci 19:548–553.

Di Cristofano A, De Acetis M, Koff A, Cordon-Cardo C, Pandolfi PP. 2001. Pten and p27KIP1 cooperate in prostate cancer tumor suppression in the mouse. Nat Genet 27:222–224.

di Sant'Agnese PA, Cockett AT. 1996. Neuroendocrine differentiation in prostatic malignancy. Cancer 78:357–361.

DiGiovanni J, Kiguchi K, Frijhoff A, Wilker E, Bol DK, Beltran L, Moats S, Ramirez A, Jorcano J, Conti C. 2000. Deregulated expression of insulin-like growth factor 1 in prostate epithelium leads to neoplasia in transgenic mice. Proc Natl Acad Sci USA 97:3455–3460.

Donehower LA, Harvey M, Slagle BL, McArthur MJ, Montgomery CA, Jr., Butel JS, Bradley A. 1992. Mice deficient for p53 are developmentally normal but susceptible to spontaneous tumours. Nature 356:215–221.

Dorkin TJ, Robinson MC, Marsh C, Bjartell A, Neal DE, Leung HY. 1999. FGF8 over-expression in prostate cancer is associated with decreased patient survival and persists in androgen independent disease. Oncogene 18:2755–2761.

Elgavish A, Wood PA, Pinkert CA, Eltoum IE, Cartee T, Wilbanks J, Mentor-Marcel R, Tian L, Scroggins SE. 2004. Transgenic mouse with human mutant p53 expression in the prostate epithelium. Prostate 61:26–34.

Ellwood-Yen K, Graeber TG, Wongvipat J, Iruela-Arispe ML, Zhang J-F, Matusik RJ, Thomas GV, Sawyers CL. 2003. Myc-driven murine prostate cancer shares molecular features with human prostate tumors. Cancer Cell 4:223–238.

Feldman BJ, Feldman D. 2001. The development of androgen-independent prostate cancer. Nat Rev Cancer 1:34–45.

Fleming WH, Hamel A, MacDonald R, Ramsey E, Pettigrew NM, Johnston B, Dodd JG, Matusik RJ. 1986. Expression of the c-myc protooncogene in human prostatic carcinoma and benign prostatic hyperplasia. Cancer Res 46:1535–1538.

Foster BA, Evangelou A, Gingrich JR, Kaplan PJ, DeMayo F, Greenberg NM. 2002. Enforced expression of FGF-7 promotes epithelial hyperplasia whereas a dominant negative FGFR2iiib promotes the emergence of neuroendocrine phenotype in prostate glands of transgenic mice. Differentiation 70:624–632.

Gabril MY, Onita T, Ji PG, Sakai H, Chan FL, Koropatnick J, Chin JL, Moussa M, Xuan JW. 2002. Prostate targeting: PSP94 gene promoter/enhancer region directed prostate tissue-specific expression in a transgenic mouse prostate cancer model. Gene Ther 9:1589–1599.

Garabedian EM, Humphrey PA, Gordon JI. 1998. A transgenic mouse model of metastatic prostate cancer originating from neuroendocrine cells. Proc Natl Acad Sci USA 95:15382–15387.

Gingrich JR, Barrios RJ, Morton RA, Boyce BF, DeMayo FJ, Finegold MJ, Angelopoulou R, Rosen JM, Greenberg NM. 1996. Metastatic prostate cancer in a transgenic mouse. Cancer Res 56:4096–4102.

Glick AB, Weinberg WC, Wu IH, Quan W, Yuspa SH. 1996. Transforming growth factor beta 1 suppresses genomic instability independent of a G1 arrest, p53, and Rb [published erratum appears in Cancer Res 1997 May 15;57(10):2079]. Cancer Res 56:3645–3650.

Greenberg NM, DeMayo FJ, Sheppard PC, Barrios R, Lebovitz M, Finegold M, Angelopoulou R, Dodd JG, Duckworth ML, Rosen JM, Matusik RJ. 1994. The rat probasin gene promoter directs hormonally and developmentally regulated expression of a heterologous gene specifically to the prostate in transgenic mice. Mol Endocrinol 8:230–239.

Greenberg NM, DeMayo FJ, Finegold MJ, Medina D, Tilley WD, Aspinall JO, Cunha GR, Donjacour AA, Matusik RJ, Rosen JM. 1995. Prostate cancer in a transgenic mouse. Proc Natl Acad Sci USA 92:3439–3443.

Hachiya M, Chumakov A, Miller CW, Akashi M, Said J, Koeffler HP. 1994. Mutant p53 proteins behave in a dominant, negative fashion in vivo. Anticancer Res 14:1853–1859.

Hennighausen L, McKnight R, Burdon T, Baik M, Wall RJ, Smith GH. 1994. Whey acidic protein extrinsically expressed from the mouse mammary tumor virus long terminal repeat results in hyperplasia of the coagulation gland epithelium and impaired mammary development. Cell Growth Differ 5:607–613.

Hobisch A, Culig Z, Radmayr C, Bartsch G, Klocker H, Hittmair A. 1995. Distant metastases from prostatic carcinoma express androgen receptor protein. Cancer Res 55:3068–3072.

Huang J, Powell WC, Khodavirdi AC, Wu J, Makita T, Cardiff RD, Cohen MB, Sucov HM, Roy-Burman P. 2002. Prostatic intraepithelial neoplasia in mice with conditional disruption of the retinoid X receptor alpha allele in the prostate epithelium. Cancer Res 62:4812–4819.

Jin RJ, Wang Y, Masumori N, Ishii K, Tsukamoto T, Shappell SB, Hayward SW, Kasper S, Matusik RJ. 2004. NE-10 neuroendocrine cancer promotes the LNCaP xenograft growth in castrated mice. Cancer Res 64: 5489–5495.

Kaplan-Lefko PJ, Chen TM, Ittmann MM, Barrios RJ, Ayala GE, Huss WJ, Maddison LA, Foster BA, Greenberg NM. 2003. Pathobiology of autochthonous prostate cancer in a pre-clinical transgenic mouse model. Prostate 55: 219–237.

Kasper S, Smith JA, Jr. 2004. Genetically modified mice and their use in developing therapeutic strategies for prostate cancer. J Urol 172:12–19.

Kasper S, Rennie PS, Bruchovsky N, Sheppard PC, Cheng H, Lin L, Shiu RPC, Snoek R, Matusik RJ. 1994. Cooperative binding of androgen receptors to two DNA sequences is required for androgen induction of the probasin gene. J Biol Chem 269:31763–31769.

Kasper S, Sheppard PC, Yan Y, Pettigrew N, Borowsky AD, Prins GS, Dodd JG, Duckworth ML, Matusik RJ. 1998. Development, progression, and androgen-dependence of prostate tumors in probasin-large T antigen transgenic mice: A model for prostate cancer. Lab Invest 78:i–xv.

Kasper S, Tu WH, Roberts RL, Shappell SB. 2003. Transgenic mouse models for prostate cancer: Indentification of an androgen dependent promoter and creation and characterization of the LPB-Tag model. Methods Mol Med 81:113–147.

Kelly WK, Scher HI. 1993. Prostate specific antigen decline after antiandrogen withdrawal: The flutamide withdrawal syndrome. J Urol 149:607–609.

Kim IY, Ahn HJ, Lang S, Oefelein MG, Oyasu R, Kozlowski JM, Lee C. 1998. Loss of expression of transforming growth factor-beta receptors is associated with poor prognosis in prostate cancer patients. Clin Cancer Res 4:1625–1630.

Kim MJ, Bhatia-Gaur R, Banach-Petrosky WA, Desai N, Wang Y, Hayward SW, Cunha GR, Cardiff RD, Shen MM, Abate-Shen C. 2002a. Nkx3.1 mutant mice recapitulate early stages of prostate carcinogenesis. Cancer Res 62:2999–3004.

Kim MJ, Cardiff RD, Desai N, Banach-Petrosky WA, Parsons R, Shen MM, Abate-Shen C. 2002b. Cooperativity

of Nkx3.1 and Pten loss of function in a mouse model of prostate carcinogenesis. Proc Natl Acad Sci USA 99: 2884–2889.

Kindblom J, Dillner K, Sahlin L, Robertson F, Ormandy C, Tornell J, Wennbo H. 2003. Prostate hyperplasia in a transgenic mouse with prostate-specific expression of prolactin. Endocrinology 144:2269–2278.

Kitsberg DI, Leder P. 1996. Keratinocyte growth factor induces mammary and prostatic hyperplasia and mammary adenocarcinoma in transgenic mice. Oncogene 13:2507–2515.

Klezovitch O, Chevillet J, Mirosevich J, Roberts RL, Matusik RJ, Vasioukhin V. 2004. Hepsin promotes prostate cancer progression and metastasis. Cancer Cell 6:185–195.

Konno-Takahashi N, Takeuchi T, Shimizu T, Nishimatsu H, Fukuhara H, Kamijo T, Moriyama N, Tejima S, Kitamura T. 2003. Engineered IGF-I expression induces glandular enlargement in the murine prostate. J Endocrinol 177:389–398.

Kulkarni AB, Huh CG, Becker D, Geiser A, Lyght M, Flanders KC, Roberts AB, Sporn MB, Ward JM, Karlsson S. 1993. Transforming growth factor beta 1 null mutation in mice causes excessive inflammatory response and early death. Proc Natl Acad Sci USA 90:770–774.

Kwabi-Addo B, Giri D, Schmidt K, Podsypanina K, Parsons R, Greenberg N, Ittmann M. 2001. Haploinsufficiency of the Pten tumor suppressor gene promotes prostate cancer progression. Proc Natl Acad Sci USA 98:11563–11568.

Lasky T, Silbergeld E. 1996. P53 mutations associated with breast, colorectal, liver, lung, and ovarian cancers. Environ Health Perspect 104:1324–1331.

Li H, Ahonen TJ, Alanen K, Xie J, LeBaron MJ, Pretlow TG, Ealley EL, Zhang Y, Nurmi M, Singh B, Martikainen PM, Nevalainen MT. 2004. Activation of signal transducer and activator of transcription 5 in human prostate cancer is associated with high histological grade. Cancer Res 64:4774–4782.

Lohnes D, Kastner P, Dierich A, Mark M, LeMeur M, Chambon P. 1993. Function of retinoic acid receptor gamma in the mouse. Cell 73:643–658.

Majumder PK, Yeh JJ, George DJ, Febbo PG, Kum J, Xue Q, Bikoff R, Ma H, Kantoff PW, Golub TR, Loda M, Sellers WR. 2003. Prostate intraepithelial neoplasia induced by prostate restricted Akt activation: The MPAKT model. Proc Natl Acad Sci USA 100:7841–7846.

Majumder PK, Febbo PG, Bikoff R, Berger R, Xue Q, McMahon LM, Manola J, Brugarolas J, McDonnell TJ, Golub TR, Loda M, Lane HA, Sellers WR. 2004. mTOR inhibition reverses Akt-dependent prostate intraepithelial neoplasia through regulation of apoptotic and HIF-1-dependent pathways. Nat Med 10:594–601.

Markowitz SD, Roberts AB. 1996. Tumor suppressor activity of the TGF-beta pathway in human cancers. Cytokine Growth Factor Rev 7:93–102.

Masumori N, Thomas TZ, Case T, Paul M, Kasper S, Chaurand P, Caprioli RM, Tsukamoto T, Shappell SB, Matusik RJ. 2001. A probasin-large T antigen transgenic mouse line develops prostate adeno and neuroendocrine carcinoma with metastatic potential. Cancer Res 61: 2239–2249.

Matusik RJ, Fleming WH, Hamel A, Westenbrink TG, Hrabarchuk B, MacDonald R, Ramsey E, Gartner JG, Pettigrew NM, Johnston B, Dodd JG. 1987. Expression of the c-myc proto-oncogene in prostatic tissue. Prog Clin Biol Res 239:91–112.

McGarvey TW, Stearns ME. 1995. Keratinocyte growth factor and receptor mRNA expression in benign and malignant human prostate. Exp Mol Pathol 63:52–62.

Nasi S, Ciarapica R, Jucker R, Rosati J, Soucek L. 2001. Making decisions through Myc. FEBS Lett 490:153–162.

Nevalainen MT, Ahonen TJ, Yamashita H, Chandrashekar V, Bartke A, Grimley PM, Robinson GW, Hennighausen L, Rui H. 2000. Epithelial defect in prostates of Stat5a-null mice. Lab Invest 80:993–1006.

Perez-Stable C, Altman NH, Brown J, Harbison M, Cray C, Roos BA. 1996. Prostate, adrenocortical, and brown adispose tumors in fetal globin T antigen transgenic mice. Lab Invest 74:363–373.

Phillips SM, Barton CM, Lee SJ, Morton DG, Wallace DM, Lemoine NR, Neoptolemos JP. 1994. Loss of the retinoblastoma susceptibility gene (RB1) is a frequent and early event in prostatic tumorigenesis. Br J Cancer 70: 1252–1257.

Pietenpol JA, Stewart ZA, 2002. Cell cycle checkpoint signaling: Cell cycle arrest versus apoptosis. Toxicology 181-182:475–481.

Pietrzkowski Z, Mulholland G, Gomella L, Jameson BA, Wernicke D, Baserga R, 1993. Inhibition of growth of prostatic cancer cell lines by peptide analogues of insulin-like growth factor 1. Cancer Res 53:1102–1106.

Pipas JM, Levine AJ, 2001. Role of T antigen interactions with p53 in tumorigenesis. Semin Cancer Biol 11:23–30.

Podsypanina K, Ellenson LH, Nemes A, Gu J, Tamura M, Yamada KM, Cordon-Cardo C, Catoretti G, Fisher PE, Parsons R, 1999. Mutation of Pten/Mmac1 in mice causes neoplasia in multiple organ systems. Proc Natl Acad Sci USA 96:1563–1568.

Polnaszek N, Kwabi-Addo B, Peterson LE, Ozen M, Greenberg NM, Ortega S, Basilico C, Ittmann M. 2003. Fibroblast growth factor 2 promotes tumor progression in an autochthonous mouse model of prostate cancer. Cancer Res 63:5754–5760.

Rennie PS, Bruchovsky N, Leco KJ, Sheppard PC, McQueen SA, Cheng H, Block ME, MacDonald BS, Nickel BE, Chang C, Liao S, Cattini PA, Matusik RJ. 1993. Characterization of two cis-acting elements involved in the androgen regulation of the probasin gene. Mol Endocrinol 7:23–36.

Scherl A, Li JF, Cardiff RD, Schreiber-Agus N. 2004. Prostatic intraepithelial neoplasia and intestinal metaplasia in prostates of probasin-RAS transgenic mice. Prostate 59:448–459.

Schneider A, Brand T, Zweigerdt R, Arnold H. 2000. Targeted disruption of the nkx3.1 gene in mice results in morphogenetic defects of minor salivary glands: Parallels to glandular duct morphogenesis in prostate. Mech Dev 95:163–174.

Shappell SB, Masumori N, Thomas TZ, Case T, Paul M, Kasper S, Matusik RJ. 2003. Transgenic mouse models of prostate carcinoma: Anatomic, histopathologic, and molecular considerations. In: Abel PD, Lalani E-N, editors. Prostate cancer: Clinical and scientific aspects—Bridging the gap. London: Imperial College Press. pp 245–319.

Shappell SB, Roberts RL, Thomas GV, Herbert R, Rubin MA, Humphrey PA, Ittmann MM, Sundbuerg JP,

Rozengurt N, Barrios R, Ward JM, Cardiff RD. 2004. Prostate pathology of genetically engineered mice: The consensus report from the Bar Harbor Meeting. Cancer Res 64:2270–2305.

Shibata MA, Maroulakou IG, Jorcyk CL, Gold LG, Ward JM, Green JE. 1996a. p53-independent apoptosis during mammary tumor progression in C3(1)/SV40 large T antigen transgenic mice: Suppression of apoptosis during the transition from preneoplasia to carcinoma. Cancer Res 56:2998–3003.

Shibata MA, Ward JM, Devor DE, Liu ML, Green JE. 1996b. Progression of prostatic intraepithelial neoplasia to invasive carcinoma in C3(1)/SV40 large T antigen transgenic mice: Histopathological and molecular biological alterations. Cancer Res 56:4894–4903.

Shim EH, Johnson L, Noh HL, Kim YJ, Sun H, Zeiss C, Zhang H. 2003. Expression of the F-box protein SKP2 induces hyperplasia, dysplasia, and low-grade carcinoma in the mouse prostate. Cancer Res 63:1583–1588.

Skalnik DG, Dorfman DM, Williams DA, Orkin SH. 1991. Restriction of neuroblastoma to the prostate gland in transgenic mice. Mol Cell Biol 11:4518–4527.

Song Z, Powell WC, Kasahara N, van Bokhoven A, Miller GJ, Roy-Burman P. 2000. The effect of fibroblast growth factor 8, isoform b, on the biology of prostate carcinoma cells and their interaction with stromal cells. Cancer Res 60:6730–6736.

Song Z, Wu X, Powell WC, Cardiff RD, Cohen MB, Tin RT, Matusik RJ, Miller GJ, Roy-Burman P. 2002. FGF8, Isoform b overexpression in prostate epithelium: A new mouse model for prostatic intraepithelial neoplasia. Cancer Res 62:5096–5105.

Stanbrough M, Leav I, Kwan PW, Bubley GJ, Balk SP. 2001. Prostatic intraepithelial neoplasia in mice expressing an androgen receptor transgene in prostate epithelium. Proc Natl Acad Sci USA 98:10823–10828.

Sun M, Wang G, Paciga JE, Feldman RI, Yuan ZQ, Ma XL, Shelley SA, Jove R, Tsichlis PN, Nicosia SV, Cheng JQ. 2001. AKT1/PKBalpha kinase is frequently elevated in human cancers and its constitutive activation is required for oncogenic transformation in NIH3T3 cells. Am J Pathol 159:431–437.

Taplin ME, Bubley GJ, Shuster TD, Frantz ME, Spooner AE, Ogata GK, Keer HN, Balk SP. 1995. Mutation of the androgen-receptor gene in metastatic androgen-independent prostate cancer [see comments]. N Engl J Med 332:1393–1398.

Tehranian A, Morris DW, Min BH, Bird DJ, Cardiff RD, Barry PA. 1996. Neoplastic transformation of prostatic and urogenital epithelium by the polyoma virus middle T gene. Am J Pathol 149:1177–1191.

Thomson AA. 2001. Role of androgens and fibroblast growth factors in prostatic development. Reproduction 121:187–195.

Treiger B, Isaacs J. 1988. Expression of a transfected v-Harvey-ras oncogene in a Dunning rat prostate adenocarcinoma and the development of high metastatic ability. J Urol 140:1580–1586.

Tu WH, Thomas TZ, Masumori N, Bhowmick NA, Gorska AE, Shyr Y, Kasper S, Case T, Roberts RL, Shappell SB, Moses HL, Matusik RJ. 2003. The loss of TGF-beta signaling promotes prostate cancer metastasis. Neoplasia 5:267–277.

van der Kwast TH, Schalken J, Ruizeveld de Winter JA, van Vroonhoven CC, Mulder E, Boersma W, Trapman J. 1991. Androgen receptors in endocrine-therapy-resistant human prostate cancer. Int J Cancer 48:189–193.

Vazquez F, Sellers WR. 2000. The PTEN tumor suppressor protein: An antagonist of phosphoinositide 3-kinase signaling. Biochim Biophys Acta 1470:M21–M35.

Voelkel-Johnson C, Voeks DJ, Greenberg NM, Barrios R, Maggouta F, Kurtz DT, Schwartz DA, Keller GM, Papenbrock T, Clawson GA, Norris JS. 2000. Genomic instability-based transgenic models of prostate cancer. Carcinogenesis 21:1623–1627.

von Wichert G, Jehle PM, Hoeflich A, Koschnick S, Dralle H, Wolf E, Wiedenmann B, Boehm BO, Adler G, Seufferlein T. 2000. Insulin-like growth factor-I is an autocrine regulator of chromogranin A secretion and growth in human neuroendocrine tumor cells. Cancer Res 60:4573–4581.

Wang Y, Hayward SW, Donjacour AA, Young P, Jacks T, Sage J, Dahiya R, Cardiff RD, Day ML, Cunha GR. 2000. Sex hormone-induced carcinogenesis in Rb-deficient prostate tissue. Cancer Res 60:6008–6017.

Wang S, Gao J, Lei Q, Rozengurt N, Pritchard C, Jiao J, Thomas GV, Li G, Roy-Burman P, Nelson PS, Liu X, Wu H. 2003. Prostate-specific deletion of the murine Pten tumor suppressor gene leads to metastatic prostate cancer. Cancer Cell 4:209–221.

Wennbo H, Kindblom J, Isaksson OG, Tornell J. 1997. Transgenic mice overexpressing the prolactin gene develop dramatic enlargement of the prostate gland. Endocrinology 138:4410–4415.

Wikstrom P, Stattin P, Franck-Lissbrant I, Damber JE, Bergh A. 1998. Transforming growth factor beta1 is associated with angiogenesis, metastasis, and poor clinical outcome in prostate cancer. Prostate 37:19–29.

Wu X, Wu J, Huang J, Powell WC, Zhang J, Matusik RJ, Sangiorgi FO, Maxson RE, Sucov HM, Roy-Burman P, 2001. Generation of a prostate epithelial cell-specific Cre transgenic mouse model for tissue-specific gene ablation. Mech Dev 101:61–69.

Yan Y, Sheppard PC, Kasper S, Lin L, Hoare S, Kapoor A, Dodd JG, Duckworth ML, Matusik RJ, 1997. A large fragment of the probasin promoter targets high levels of transgene expression to the prostate of transgenic mice. Prostate 32:129–139.

Zhang X, Chen MW, Ng A, Ng PY, Lee C, Rubin M, Olsson CA, Buttyan R. 1997. Abnormal prostate development in C3(1)-bcl-2 transgenic mice. Prostate 32:16–26.

Zhang ZF, Thomas TZ, Kasper S, Matusik RJ. 2000a. A small composite probasin promoter confers high levels of prostate-specific gene expression through regulation by androgens and glucocorticoid in vitro and in vivo. Endocrinology 141:4698–4710.

Zhang X, Lee C, Ng PY, Rubin M, Shabsigh A, Buttyan R. 2000b. Prostatic neoplasia in transgenic mice with prostate-directed overexpression of the c-myc oncoprotein. Prostate 43:278–285.