[Frontiers in Bioscience 5, d372-390, April 1, 2000]

CELL-CYCLE DYSREGULATION AND THE MOLECULAR MECHANISMS OF PROSTATE CANCER

Derek F. Amanatullah, Anne T. Reutens, Brian T. Zafonte, Maofu Fu, Sridhar Mani, and Richard G. Pestell

The Albert Einstein Cancer Center, Department of Developmental and Molecular Biology, Department of Medicine, Albert Einstein College of Medicine, 1300 Morris Park Ave., Bronx, NY 10461

TABLE OF CONTENTS

1. ABSTRACT

Prostate cancer is the most common cause of non-cutaneous cancer in men and although frequently latent is the second commonest cause of death. Screening for the disease was historically based on symptoms of urethral obstruction, clinical examination of the prostate gland and serum measurements of prostate specific antigen. As prostate cancer growth in the early stages is enhanced by androgens, the mainstay of therapy has been androgen ablation by pharmaco-therapeutic or surgical means. The subsequent development of androgen therapy resistant prostate cancer in many patients, for whom therapeutic options remain limited, has led researchers to focus attention on understanding the molecular genetics of prostate cancer. The array of genetic abnormalities observed in prostate tumors, which include changes in components of the cell cycle, suggest the disease is quite heterogeneous and may require further sub-classification based on genetic markers. Such analyses may lead to identification of relevant new prognostic and therapeutic indicators. The advent of transgenic mouse models of prostate cancer may provide a critical tool for the implementation of rational genetic based therapeutics and alternate drug design.

2. INTRODUCTION

2.1. Prostate Cancer: The Pathology

The adult male prostate is a twenty-gram retroperitoneal organ that surrounds the neck of the bladder and prostatic urethra. The prostate can be divided into several zones: peripheral, central, transitional, and periurethral. Clinically, these zones are important because 70% of prostatic adenocarcinomas develop from the peripheral zone. Normal prostate consists of tubuloalveolar glands embedded in fibromuscular stroma. The prostate glands consist of a well-defined basement membrane covered by low cuboidal basal epithelia and mucous-secreting columnar cells that rest on top of the basal cells.

Although an area of controversy, it is likely that prostate cancers arise from prostatic intraepithelial neoplasia (PIN) but not benign prostatic hyperplasia (BPH) (1,2). Prostate adenocarcinoma arises in 75- to 80% of cases from the peripheral zone, whereas BPH occurs in the periurethral transitional region of the human prostate (1,2). PIN by contrast typically co-localizes with and shares similar genetic lesions to prostate cancer (3-5).

Table 1. Distribution of various types of cancer

Cancer	Estimated distribution of male cancer incidence, U.S.A., 1999 (%)	Estimated distribution of male cancer deaths U.S.A, 1999 (%)
Lung and bronchus	15	31%
Oral cavity and pharynx	4	
Esophagus		3
Stomach		3
Pancreas	2	5
Liver and intrahepatic bile duct		3
Colon and rectum	10	10
Prostate	29	13
Non-Hodgkin's lymphoma	6	5
Melanoma of the skin	5	
Kidney and renal pelvis	3	
Leukemia	3	4
All other sites	20	20

Reference (186)

Table 2. Two highest reported cancer sites leading to death in males, U.S.A., 1995

Age	First	Second	Total deaths all types
<19	Leukemia (465)	Nervous system (300)	1,341
29-39	Non-Hodgkin's lymphoma (800)	Leukemia (686)	5,683
40-59	Lung and bronchus (15, 606)	Colon and Rectum (4,275)	46,081
60-79	Lung and bronchus (60,721)	Prostate (17,773)	164,796 3,7054
<80	Prostate (15,657)	Lung and bronchus (14,475)	281,611
All ages	Lung and bronchus (91,800)	Prostate (34,475)	

The number in parenthesis refer to number of deaths

2.2. Prostate Cancer Screening

Patients may present with symptoms related to urethral obstruction (urinary frequency, hematuria, difficulty initiating urination, or dysuria) or more commonly may be asymptomatic. Prostate cancer can be detected by digital rectal exam, transrectal ultrasonography and prostate-specific antigen (PSA) screening, but biopsy is required for absolute diagnosis. Biological markers for prostate cancer have been identified and include prostate acid phosphatase (PAP) and PSA. PSA is normally secreted by the prostatic epithelium into the semen and helps to liquefy the seminal coagulum before ejaculation. However, elevated blood levels of PSA (greater than 4 mg/ml) correlate with prostate cancer, but false positive results can occur. Constant PSA monitoring can be used to access the outcome of previous treatment or surgery and monitor current prostate cancers for progression (3). PSA is believed to augment the cleavage and activation of growth factors like transforming growth factor-β (TGF-β). This function may explain why prostate cancer tends to invade diffusely due to the growth advantage provided by the influx of growth factors, and emphasizes the importance of PSA control (6). Other predictors of prostate cancer have been derived from the serum PSA concentrations like PSA density (serum PSA/volume of prostate), PSA velocity (change in serum PSA with time), and percentage of free PSA (serum PSA not bound to α1-antichymotrypsin /total serum PSA) in an attempt to improve prediction of prostate cancer.

2.3. Epidemiology

Prostatic adenocarcinoma is the most common form of non-cutaneous cancer in men with an incidence of 179,300 (29%) new cases per year and is the second commonest cause of cancer death in males after lung cancer in the United States with 37,000 (13%) deaths in 1999 (Table 1) (7). The rising age of American men and early detection programs with improved screening for prostate cancer have caused the incidence rate of prostate cancer to increase dramatically. However, the mortality rate from prostate cancer has remained constant (8,9). The prevalence of latent clinically undetectable prostate cancer is thought to be as high as 80% of patients over 80 years of age. The majority of men that have prostate cancer will not be diagnosed with the disease however, and death rates are low compared with the number of cases diagnosed each year (8,9).

The etiology of prostate cancer is likely multifactorial involving environmental and genetic factors. Prostate cancer tends to be a disease of older men with greater than 75% of the diagnoses being in men over 65 years of age (Table 2). There are racial and ethnic differences in both incidence and mortality rates (2). The incidence and mortality rates of African-American men are twice those of Caucasian men, and African-American men present earlier in life with higher grade cancer (wwwosp.nci.nih.gov/planning/prg/default.htm). The incidence and mortality rates of Asian/Pacific Islander men are half those of Caucasian men. Other risk factors for the development of prostate cancer include high-fat diet and obesity, high androgen levels, smoking, sexual behavior and genetic mutations. Castration and high levels of estrogen may be protective factors. These genetic factors are complicated by additional familial, occupational, socioeconomic, and environmental influences.

2.4. Molecular genetics

Analyses of pedigrees in the Mormon population support a role for heredity in prostate cancer. Men who had a father or brother with prostate cancer were twice as likely to develop the cancer themselves. This is consistent with a highly penetrant rare autosomal dominant inheritance pattern (8,9). Several different chromosomal rearrangements or deletions have been observed in prostate

cancer, including loss of sequences within the short arm of chromosome 8, chromosome 6q, 8p, 10q, 12ql3.3-14.1, 13q and 16q (10). The *c-myc* oncogene (locus 8q24) and the *androgen receptor* gene (Xq11--13) have been implicated in prostate cancer through the finding of frequent gain of sequences on chromosome 8q and the X chromosome respectively (2,11). Cloning of regions within translocations led to the identification of candidate tumor suppressor genes including a nuclear co-repressor *NAB2* (12), a dual specificity phosphatase *PTEN* (13) *MXI1, RB* on 13q and *BCL2* on 18q. Linkage studies identified breast cancer 1 *(BRCA1),* a gene involved in DNA damage repair, with a familial history of prostate cancer in addition to breast and ovarian cancer. Also, hereditary prostate cancer-1 *(HPC-1)* located on 1q24-25 was found to be a prostate cancer susceptibility gene due to its linkage in 66% of families tested. Both *BRCA1* and *HPC-1* represent excellent predictive candidates for genetic prostate cancer susceptibility.

Murine *Nkx3.1,* a homeobox gene, is involved in the normal genesis of the prostate and is the earliest marker of prostatic epithelium. Homozygous and heterozygous *Nkx3.1* mutant mice develop prostatic hyperplasia and dysplasia, evidence that supports a role for *Nkx3.1* as an early developmental tumor suppressor gene (14). Recently, human papillomavirus (HPV) has been implicated in the promotion of prostate cancer, because HPV DNA has been identified in both benign hyperplasia and adenocarcinoma of the prostate. One caveat to this observation is that HPV is thought to infect only squamous tissue such as in cervical squamous carcinoma.

Clinically, it is impossible to distinguish the hereditary form of prostate cancer from the sporadic form, because the tumors are multifocal. This is a phenomenon whereby carcinogens give rise to a "field effect" and spontaneously transform several cells that consequently undergo clonal expansion. This induction of multiple lesions in the prostate makes it impossible to separate the inherited and acquired mutations, making prostate cancer difficult to study due to the heterogeneous population of cancer cells. Laser capture micro-dissection may allow investigators to select precisely cell populations within the adenocarcinoma (15).

3.CELLULAR BIOLOGY AND MOLECULAR MECHANISMS

3.1. The HER-2/Neu receptor in prostate cancer

Neu is a member of a growth factor receptor family that includes the epidermal growth factor receptor (EGFR), and is also known as HER-2, for human EGF receptor 2, or c-ErbB-2. The ligands for the Neu receptor are distinct from those for the structurally related EGFR (16). However, the EGF receptor is capable of dimerizing with Neu and this heterodimer can form when only one member of the pair binds ligand (16). HER-2/Neu is normally expressed in prostate epithelial cells (17,18) and the heregulin ligand is expressed in the stroma and basal epithelium of the normal prostate gland (19). The *Neu* proto-oncogene encodes a tyrosine kinase receptor that is amplified and overexpressed in a significant proportion of prostate cancers. Overexpression of ErbB-2/neu and ErbB-3 has been implicated in the neoplastic transformation of prostate carcinoma (20,21). The serum levels of Neu correlated with poor prognosis since patients with elevated levels had a significantly shorter interval to disease progression than those with normal level (22). The predictive role of Neu in prostatic tumors remains controversial. Increased Neu correlated with worse histochemical features in some (23,24) but not all studies (25). In recent studies, ErbB-2 overexpression correlated with a metastatic phenotype and poor prognosis (26).

Monoclonal antibodies to ErbB-2 inhibit cellular proliferation of prostate cancer cell lines (PCCL) (27), suggesting ErbB-2 contributes to proliferative signaling. EGF treatment of PCCL induces cellular proliferation (28) and recently, Neu overexpression was shown to promote androgen-independent prostate cancer cell growth (29). Oncogenic activation by Neu can occur through overexpression, point mutation within the transmembrane domain or deletion of the extracellular domain (30). A variety of mutations within the transmembrane domain of the coding sequence of the rat Neu gene, for example NeuT, are highly oncogenic (30). Introduction of the NeuT oncogene induced a malignant phenotype in the rat ventral prostate line NbE2 (31), and in PC3 cells induced anchorage-independent growth and a highly metastatic phenotype (32). Overexpression of ErbB-2 induced androgen-independent growth in LNCaP cells (29). Neu activates several signaling pathways in prostate cancer cell lines including the GTPase activating protein, phospholipase C-γ1 (21) and the tyrosine kinase c-Src. In LNCaP cells, ErbB-2 activates MAPK signaling. Interleukins, which induce MAPK activation, do so at least in part through the ErbB-2 receptor (33). Inhibitors of ErbB-1 activity block cellular proliferation in PCCL (DU145) (34).

Induction of cyclin D1 or reduction in $p27^{Kip1}$ levels may be important in the ErbB-2 induced tumorigenic function. EGF induces cyclin D1 in PCCL (35). We have previously shown that both interleukins and STATs (36,37) and ErbB-2 (38) induce cyclin D1 expression in several cell types. Several other growth factors that have been shown to stimulate prostate cancer cellular proliferation also induce cyclin D1 abundance in other cell types. The growth factors that have been shown to induce prostate cellular proliferation include epidermal growth factor (EGF) (39), insulin-like growth factor-1 (IGF-1) (40), transforming growth factor-α (TGFα), (41), platelet derived growth factor (PDGF), nerve growth factor (NGF) (42) and fibroblast growth factor (FGF) (43). These agents have also been shown to induce cyclin D1 expression in other cell types (44). Cyclin D1 anti-sense constructs were shown to inhibit ErbB-2-induced tumor growth in nude mice and cell transformation in Rat-1 assays. Together, these findings suggest the possibility that ErbB-2 induction of cyclin D1 may also contribute to tumor growth and survival in PCCL.

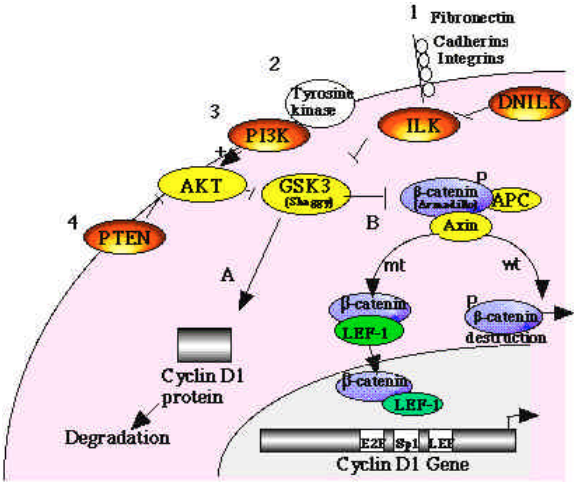

Figure 1. The β-catenin signaling pathway in prostate cancer. Activation of (1) integrin signaling and integrin linked kinase activity, (2) tyrosine kinase receptors, (3) PI3-kinase and (4) loss of the phosphatase PTEN, each contribute to the inhibition of GSK-3β. Inhibition of GSK-3β increases cyclin D1 levels through reduced protein degradation and increased transcriptional activation. The targets of GSK3β, whose dephosphorylation propagates Wnt signaling, may include the β-catenin homolog Armadillo and Armadillo-interacting proteins such as the Adenomatous Polyposis Coli (*APC)* gene product. APC associates with the α and β catenins and triggers the degradation of free β-catenin.

3.2. PTEN in prostate cancer

The *PTEN/MMAC1* phosphatase is a tumor suppressor gene implicated in a wide range of human cancers. Homozygous deletion and point mutations were observed in a set of prostate cancer samples and cell lines (45,46) and PTEN expression levels are reduced in prostate cancer xenografts derived from patients with advanced prostate cancer (47). PTEN/MMAC1 acts a negative regulator of the phosphoinositide 3-kinase (PI3-kinase)/Akt pathway (48). PTEN/MMAC1 impairs activation of endogenous Akt in cells (47). Higher levels of Akt activation are observed in human prostate cancer cell lines and xenografts lacking PTEN/MMAC1 expression when compared with PTEN/MMAC1-positive prostate tumors or normal prostate tissue (49). Induction of Akt activity provides an antiapoptotic signal (50). In addition, PTEN/MMAC1 represses gene expression in a manner that is rescued by Akt but not PI3-kinase (48) and inhibits G_1 phase progression in cells that lack PTEN (51). Because constitutive activation of either PI3-kinase or Akt is known to induce cellular transformation, an increase in the activation of this pathway caused by mutations in PTEN/MMAC1 provides a potential mechanism for its tumor suppressor function.

The cell cycle targets of PTEN regulating cellular proliferation and transformation are an area of active investigation and may include cyclin D1. PTEN inhibits PI3-kinase signaling and cyclin D1 is induced by the PI3-kinase signaling pathway through direct transcriptional induction of the promoter in one study (52). Another study using chemical inhibitors implicated a post-translational mechanism in PI3-kinase-dependent induction of cyclin D1, although the authors also stated that a direct transcriptional induction was not precluded from the studies nor directly assessed (53). Glycogen synthase kinase-3β (GSK-3β)-dependent mechanisms regulate post-translational stability of cyclin D1. GSK-3β phosphorylation of cyclin D1 enhances its proteolysis (54). Therefore, inhibition of GSK-3β would be expected to increase cyclin D1 levels (Figure 1).

3.3. Cell adhesion molecules and the b-catenin signaling pathway

The Wnt-1 protein is a secreted protein that plays a role in tumorigenesis (reviewed in: (55,56)). Wnt family members are widely expressed and the Wnt receptors belong to the *frizzled* seven transmembrane domain family. Several components of the Wnt pathway have been implicated in cancer (55). Mammalian GSK-3β normally inhibits the Wnt pathway. The targets of GSK-3β, whose dephosphorylation propagates Wnt signaling, include β-catenin and the Adenomatous Polyposis Coli (APC) gene product which is mutated in many colon cancers.

APC associates with α– and β-catenins and triggers the degradation of free β-catenin, keeping its levels low (57) (Figure 1). Increased free β-catenin levels in many cancers lead to the activation of gene transcription by the association of β-catenin with transcription factors of the LEF-1 family. Mutant forms of β-catenin have been discovered in a variety of tumors including prostate cancer (58). The nature of the target genes of the β-catenin/LEF complex which may promote cellular proliferation and tumorigenesis were largely unknown. Recent studies however, identified the c-*MYC* gene (59), and in our laboratory the *cyclin D1* gene, as transcriptional targets activated by the β-catenin signaling pathway (60,61).

Loss of E-cadherin correlates with poor prognosis in prostate cancer (2). E-cadherins are tumor suppressor genes and when their expression is low, cell-cell adhesion is reduced, differentiated morphology is lost and a more invasive phenotype results. This could result from the loss of E-cadherin expression or mutation (i.e. "two hit" model requiring two different mutations for tumor initiation (62)) as well as mutations in α-catenin, APC or β-catenin. When α-catenin is mutated, cell-cell contact is also reduced since the cytoskeleton cannot link to the cadherin. *KAI1* on 11p and *CD44*, both of which participate in the regulation of cell-extracellular matrix interaction and cell-cell interactions, are lost in metastatic prostate cancer and have thereby become implicated in prostate cancer progression.

3.4. Androgen receptor mutations in prostate cancer

The androgen signaling cascade is believed to play a major role in the pathogenesis and treatment of

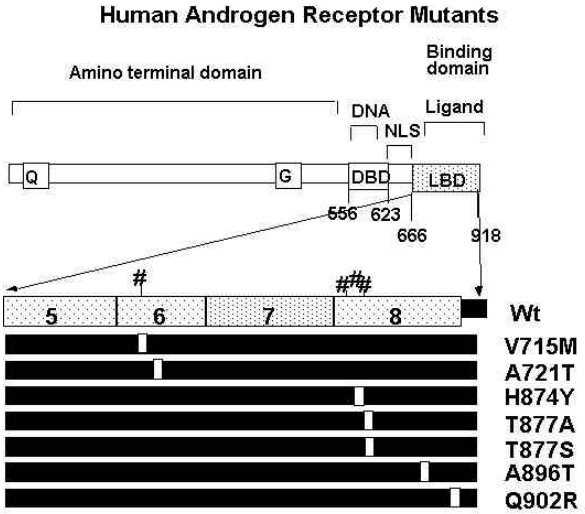

Figure 2. Mutations of the androgen receptor identified in patients with androgen therapy resistant prostate cancer. Schematic representation of the human AR indicating the DNA binding domains (DBD) and ligand binding domains (LBD). The LBD has been expanded to show the site of somatic mutations identified in human prostate cancer.

prostatic adenocarcinoma, because androgens are necessary for the maintenance of the normal and neoplastic prostatic epithelium. Androgens circulate in the serum bound to steroid-binding globulin and can readily diffuse into cells. 90% of the testosterone is converted to a more potent androgen, dihydrotestosterone (DHT), by 5α-reductase (5αR). DHT or testosterone can bind the intracellular androgen receptor (AR). The AR is a member of the steroid hormone receptor superfamily and contains two conserved N-terminal transactivation domains, a conserved cysteine-rich DNA binding domain with two zinc fingers, a variable hinge region, a conserved hormone-binding domain (HBD), and a variable C-terminal tail region (Figure 2). Normally, heat-shock proteins bind to the HBD and keep the AR inactive, but when an androgen displaces the heat-shock protein and binds the HBD, the AR undergoes a conformational change. Two active androgen/AR complexes then dimerize to form a functional unit that binds DNA containing an androgen response element (ARE) and stimulate gene transcription. AR function is also regulated through the recruitment of p160 co-activator proteins at the amino-terminal domain (63). Peptide growth factors (IGF-1 and EGF) can interact with the AR pathways via a ligand-independent tyrosine kinase cross talk.

The majority of patient deaths from metastatic prostate cancer result from androgen-independent tumors. Since most prostate cancers express the AR regardless of androgen sensitivity, changes in the AR other than loss of expression must be present. Alterations in the AR cascade could be caused by changes in testosterone metabolism or binding, mutations in the AR (DNA binding, transactivation, or HBD), mutation of an ARE, amplification of the AR, cross-talk due to androgen receptor homology, or dysregulated growth factor pathways. Several of these mutations could reduce the efficacy of androgen ablation therapy and lead to androgen insensitivity, while conferring a selective advantage that would allow the progression of the cancer even with treatment. As many as half of metastatic prostate tumors that had escaped androgen ablation therapy had somatic mutations in the AR gene (Figure 2) (64). Several subsequent studies have confirmed these observations and mutations are clustered within the carboxyl-terminus of the AR (65). Although the carboxyl-terminus binds ligand, deletion within amino acids 80-93 of the AR amino-terminal activation domain enhanced basal level activation and abolished ligand-dependent regulation (66). The AR mutations that are found in the tumors of patients with androgen-resistant prostate cancer are typically somatic point mutations causing amino acid substitutions in the hormone-binding domain. Like the wild type AR, the mutant ARs found in patients with prostate cancer are induced by ligand. In contrast with the wild type AR, mutant ARs responded promiscuously to a variety of hormones (estrogen, progesterone) that would normally have no effect on the receptor (64,65). It has been proposed that these somatic AR mutations contribute to autonomous prostate cancer cellular growth (64,65,67). The mechanisms governing AR induction of cellular proliferation are unclear. However, recent studies suggest that the G_1 phase cyclins are induced by androgen (68).

LNCaP tumor cells are derived from a metastatic human prostate cancer. They contain the AR and proliferate in response to androgens. However, LNCaP cells, which lack estrogen and progesterone receptors, also proliferate when exposed to estrogen, progesterone and some anti-androgens (Flutamide). A threonine to alanine mutation (codon 877: ACT(GCT) identified in the HBD of the AR in LNCaP allowed the AR to cross react with anti-androgens, estrogens and progestins (69). Therefore, a single point mutation in the AR could reverse the effects of anti-androgen therapies, assuming it was present in the LNCaP cells prior to establishment of the cell line. Indeed, 6 of 24 transurethral prostatic resections from patients with metastatic prostate cancer showed the same LNCaP codon 877 mutation. Codon 877 represents the first mutational "hot spot" in the AR gene that could contribute to the aggressive hormone-refractory phenotype of advanced prostate cancer (70). A valine to methionine mutation (codon 730: GTG(ATG) was identified in the HBD from 1 of 26 radical prostatectomies (71). A similar valine to methionine mutation was found in codon 715 of an androgen-resistant specimen. This mutation showed a different spectrum of transactivation than the LNCaP cells, but serves to highlight the promiscuity of the AR for several ligands and the potential role of AR mutations in androgen ablation therapy resistance (67). Another study identified the codon 877 mutation and codon 701 (CAC(CAC) resulting in the substitution of histidine for leucine (72). Interestingly, AR mutations were found in only 12.5% of the hormone-resistant cases, suggesting that mechanisms other than AR mutation are involved in hormone-resistant prostate cancer (72). Reverse transcriptase polymerase chain reaction studies

demonstrated that AR mutations were present in 50% of metastatic androgen-insensitive tumors. Mutations in codon 877 and 715 were present in these metastatic androgen-insensitive tumors and highlighted the mutation of conserved amino acids of the AR in the progression of prostate cancer even in the presence of androgen ablation therapy (64,73).

The steroid binding capacity of the AR is not a completely accurate measure of AR function, since deletion of the N-terminal domain or the DNA binding domain renders the AR transcriptionally inactive but able to bind androgens. However, deletion of the HBD renders the AR unable to bind steroid but constitutively active even in the absence of androgens (74,75). Point mutations in the DNA binding domain and transactivation domain of the AR (1832: G/A, 1883: T(C, 1945: A/G, and 2006: G/C are often the source of receptor-binding positive androgen resistance. Thus, even though the androgen-AR complex can be formed, it cannot bind the ARE (76).

Besides regulating the survival and growth of the prostatic epithelium, the AR also has several novel functions that indicate how it could contribute to cancer progression in other ways. The AR can negatively regulate the expression of matrix metalloproteinases (MMPs) and may play a role in tumor metastasis. MMPs are metal containing enzymes that degrade the extracellular matrix (77). One member of the serpin family, mapsin, is normally expressed in the prostatic epithelium. Lower levels of mapsin correlate with a higher metastatic potential (78). Mapsin expression is induced through an Ets domain and is inhibited by a hormone response element (HRE) that is recognized by the androgen/AR complex. The reduced Ets abundance in prostate tumors may contribute to reduced mapsin expression in prostate cancer. The AR regulation of MMPs and mapsin may contribute to prostatic cancer invasiveness and metastasis.

3.5. Cyclins and Cyclin Dependent Kinases

The cyclin dependent kinases (CDKs) are a family of serine/threonine kinases that play a pivotal role in controlling progression through the cell cycle (44). The regulatory subunits of the CDKs, known as cyclins, form complexes with their catalytic partner to function as kinases of specific proteins at different phases of the cell cycle (44,79-81). The D cyclins are involved in regulation of the G_1 phase of the cell cycle. Our laboratory and others have shown a pivotal role for cyclin D1 in cell-cycle progression in fibroblasts (82,83), myocytes (84) and breast epithelial cells (85). Inhibition of cyclin D1 expression results in cell-cycle arrest whereas moderate overexpression accelerates G_1 phase progression (83,86,87). Cyclin D1 is rate-limiting in growth factor or estrogen-induced mammary epithelial cell proliferation (85) and is therefore a critical target for proliferative signals in G_1. Homozygous deletion of the *cyclin D1* gene (88,89) and the *D2* gene (90) resulted in mice with distinct phenotypes. The *cyclin D1*$^{-/-}$ mice have failure of normal mammary gland development and retinal apoptosis (88,89), indicating a specific and important role for cyclin D1 in normal mammary gland development.

The D cyclins are found associated with several different intracellular proteins. The three D type cyclins share conserved domains (D1-3, (91)), and form physical complexes with pRB (80,92,93). However, cyclin D1 overexpression is selectively associated with prostate and breast tumorigenesis. Unlike the mechanisms of transformation for several other oncogenes in which mutant proteins are responsible, the cyclin D1 protein and coding sequence from tumors examined to date are normal (94), suggesting it is the overexpression of cyclin D1 *per se* that is linked to the formation of tumors. Overwhelming evidence suggests it is the overexpression of cyclin D1 rather than D2 or D3 which is important in breast tumorigenesis. The main region of structural divergence between cyclin D1, D2 and D3 lies in the carboxyl-terminus, though the function of the cyclin D1 carboxyl-terminus remains to be determined. Together these findings suggest that overexpression of cyclin D1 can promote tumor formation either by collaborating in cellular transformation with known transforming factors or by antagonizing the action of tumor suppressor genes.

3.6. Cyclin D1 in prostate cancer

Cyclin D1 is widely expressed in normal tissues including the normal prostate. In a recent analysis, cyclin D1 mRNA levels were increased in six prostate cancer cell lines (PCCL) examined and 25% of prostate cancer samples examined (95). The frequency of cyclin D1 overexpression in human prostate cancer samples demonstrated was 30% in one study (96) but lower in another (97). Overexpression of cyclin D1 increases cell growth and tumorigenicity in LNCaP cells (68). Several lines of evidence are consistent with a model in which abnormalities previously identified in prostate cancer, may contribute to cellular proliferation through the induction of cyclin D1 abundance. Abnormalities that have been identified in prostate cancer include overexpression of ErbB-2, loss of PTEN, increased β-catenin levels and mutations of the AR. LNCaP cells overexpressing cyclin D1 develop tumors faster than controls and do not regress with castration (68). Overexpression of components of the cell cycle machinery may therefore contribute to androgen-independent growth and androgen ablation therapy resistance (68).

The cyclins may function to link androgens and their proliferative effects on the prostatic epithelium. In castrated rats exposed to androgens, cyclins D1, D2, D3 and E are induced and correlate with an increase in cellular proliferation. Cyclin D1 mRNA and protein peaked early while cyclin D3 and cyclin E mRNA and protein peaked later. Although cyclin D2 mRNA was induced, there was no change in protein level. Cdk6 was induced early followed by the induction of Cdk2. Cdk4 mRNA level was constant over the treatment period. The cyclin and Cdk expressions were consistent with their role in the cell cycle:

cyclin D1/Cdk4 or Cdk6 in early G_1 and cyclin E/Cdk2 in late G_1-S transition. These data suggest that androgens activate several cell cycle components governing the G_1 phase of the cell cycle (98).

3.7. Cyclin Dependent Kinase Inhibitors (CKIs)

The G1 phase of the cell cycle is inhibited by two families of CKIs. The two CKI families, Cip/Kip and INK4, have previously been shown to inactivate the cyclin–Cdk holoenzyme complexes (80,81,99,100). The INK4a/ARF locus encodes two distinct gene products, p16INK4a and human p14ARF (murine p19ARF) which are modulators of the pRB and p53 pathways respectively (101). Members of the Cip/Kip family include $p21^{Cip1/WAF1}$, $p27^{Kip1}$ and $p57^{Kip2}$, whereas p16Ink4a, p15ink4b, p18ink4c, and p19ink4d comprise the INK4 inhibitor group. The INK4 CKIs specifically inhibit the catalytic domains of Cdk4 and Cdk6. The broader acting Cip/Kip "inhibitors" can function as either inhibitors or activators of Cdk function. Historically, the Cip/Kip "inhibitors" were considered universal inhibitors that at high concentrations/signal intensities blocked cyclin D-, E-, and A-dependent kinase activities (102,103). More recently the "activator" function of the Cip/Kip proteins has been examined. In immunodepletion studies, p21Cip1/WAF1 was shown to function in a dose-dependent manner, inhibiting Cdk activity only at high concentrations (104).

p27Kip1 levels decrease in response to mitogens and increase in quiescence. Increasing levels of p27Kip1 serve as a barrier to G1-S transition and may promote cell cycle exit. p27Kip1 is rarely mutated, but its reduced expression has been seen in several cancers including prostate. Benign prostatic hyperplasia, prostatic intraepithelial neoplasia (PIN), and prostatic carcinoma all exhibit decreased levels of p27Kip1, likely contributing to their high mitotic rates (105). Recently, reduced p27Kip1 has shown promise as a marker of malignant prostate cancer (106-108). Decreased expression of p27Kip1 has been correlated with androgen ablation failure, high Gleason grade, aggressive metastases and poor prognosis (105,109).

4. OTHER CANDIDATES IN PROSTATE CANCER

4.1. Transforming Growth Factor-b

TGF-β has been shown to stimulate growth in LNCaP cells (probably due to the expression of a TGF-β receptor) and inhibit growth in DU145 and PC3 cells. TGF-β is expressed after castration and inhibits prostatic growth even in the presence of DHT. The actions of the TGF-β family are complex and may depend on the extra- and intracellular environments (110). Activins are a member of the transforming growth factor-β (TGF-β) family and regulate cell growth and differentiation. Activin inhibits androgen-responsive prostate cells (LNCaP) but not androgen-resistant prostate cells (PC3) (111). This indicates a possible growth advantage conferred by activin on cells for the progression to androgen resistance (112).

4.2. Fibroblast Growth Factor

Changes in the expression of fibroblast growth factor receptor (FGFR) have been seen in prostate cancer. FGFR2 splice variant IIIb switches to FGFR2 variant IIIc in malignant metastatic tumors and can no longer respond to FGF-7. FGF-7 is normally secreted by the stromal cells, and has been implicated in triggering prostate oncogenesis (113,114). These tumors also express FGFR1, which is normally expressed only by stromal cells. In this tumor cell type, FGFR1 supports the mitogenic response to FGF-7, whereas restoration of FGFR2IIIb to malignant cells expressing FGFR1 decreases tumor growth and restores the differentiated phenotype (115).

4.3. Myc, Ras, p53, Bcl-2 and telomerase

c-Myc mRNA is expressed in hyperplastic and malignant prostate. However, the role of c-Myc in prostate cancer remains controversial. As noted above, gains of sequences on the long arm of chromosome 8 containing the 8q24 *c-myc* locus are common events in prostate cancer (11). Amplifications are more frequent in hormone-refractory prostate cancer. In fluorescence in-situ hybridization (FISH) and microarray analyses of hormone-refractory prostate cancer metastases, 22% had *AR*, 11% had *MYC*, 5% had *CCND1*, and none had *ERBB2* amplifications. These amplifications suggest the failure of hormone therapy for prostate cancer and implicate Myc in the metastatic progression of this disease.

Following castration of adult rats, the steady state mRNA levels of TGF-β, c-Myc, c-Fos, testosterone-repressed prostate mRNA 2 (TPRM-2) and tissue plasminogen activator tend to increase and correspond with cell loss. However, following administration of testosterone post-castration, the steady state mRNA levels of c-Myc, c-Fos, c-Ki-Ras, and fibroblast growth factor tend to increase and correspond with cellular proliferation. Using the mouse prostate reconstruction (MPR) model, Ras overexpression induced a dysplastic phenotype with strong angiogenesis, Myc overexpression induced epithelial hyperplasia, and Ras and Myc overexpressed in combination demonstrated the progression of hyperplasia to cancer (116).

p53 protein is important for growth suppression and induction of apoptosis. In human PCCLs (DU145, LNCaP, PC3, 1LN and DUPro-1), infection with an adenovirus expressing wild-type p53 inhibits cell proliferation (117). In normal rat ventral prostate cells, androgen ablation by castration did not increase p53 mRNA or protein levels and did not produce any changes in $p21^{CIP1/WAF1}$ levels (118). These findings were interpreted to demonstrate that apoptosis following androgen withdrawal in normal prostate cells did not require an increase in p53 levels and was not associated with recruitment into an abnormal cell cycle preceding initiation of apoptosis (118). The frequency of mutations of the *TP53* gene in prostate cancer has been reported to be from 3% up to 79% (119). This wide range may reflect different detection methods, analysis of different stages of the disease and also the inherent heterogeneity of the disease. For example, p53 mutations are uncommon (3%) in clinically localized prostate carcinoma in some studies (120) but up to 40% in another study which combined several techniques to identify mutations (121). p53 mutations are detected with increased frequency in more advanced metastatic prostate cancers (122), occurring at up

to twice the frequency in metastases compared to primary tumors (123,124). Furthermore, multifocal tumors within a prostate differ in whether or not they harbor the mutant *TP53* gene (123), prostate tumors have been documented to contain more than one p53 mutation (119), and within a single tumor, there is heterogeneity of distribution of mutant p53 (123). The increased p53 mutation frequency found post-androgen ablation (124) and low p53 levels found after radical radiotherapy (125) indicate that abnormalities in p53 may lead to tumor progression, therapy resistance and association with a more invasive phenotype.

Bcl-2 protein mediates survival by dimerizing with Bax and inhibiting apoptosis. Although expression of Bcl-2 is low or absent in normal adult prostate and restricted in expression to the basal cells, Bcl-2 expression increases in PIN and locally invasive prostate carcinomas, and is found in up to 100% of hormone-refractory primary and metastatic tumor samples (126). This finding implies that upregulation of Bcl-2 confers a survival advantage in androgen-deprived conditions, and in keeping with this, overexpression of Bcl-2 in human LNCaP cells enables continued growth in androgen-depleted media and protection from a variety of apoptotic stimuli (127). Strategies that decrease Bcl-2 levels by anti-sense oligonucleotides and hammerhead ribozymes inhibit progression to androgen independence in vitro (128,129).

Telomeres at the end of chromosomes are responsible for the maintenance of structural integrity and genomic stability, and consist of multiple repeats of a hexameric DNA sequence (reviewed in (130)). Repeated cell division results in telomeric shortening, because DNA polymerases are unable to replicate the ends of DNA. Telomerase, a reverse transcriptase, catalyzes the addition of DNA to the terminal stretch of unreplicated DNA at the telomere, and thereby maintains telomere length and protects the cell against senescence. Telomerase activity is downregulated during prostate differentiation, and is normally absent in the epithelial cells (131). Telomerase is absent from BPH samples that are not adjacent to cancerous tissue (132,133). Examination of prostate tissue samples demonstrates telomerase activity in ~75% of PINs and ~90% of prostatic carcinomas (131,132). Within a single prostatic tumor, heterogeneous telomerase activation is observed in tumors with Gleason score < 7 and homogeneity of telomerase expression increases with increasing severity of the Gleason score (134). The preceding findings implicate upregulated telomerase activity in maintenance of continuing neoplastic cell proliferation. Telomerase inhibition has been reported to cause death of tumor cells. However, telomerase-deficient (mTR-null) mice crossed into INK4A-null or p53-null backgrounds provide evidence that the role of telomerase is dependent on genetic context and stage in pathogenesis of the tumor (135-137). Short telomeres, resulting from telomerase deficiency, can lead to apoptosis in p53-containing cells but may result in chromosome instability and an increase in other mutations and neoplastic transformation when p53 is absent (135). In this setting, telomerase inhibition is pro-tumorigenic. In later stage tumorigenesis in which neoplastic transformation is already high, telomerase deficiency inhibits tumor development (136).

4.4. Vitamin D Receptor

The vitamin D signaling pathway may also play a role in prostate cancer. $1\alpha,25\text{-}(OH)_2D_3$ (calcitriol) acts through the vitamin D receptor (VDR) to stimulate intracellular signaling that mediates calcium homeostasis (increases serum calcium). The $1\alpha,25\text{-}(OH)_2D_3$/VDR complex binds a vitamin D response element as a heterodimer with the retinoid X receptor, and ligand binding displaces co-repressors bound to the nuclear receptor. VDR is detected in human prostate cancer cell lines and in primary human prostate cultures obtained from BPH and carcinoma specimens (reviewed in (138). Calcitriol and its analogs inhibit cell growth in PCCLs and primary cultures of prostate cells by causing accumulation in G_1 phase of the cell cycle (138,139), and induce a differentiated phenotype (140).

In LNCaP cells, calcitriol treatment produced G_1 phase arrest associated with decreased pRB phosphorylation, decreased E2F transcriptional activity, increased $p21^{CIP1/WAF1}$ levels and decreased Cdk2 activity (141). Calcitriol induced apoptosis in LNCaP cells associated with decreased levels of Bcl-2 and Bcl-XL proteins, and this effect was abrogated by overexpression of Bcl-2 (142). Therefore, the VDR is involved in control of prostate cell proliferation and death. Although an area of controversy, in some studies, polymorphisms in the VDR have been shown to correlate with prostate cancer risk. Both the elderly and African-American populations who are at risk of prostate cancer tend to have low serum vitamin D concentrations (138). The use of calcitriol in the treatment of prostate cancer may be limited by the induction of hypercalcemia. However, newer vitamin D3 analogs such as 1,25(OH)2-16-ene-5,6- trans-D3 tested in other malignancies have greatly reduced calcemic activity compared to calcitriol while retaining potent growth inhibitory and telomerase inhibitory activity (143), and may have therapeutic potential in prostate cancer.

4.5. Microsatellite Instability (MSI)

Microsatellites are short repetitive sequences found at various genetic loci, and polymorphisms within these microsatellites (referred to as MSI) are an indication of overall genomic instability. A high degree of MSI is a marker for a DNA replication error phenotype, first described in hereditary nonpolyposis colorectal carcinoma (144). Microsatellite analyses have been performed on human prostatic tissue samples to determine whether MSI plays a role in prostate tumorigenesis. The results vary depending on type of sample, site and number of microsatellite loci examined. Most studies show a high frequency of MSI in prostate carcinomas: 45% in the study by Dahiya et al. (145) and 35% of cases of advanced prostate cancer in the study by Rohrbach et al. (146). MSI is also seen in early prostate tumors discovered incidentally at TURP (31%, (147) but the role of MSI in promotion of prostate tumor progression remains controversial (146,147).

MSI is thought to arise from defective DNA replication error repair under the control of several genes regulating mismatch repair such as *hMSH2* and *hMLH1* (148). Germline mutations in mismatch repair genes are hypothesized to lead to a mutator phenotype in which increased chromosomal instability leads to mutations that cause neoplastic transformation. However, there is currently no clear evidence that mutations in mismatch repair genes cause prostate tumors in humans. However, the PCCL DU145 derived from human prostate cancer displays substantial MSI, is defective in mismatch repair and has mutations in two mismatch repair genes, *hMLH1* and *hPMS2* (149).

Both the AR and VDR genes are affected by microsatellite instability (MSI). Epidemiological studies have identified correlations between different inherited AR and VDR gene polymorphisms and risk of developing prostate cancer. However the role of these polymorphisms in the development of prostate cancer has not been fully established. Polymorphic (CAG)n (encoding a glutamine repeat region) and (GGN)n (glycine) repeats are present in the first exon of the AR. The glutamine-rich region usually contains 20-25 direct repeats and the glycine-rich region has ~23 repeats. A long (CAG)n (where n=40-62) microsatellite is associated with Kennedy's disease (spinal and bulbar muscular atrophy), while a short (n<16) (CAG)n microsatellite is associated with an increased risk for prostate cancer (reviewed in (8)). The effect of contraction of this repeat on AR function is poorly understood. Expansion of the glutamine repeats leads to reduced transactivation (150). The glutamine-rich region is also the site of AR (p160) co-activator binding (150). The distribution of (CAG)n microsatellite length polymorphism is different among racial/ethnic groups and may contribute to the differences in prostate cancer incidence and mortality. African-Americans tend to have the shortest alleles while Asians and Caucasians tend to have long alleles (8). The VDR contains a poly-(A)n microsatellite in its 3'-untranslated region and other polymorphisms that introduce restriction length polymorphisms when the DNA is restricted with Bsm I, Apa I and Taq I enzymes (reviewed in (8)). Although several groups have identified linkages between different VDR haplotypes and prostate cancer relative risks (e.g. increased risk with at least one long poly A allele (151); decreased risk if homozygous for the t allele (152); other studies have not identified any associations (153), or risk reductions are stratified according to serum vitamin D levels (as in (154), where reduced relative risk associated with the BB genotype was found only in men with low serum vitamin D levels).

5. TREATMENT

The formation of prostate cancer is a multi-step process requiring multiple mutations in several genes to initiate, promote and allow a tumor to progress. This implies that there is a precursor lesion (PIN) that has only a subset of the mutations needed to become cancer. Clinically undetectable, PIN is thought to represent this precancerous dysplastic lesion, because they are anaplastic although their basement membrane is intact, and they have many of the genetic changes that prostate cancers have (including chromosomal abnormalities and telomerase activity). Through the accumulation of additional mutations, PIN is thought to progress to malignant prostate cancer over a span of about ten years. Therefore, it is important to detect prostate cancer early so that it can be treated in a benign or close to benign state.

A first line of treatment is androgen ablation. Surgical castration (orchiectomy), administration of anti-androgens (chemical castration), or a combination of these methods removes the androgens necessary for prostatic epithelial growth and induces apoptosis (155,156). Nonsteroidal anti-androgens (flutamide, nilutamide, and bicalutamide) act by competing with androgens for the androgen receptor. Because they inhibit the negative feedback of testosterone on the hypothalamus, testosterone levels increase. Steroidal anti-androgens (cyproterone acetate) inhibit both the androgen receptor (AR) and reduce testosterone levels. Chemicals that inhibit the metabolism of testosterone (ketoconazole and aminoglutethimide) interfere with the formation of steroids that are the precursors of testosterone as well as the formation of other steroids, namely glucocorticoids, mineralocorticoids and estrogens. 5α–reductase inhibitors act by blocking DHT formation. While 70% to 80% of patients respond to this form of treatment, androgen-resistant (also termed androgen-refractory, -independent or hormone-escaped) prostate cancer arises, associated with additional mutations including the *AR* gene. Intermittent androgen ablation has been used to slow this selection process and increase the effectiveness of treatment, but inevitably androgen-resistant prostate cancer cells arise within about two years (155,157,158).

Once androgen-independent prostate cancer has developed, the prognosis is poor. Several options are available, but few are very effective. Radical prostatectomy rarely results in cure at this stage. Supportive care, external beam radiation, and radioisotopes (phosphorus-32, samarium-153, and strontium-89) provide palliation. Second-line hormonal therapy or the administration of a different anti-androgen has had some success, presumably because the mutations in the AR are specific for a given anti-androgen. Most chemotherapeutic agents (antimetabolites, alkylating agents, vinca alkaloids, and nitrosoureas) alone have little effect on androgen-independent prostate cancer. However, 9-amino-camptothecin has showed promising results and is in phase II clinical trials (155). In addition, combinations of traditional chemotherapeutic agents with cytotoxic agents have had some success. Paclitaxel, an agent that stabilizes the formation of microtubules, combined with estramustine, an agent that destabilizes microtubule assemblies, have synergistic cytotoxicity on androgen-independent prostate cancers (159). Other promising chemotherapeutic combinations include doxorubicin/5-fluorouracil/cisplatin, hydrocortisone/mitoxantrone, mitoantrone/prednisone, paclitaxel/estramustine/etoposide, and paclitaxel/interferon-α/cis-retinoic acid regimens which work on androgen-therapy resistant p53- and Bcl-2 mutant cells (155,160).

Recently, the flavinoid antioxidant, silibinin, has been proposed as a possible therapy for hormone-refractory prostate cancer. It decreases PSA and inhibits both serum-and

Table 3. Transgenic mouse models of prostate cancer

Promoter	Transgene	Pathology
Probasin (-454)	SV40 T antigen	metastatic adenocarcinoma
Probasin (-454)	Ras	Epithelial and stromal hyperplasia
Large Probasin	SV40 T antigen	Invasive adenocarcinoma
C3(1)	SV40 T antigen	Invasive adenocarcinoma, rare metastasis
C3(1)	Polyoma middle T	Invasive adenocarcinoma
C3(1)	bcl-2	Epithelial and stromal hyperplasia
MMTV	SV40 T antigen	prostate and/or seminal vesicle adenocarcinoma
MMTV	int-2	Hyperplasia of urogenital organ epithelium
MMTV	kgf	Hyperplasia of urogenital organ epithelium
Cryptdin	SV40 T antigen	Androgen-independent metastatic prostate adenocarcinoma
Fetal gamma globin	SV40 T antigen	Androgen-independent metastatic prostate adenocarcinoma
gp91-phox	SV40 T antigen	Neuroblastoma of neuroectodermal origin

The promoter and the transgene used to drive prostatic expression are detailed.

androgen-induced proliferation by stimulating G1 arrest. Silibinin treatment decreased levels of cyclin D1, Cdk4, and Cdk6 coupled with increases in p27Kip1 and p21Cip1/WAF1. Together these changes resulted in decreased cyclin E and Cdk2 and G1 arrest. Silibinin's effect is mostly mediated through the AR (161). Other therapies like tumor-associated antigen expression therapy, cyclin-dependent kinase inhibitors, matrix metalloproteinase inhibitors, angiogenesis inhibitors, apoptosis inducers and gene therapy are all under development and might hold promise in the future (155). Due to the tissue specificity of PSA for the prostate, researchers were able to generate a vaccinia virus expressing PSA as an antigen to immunize against prostate cancer. This therapy was effective in rhesus monkeys, but the vaccine cross reacts with other kallikrein family members and only elicited a low titer IgM response (no IgG) (162).

6. FUTURE PERSPECTIVES AND THE DEVELOPMENT OF BETTER TRANSGENIC MODELS OF PROSTATE CANCER

Alternative therapies for androgen-resistant tumors are predicated upon knowledge of the molecular basis of the disease and improved animal models for assessing novel therapeutics. Prostate cancer models have included models in the dog, rat models, heterotopic transplantation of cell lines into immunodeficient mice and use of transgenic mice (163).

Canine prostate cancer resembles human prostate pathology and becomes both metastatic, and at a later stage, androgen-independent. The low incidence of spontaneous disease in the dog, the high cost of animal maintenance and the inability to manipulate genetically the dog model has limited the use of this model (164,165). Prostate cancer can be induced in rats through treatment with chemical carcinogens or hormones (androgens or estrogens) (164,166); however reproducibility varies in these models. Sublines derived from the Dunning rat (R3327) are more widely used. Xenograft models implanting human cell lines (LNCaP, CWR-22 and LuCap-23) into immunodeficient or SCID mice have the limitations that the role of the immune system in tumor progression is ignored (167,168).

The mouse can be genetically manipulated and the role of tumor suppressors/inducers can be integrated with readily available genetic models. The limitations of the mouse as a model include the structural differences between the human and murine prostate and the lack of spontaneous prostate cancer in the mouse. The rodent prostate consists of three lobes (dorsolateral, ventral and anterior) which are arranged circumferentially around the urethra. In contrast, the human prostate is alobular and is divided into three zones (central, peripheral, transitional). Nonetheless, prostatic hyperplasia has been observed in mice homozygously deleted of candidate tumor suppressor genes including *Mxi1* (169), *estrogen receptor b* (170), *pten* (171,172), *p27Kip1* (173), *nkx3.1* (174)

Genetically engineered strains of murine prostate cancer have been made and studied (Table 3) (163,175). The promoters used to induce prostate-directed expression include the probasin promoter (either a minimal 454 bp (176) or 1.5 kb 5' flanking sequence (177)), the C3(1) promoter (178), the mouse mammary tumor virus (MMTV) LTR (179,180), Cryptdin-2 (CR2) (181), fetal Gγglobin (182) and gp91-phox (183).

The rat probasin gene is expressed in the epithelial cells and ducts of the dorsolateral and ventral prostate. Expression of the probasin promoter is regulated by steroids and during development. Two probasin derived fragments have been analyzed in transgenics. The minimal probasin promoter consists of a 454 bp fragment and the large probasin promoter fragment consists of 11.5 kb of 5' flanking sequence (177). When a transgene consisting of the minimal probasin promoter linked to the SV40 early region was coinjected with the chicken lysozyme MAR sequence, a metastatic prostate cancer mouse model resulted, known as the TRAMP model (transgenic adenocarcinoma mouse prostate) (184). The local tumor arose primarily in the dorsolateral lobes of the mouse prostate. The probasin minimal promoter was also sufficient to drive expression of the SV40 T antigen and Ras transgenes in mice (Table 1). The large probasin promoter directed expression of SV40 large T antigen to the dorsolateral and ventral prostate resulting in androgen-dependent adenocarcinoma (185).

The C3(1) promoter is also androgen-regulated and its expression normally contributes to a component of a rat ventral secretory protein. The C3(1) promoter in

transgenic mice directed expression of SV 40 large T antigen to the ventral prostate in the male and the mammary epithelium in the female (178) and has provided a useful model of prostate tumor progression from PIN to invasive adenocarcinoma. The MMTV LTR promoter is expressed in a variety of epithelial tissues including the prostate. Prostatic phenotypes have been observed in the offspring of mice with MMTV-directed expression of SV40 T antigen (186), *int2* (179), and *kgf* (180), although hyperplasia was the predominant phenotype. The cryptdin-2 promoter drove expression of the SV40 T antigen to the prostatic neuroendocrine cells, resulting in a highly aggressive tumor that evolved through PIN to become androgen-independent and metastatic (181). The fetal γ globin/SV40 transgenic mice developed androgen-independent metastatic prostate tumors with neuroendocrine and epithelial features (182). Neuroblastomas, presumably arising from neuroectodermal cells in the prostate, were noted in gp91-phox-SV40 T antigen transgenic mice (183).

7. CONCLUSION

With the high incidence of prostate cancer, due in part to the advances in screening methods, many tumors that are detected will have no adverse effect on the patient within their normal life span. Therefore, a major challenge in prostate cancer management is the ability to distinguish prostate cancers that will kill from those that will have little impact on the patient. The identification of malignant markers holds the key to better treatments. In addition, the development of new therapeutics that slow the metastatic process and palliative treatments would greatly improve the quality of life for many patients in the absence of a chemotoxic cure. An improved understanding of the molecular mechanisms governing prostate cancer growth may lead to the identification of better diagnostic markers and treatments.

8. ACKNOWLEDGMENTS

The Authors thank Dr. Nicole Schreiber Agus for critical reading of this manuscript. We apologize for the citations that were omitted due to publication restrictions. This work was supported in part by R29CA70897, RO1CA75503, RO1 CA77552 (to R.G.P.); R.G.P. is a recipient of the Irma T. Hirschl award and an award from the Susan G. Komen Breast Cancer Foundation. Work conducted at the Albert Einstein College of Medicine was supported by Cancer Center Core National Institute of Health grant 5-P30-CA13330-26.

9. REFERENCES

1. De Marzo A.M., D. Coffey, W.G. Nelson: New concepts in tissue specificity for prostate cancer and benign prostatic hyperplasia. *Urology* 53, 29-39 (1999)

2. Isaacs W.B., G.B. Bova: Prostate cancer. In: The Genetic Basis of Human Cancer. Eds: Vogelstein B., K.W. Kinzler, McGraw-Hill, NY 653-660 (1998)

3. Zlotta A.R., C.C. Schulman: Clinical evolution of prostatic intraepithelial neoplasia. *Eur Urol* 35, 498-503 (1999)

4. Haggman M.J., J.A. Macoska, K.J. Wojno, J.E. Oesterling: The relationship between prostate intraepithelial neoplasia and prostate cancer: critical issues. *J Urol* 158, 12-22 (1997)

5. Qian J., R.B. Jenkins, D.G. Bostwick: Genetic and chromosomal alterations in prostatic intraepithelial neoplasia and carcinoma detected by fluorescence in situ hybridization. *Eur Urol* 35, 479-483 (1999)

6. Wang L.G., X.M. Liu, W. Kreis, D.R. Budman: Down-regulation of prostate-specific antigen expression by finasteride through inhibition of complex formation between androgen receptor and steroid receptor-binding consensus in the promoter of the PSA gene in LNCaP cells. *Cancer Res* 57, 714-719 (1997)

7. Landis S.H., T. Murray, S. Bolden, P.A. Wingo: Cancer Statistics, 1998. *CA Cancer J Clin* 48, 6-29 (1998)

8. Ruijter E., C.V.D. Kaa, G. Miller, D. Ruiter, F. Debruyne, J. Schalken: Molecular Genetics and Epidemiology of Prostate Cancer. *Endocr Rev* 20, 22-45 (1999)

9. Brawley O.W., K. Knopf, R. Merrill: The epidemiology of prostate cancer Part I: Descriptive epidemiology. *Semin Urol Oncol* 16, 187-192 (1998)

10. Bova G.S., W.B. Isaacs: Review of allelic loss and gain in prostate cancer. *World J Urol* 14, 338-346 (1996)

11. Brothman A.R., T.M. Maxwell, J. Cui, D.A. Deubler, X.L. Zhu: Chromosomal clues to the development of prostate tumors. *Prostate* 38, 303-312 (1999)

12. Svaren J., B.R. Sevetson, E.D. Apel, D.B. Zimonjic, N.C. Popescu, J. Milbrandt: NAB2, a corepressor of NGFI-A (Egr-1) and Krox20, is induced by proliferative and differentiative stimuli. *Mol Cell Biol* 16, 3545-3553 (1996)

13. Li J., C. Yen, D. Liaw, K. Podsypanina, S. Bosse, S.I. Wang, J. Puc, C. Miliaresis, L. Rodgers, R. McCombie, S.H. Bigner, B.C. Giovanella, M. Ittmann, B. Tycko, H. Hibshoosh, M.H. Wigler, R. Parsons: PTEN, a putative protein tyrosine phosphatase gene mutated in human brain, breast, and prostate cancer. *Science* 275, 1943-1947 (1997)

14. Bhatia-Gaur R., A.A. Donjacour, P.J. Sciavolino, M. Kim, N. Desai, P. Young, C.R. Norton, T. Gridley, R.D. Cardiff, G.R. Cunha, C. Abate-Shen, M.M. Shen: Role for Nxk3.1 in prostate development and cancer. *Genes Dev* 13, 966-977 (1999)

15. Emmert-Buck M.R., R.F. Bonner, P.D. Smith, R.F. Chuaqui, Z. Zhua L.A. Liotta: Laser capture microdissection. *Science* 274, 998-1001 (1996)

16. Earp H.S., T.L. Dawson, X. Li, H. Yu: Heterodimerization and functional interaction between EGF receptor family members: A new signaling paradigm with implications for breast cancer research. *Breast Cancer Res. Treat.* 35, 115-132 (1995)

17. Ware J.L., S.J. Maygarden, W.W. Koontz, S.C. Strom: Immunohistochemical detection of c-erbB-2 protein in human benign and neoplastic prostate. *Hum. Pathol* 22, 254-258 (1991)

18. Robinson D., F. He, T. Preglow, H.J. Kung: A tyrosine kinase profile of prostate carcinoma. *Proc Natl Acad Sci USA* 93, 5958-5962 (1996)

19. Lyne J.C., M.F. Melhem, G.G. Finley, D. Wen, N Liu, D.H. Deng, R. Salup: Tissue expression of neu differentiation factor/heregulin and its receptor complex in prostate cancer and its biological effects on prostate cancer cells in vitro. *Cancer J Sci Am* 3, 21-30 (1997)

20. Myers R.B., S. Srivasatava, D.K. Oelschlager, W.E. Grizzle: Expression of p130erbB-3 and p185erbB-2 in prostatic intraepithelial neoplasia and prostatic adenocarcinoma. *J Natl Cancer Inst* 86, 1140-1145 (1994)

21. Grasso A.W., D. Wen, C.M. Miller, J.S. Rhim, T.G. Pretlow, H.J. Kung: ErbB kinases and NDF signaling in human prostate cancer cells. *Oncogene* 15, 2705-2716 (1997)

22. Arai Y., T. Yoshiki, O. Yoshida: c-erbB-2 oncoprotein: a potential biomarker of advanced prostate cancer. *Prostate* 30, 195-201 (1997)

23. Harper M., E. Glynne-Jones, L. Goddard, P. Mathews, R.I. Nicholson: Expression of androgen receptor and growth factors in premalignant lesions of the prostate. *J. Pathol* 186, 169-177 (1998)

24. Fox S.B., R.A. Persad, N. Coleman, C.A. Day, P.B. Silcocks, C.C. Collins: Prognostic value of c-erbB-2 and epidermal growth factor receptor in stage A1 (T1a) prostatic adenocarcinoma. *Br J Urol* 74, 214-220 (1994)

25. Kuhn E.J., R.A. Kurnot, I.A. Sesterhenn, E.H. Chang, J.W. Moul: Expression of the c-erbB-2 (HER-2/neu) oncoprotein in human prostatic carcinoma. *J Urol* 1427-1433 (1993)

26. Schwartz S.J., C. Caceres, J. Morote, I. De Torres, J.M. Rodriguez-Vallejo, J. Gonzalez, J. Reventos: Gains of the relative genomic content of erbB-1 and erbB-2 in prostate carcinoma and their association with metastasis. *Int J Oncol* 14, 367-371 (1999)

27. Ching K.Z.: Role of c-erbB gene family in prostate cancer. *Diss Abstr Int [B]* 55, 4738 (1995)

28. Meng T.C., M.F. Lin: Tyrosine phosphorylation of c-ErbB-2 is regulated by the cellular form of prostatic acid phosphatase in human prostate cancer cells. *J Biol Chem* 273, 22096-22104 (1998)

29. Craft N., Y Shostak, M. Carey, C.L. Sawyers: A mechanism for hormone-independent prostate cancer through modulation of androgen receptor signaling by the HER-2/neu tyrosine kinase. *Nat Med* 5, 280-285 (1999)

30. Bargmann C.I., R.A. Weinberg: Oncogenic activation of the neu-encoded receptor protein by point mutation and deletion. *EMBO J* 7, 2043-2052 (1988)

31. Sikes R.A., L.W. Chung: Acquisition of a tumorigenic phenotype by a rat ventral prostate epithelial cell line expressing a transfected activated neu oncogene. *Cancer Res* 52, 3174-3181 (1992)

32. Zhau H.Y., J. Zhou, W.F. Symmans, B.Q. Chen, S.M. Chang, R.A. Sikes, L.W. Chung: Transfected neu oncogene induces human prostate cancer metastasis. *Prostate* 28, 73-83 (1996)

33. Qiu Y., L. Ravi, H.J. Kung: Requirement of ErbB2 for signalling by interleukin-6 in prostate carcinoma cells. *Nature* 393, 83-85 (1998)

34. Zi X., A.W. Grasso, H.J. Kung, R. Agarwal: A flavonoid antioxidant, silymarin, inhibits activation of erbB1 signaling and induces cyclin-dependent kinase inhibitors, G1 arrest, and anticarcinogenic effects in human prostate carcinoma DU145 cells. *Cancer Res* 58, 1920-1929 (1998)

35. Perry J.E., M.E. Grossmann, D.J. Tindall: Epidermal growth factor induces cyclin D1 in a human prostate cancer cell line. *Prostate* 35, 117-124 (1998)

36. Matsumura I., T. Kitamura, H. Wakao, H. Tanaka, K. Hashimoto, C. Albanese, J. Downward, R.G. Pestell, Y. Kanakura: Transcriptional regulation of cyclin D1 promoter by STAT5: its involvement in cytokine-dependent growth of hematopoietic cells. *EMBO J* 18, 1367-1377 (1999)

37. Bromberg J.F., M.H. Wrzeszczynska, G. Devgan, Y. Zhao, R.G. Pestell, C. Albanese, J.E.J. Darnell: Stat3 as an oncogene. *Cell* 98, 295-303 (1999)

38. Lee R.J., C. Albanese, M. Fu, M. D'Amico, B. Lin, G. Watanabe, G.K.I. Haines, P.M. Siegel, M.C. Hung, Y. Yarden, J.M. Horowitz, W.J. Muller, R.G. Pestell: Cyclin D1 is required for transformation by activated Neu and is induced through an E2F-dependent signaling pathway. *Mol Cell Biol* 20, 672-683 (2000)

39. Sherwood E.R., C. Lee: Epidermal growth factor-related peptides and the epidermal growth factor receptor in normal and malignant prostate. *World J Urol* 13, 290-296 (1995)

40. Peehl D.M., P. Cohen, R.G. Rosenfield: The insulin like growth factor system in the prostate. *World J Urol* 13, 306-311 (1996)

41. Hofer D., E. Sherwood, W. Bromberg, J. Mendelsohn, C. Lee, J.M. Kozlowski: Autonomous growth of androgen independent prostate carcinoma cells: Role of TGFα. *Cancer Res* 2780-2785 (1991)

42. Djakiew D.: Role of growth factor like protein in the paracrine regulation of prostate growth. *J Androl* 13, 476-487 (1992)

43. Story M.: Regulation of prostate growth by fibroblast growth factors. *World J Urol* 13, 297-305 (1996)

44. Pestell R.G., C. Albanese, A.T. Reutens, J.E. Segall, R.J. Lee, A. Arnold: The cyclins and cyclin-dependent kinase inhibitors in hormonal regulation of proliferation and differentiation. *Endocrine Rev* 20, 501-534 (1999)

45. Li J., C. Yen, D. Liaw, K. Podsypanina, S. Bose, S.I. Wang, J. Puc, C. Miliaresis, L. Rodgers, R. McCombie, S.H. Bigner, B.C. Giovanella, M. Ittmann, B. Tycko, H. Hibshoosh, M.H. Wigler, R. Parsons: PTEN, a putative protein tyrosine phosphatase gene mutated in human brain, breast, and prostate cancer. *Science* 275, 1943-1947 (1997)

46. Steck P.A., M.A. Pershouse, S.A. Jasser, W.K. Yung, H. Lin, A.H. Ligon, L.A. Langford, M.L. Baumgard, T. Hattier, T. Davis, C. Frye, R. Hu, B. Swedlund, D.H. Teng, S.V. Tavtigian: Identification of a candidate tumour suppressor gene, MMAC1, at chromosome 10q23.3 that is mutated in multiple advanced cancers. *Nat Genet* 15, 356-362 (1997)

47. Whang Y.E., X. Wu, H. Suzuki, R.E. Reiter, C. Tran, R.L. Vessella, J.W. Said, W.B. Isaacs, C.L. Sawyers: Inactivation of the tumor suppressor PTEN/MMAC1 in advanced human prostate cancer through loss of expression. *Proc Natl Acad Sci USA* 95, 5246-5250 (1998)

48. Li J., L. Simpson, M. Takahashi, C. Miliaresis, M.P. Myers, N. Tonks, R. Parsons: The PTEN/MMAC1 tumor suppressor induces cell death that is rescued by the AKT/protein kinase B oncogene. *Cancer Res* 58, 5667-5672 (1998)

49. Wu X., K. Senechal, M.S. Neshat, Y.E. Whang, C.L. Sawyers: The PTEN/MMAC1 tumor suppressor phosphatase functions as a negative regulator of the phosphoinositide 3-kinase/Akt pathway. *Proc Natl Acad Sci USA* 95, 15587-15591 (1998)

50. Kennedy S.G., A.J. Wagner, S.D. Conzen, J. Jordan, A. Bellacosa, P.N. Tsichlis, N. Hay: The PI 3-kinase/Akt signaling pathway delivers an anti-apoptotic signal. *Genes Dev* 11, 701-712 (1997)

51. Ramaswamy S., N. Nakamura, F. Vazquez, D.B. Batt, S. Perera, T.M. Roberts, W.R. Sellers: Regulation of G1 progression by the PTEN tumor suppressor protein is linked to inhibition of the phosphatidylinositol 3-kinase/Akt pathway. *Proc Natl Acad Sci USA* 96, 2110-2115 (1999)

52. Takuwa N., Y Fukui, Y. Takuwa: Cyclin D1 expression mediated by phosphatidyl inositol 3-kinase through mTOR-p70S6k-independent signaling in growth factor-stimulated NIH3T3 fibroblasts. *Mol Cell Biol* 19, 1346-1358 (1999)

53. Muise-Helmericks R.C., H.L. Grimes, A. Bellacosa, S.E. Malstrom, P.N. Tsichlis, N. Rosen: Cyclin D expression is controlled post-transcriptionally via a phosphatidylinositol 3-kinase/Akt-dependent pathway. *J Biol Chem* 273, 29864-29872 (1998)

54. Diehl J.A., M. Cheng, M.F. Roussel, C.J. Sherr: Glycogen synthase kinase-3 beta regulates cyclin D1 proteolysis and subcellular localization. *Genes Dev* 12, 3499-3511 (1998)

55. Hunter T.: Oncoprotein networks. *Cell* 88, 333-346 (1997)

56. Cadigan K.M., R. Nusse: Wnt signaling: a common theme in animal development. *Genes Dev* 11, 3286-3305 (1997)

57. Su L.K., B. Vogelstein, K.W. Kinzler: Association of the APC tumor suppressor protein with catenins. *Science* 262, 1734-7 (1993)

58. Voeller H., C. Truica, E. Gelman: β-catenin mutations in prostate cancer. *Cancer Res* 58, 2520-2523 (1998)

59. He T.C., A.B. Sparks, C. Rago, H. Hermeking, L. Zawel, L.T. da Costa, P.J. Morin, B. Vogelstein, K.W. Kinzler: Identification of c-MYC as a target of the APC pathway. *Science* 281, 1509-1512 (1998)

60. Shtutman M., J. Zhurinsky, I. Simcha, C. Albanese, M. D'Amico, R. Pestell, A. Ben-Ze'ev: The cyclin D1 gene is a target of the β-catenin/LEF-1 pathway. *Proc Natl Acad Sci USA* 96, 5522-5527 (1999)

61. Tetsu O., F. McCormick: β-catenin regulates expression of cyclin D1 in colon carcinoma cells. *Nature* 398, 422-424 (1999)

62. Knudson A.G.J.: Genetics of human cancer. *Genetics* 79, 305-16 (1975)

63. Alen P., F. Claessens, G. Verhoeven, W. Rombauts, B. Peeters: The androgen receptor amino-terminal domain plays a key role in p160 coactivator-stimulated gene transcription. *Mol Cell Biol* 19, 6085-6097 (1999)

64. Taplin M.E., G.J. Bubley, T.D. Shuster, M.E. Frantz, A.E. Spooner, G.K. Ogata, H.N. Keer, S.P. Balk: Mutation of the androgen-receptor gene in metastatic androgen-independent prostate cancer. *N Engl J Med* 332, 1393-1398 (1995)

65. Tilley W.D., G. Buchanan, T.E. Hickey, J.M. Bentel: Mutations in the androgen receptor gene are associated

with progression of human prostate cancer to androgen independence. *Clin Cancer Res* 2, 277-285 (1996)

activation and transient expression of the human androgen receptor. *J Steroid Biochem Molec Biol* 59, 9-20 (1996)

67. Culig Z., A. Hobisch, M.V. Cronauer, A.C.B. Cato, A. Hittmair, C. Radmayr, J. Eberle, G. Bartsch, H. Klocker: Mutant androgen receptor detected in an advanced-stage prostatic carcinoma is activated by adrenal androgens and progesterone. *Molec Endoc* 7, 1541-1550 (1993)

68. Chen Y., L.A. Martinez, M. LaCava, L. Coghlan, C.J. Conti: Increased cell growth and tumorigenicity in human prostate LNCaP cells by overexpression of cyclin D1. *Oncogene* 16, 1913-1920 (1998)

69. Veldscholte J., C. Ris-Stalpers, G.G.J.M. Kuiper, G. Jenster, C. Berrevoets, E. Claasen, H.C.J. van Rooij, J. Trapman, A.O. Brinkman, E. Mulder: A mutation in the ligand binding domain of the androgen receptor of human LNCaP cells affects steroid binding characteristics and response to anti-androgens. *Biochem Biophys Res Comm* 173, 534-540 (1990)

70. Gaddipati J.P., D.G. McLeod, H.B. Heidenberg, I.A. Sesterhenn, M.J. Finger, J.W. Moul, S. Srivastava: Frequent detection of codon 877 mutation in the androgen receptor gene in advanced prostate cancer. *Cancer Res* 54, 2861-2864 (1994)

71. Newmark J.R., D.O. Hardy, D.C. Tonb, B.S. Carter, J.I. Epstein, W.B. Isaacs, T.R. Brown, E.R. Barrack: Androgen receptor gene mutations in human prostate cancer. *Proc Natl Acad Sci USA* 89, 6319-6323 (1992)

72. Suzuki H., N. Sato, Y. Watabe, M. Masai, S. Seino, J. Shimazaki: Androgen receptor gene mutations in human prostate cancer. *J Steroid Biochem Molec Biol* 46, 759-765 (1993)

73. Taplin M.-E., G.J. Bubley, Y.-J. Ko, E.J. Small, M. Upton, B. Rajeshkumar, S.P. Balk: Selection for androgen receptor mutations in prostate cancers treated with androgen antagonist. *Cancer Res* 59, 2511-2515 (1999)

74. Simental J.A., M. Sar, M.V. Lane, F.S. French, E.M. Wilson: Transcriptional activation and nuclear targeting signals of the human androgen receptor. *J Biol Chem* 266, 510-518 (1991)

75. Jenster G., J. Trapman, A.O. Brinkmann: Nuclear import of the human androgen receptor. *Biochem J* 293, 761-768 (1993)

76. Zoppi S., M. Marcelli, J.-P. Deslypere, J.E. Griffin, J.D. Wilson, M.J. McPhaul: Amino acid substitutions in the DNA-binding domain of the human androgen receptor are a frequent cause of receptor-binding positive androgen resistance. *Molec Endoc* 6, 409-415 (1992)

77. Schneikert J., H. Peterzeil, P.-A. Defossez, H. Klocker, Y. de Launoit, A.C.B. Cato: Androgen receptor-Ets protein interaction is a novel mechanism for steroid hormone-mediated down-modulation of matrix metalloproteinase expression. *J Biol Chem* 271, 23907-23913 (1996)

78. Zhang M., D. Magit, R. Sanger: Expression of mapsin in prostate calls is regulated by a prostate Ets element and a negative hormonal responsive element site recognized by androgen receptor. *Proc Natl Acad Sci* 94, 5673-5678 (1997)

79. Hunter T., J. Pines: Cyclins and cancer II: cyclin D and CDK inhibitors come of age. *Cell* 79, 573-582 (1994)

80. Weinberg R.A.: The retinoblastoma protein and cell cycle control. *Cell* 81, 323-330 (1995)

81. Sherr C.J.: Cancer cell cycles. *Science* 274, 1672-1677 (1996)

82. Baldin V., J. Lukas, M.J. Marcote, M. Pagano, G. Draetta: Cyclin D1 is a nuclear protein required for cell cycle progression in G1. *Genes Dev* 7, 812-821 (1993)

83. Quelle D.E., R.A. Ashmun, S.A. Shurtleff, J.-Y. Kato, D. Bar-Sagi, M.F. Roussel, C.J. Sherr: Overexpression of mouse D-type cyclins accelerates G1 phase in rodent fibroblasts. *Genes Dev* 7, 1559-1571 (1993)

84. Xiong W., R.G. Pestell, G. Watanabe, J. Li, M.R. Rosner, M.B. Hershenson: Cyclin D1 is required for S-phase traversal in bovine tracheal myocytes. *Am J Physiol* 272, L1205-L1210 (1997)

85. Lukas J., J. Bartkova, J. Bartek: Convergence of mitogenic signalling cascades from diverse classes of receptors at the cyclin D-cyclin dependent kinase-pRb-controlled G1 checkpoint. *Mol Cell Biol* 16, 6917-6925 (1996)

86. Jiang W., S.M. Kahn, P. Zhou, Y.-J. Zhang, A.M. Cacace, A.S. Infante, S. Doi, R.M. Santella, I.B. Weinstein: Overexpression of cyclin D1 in rat fibroblasts causes abnormalities in growth control, cell cycle progression and gene expression. *Oncogene* 8, 3447-3457 (1993)

87. Resnitzky D., M. Gossen, H. Bujard, S.I. Reed: Acceleration of the G1/S phase transition by expression of cyclins D1 and E with an inducible system. *Mol Cell Biol* 14, 1669-1679. (1994)

88. Fantl V., G. Stamp, A. Andrews, I. Rosewell, C. Dickson: Mice lacking cyclin D1 are small and show defects in eye and mammary gland development. *Genes Dev* 9, 2364-2372 (1995)

89. Sicinski P., J.L. Donaher, S.B. Parker, T. Li, A. Fazeli, H. Gardner, S.Z. Haslam, R.T. Bronson, S.J. Elledge, R.A. Weinberg: Cyclin D1 provides a link between development

and oncogenesis in the retina and breast. *Cell* 82, 621-630 (1995)

90. Sicinski P., J.L. Donaher, Y. Geng, S.B. Parker, H. Gardner, M.Y. Park, R.L. Robker, J.S. Richards, L.K. McGinnis, J.D. Biggers, J.J. Eppig, R.T. Bronson, S.J. Elledge, R.A. Weinberg: Cyclin D2 is an FSH-responsive gene involved in gonadal cell proliferation and oncogenesis. *Nature* 384, 470-474 (1996)

91. Sherr C.J.: Mammalian G1 cyclins. *Cell* 73, 1059-1065 (1993)

92. Ewen M.E., H.K. Sluss, C.J. Sherr, H. Matsushime, J.-Y. Kato, D.M. Livingston: Functional interactions of the retinoblastoma protein with mammalian D-type cyclins. *Cell* 73, 487-497 (1993)

93. Dowdy S.F., P.W. Hinds, K. Louie, S.I. Reed, A. Arnold, R.A. Weinberg: Physical interaction of the retinoblastoma protein with human D cyclins. *Cell* 73, 499-511 (1993)

94. Rosenberg C.L., T. Motokura, H.M. Kronenberg, A. Arnold: Coding sequence of the overexpressed transcript of the putative oncogene PRAD1/cyclin D1 in two primary human tumors. *Oncogene* 8, 519-521 (1993)

95. Han E., J.T. Lim, N. Arber, M.A. Rubin, W.Q. Xing, I.B. Weinstein: Cyclin D1 expression in human prostate carcinoma cell lines and primary tumors. *Prostate* 35, 95-101 (1998)

96. Shiraishi T., M. Watanabe, T. Muneyuki, T. Nakayama, J. Morita, H. Ito, T. Kotake, R. Yatani: A clinicopathological study of p53, p21 (WAF1/CIP1) and cyclin D1 expression in human prostate cancers. *Urol Int* 61, 90-94 (1998)

97. Gumbiner L.M., P.H. Gumerlock, P.C. Mack, S.G. Chi, R.W. deVere White, J.L. Mohler, T.G. Pretlow, J.V. Tricoli: Overexpression of cyclin D1 is rare in human prostate carcinoma. *Prostate* 38, 40-45 (1999)

98. Chen Y., A.I. Robles, L.A. Martinez, I.B. Gimenez-Conti, C.J. Conti: Expression of the G1 cyclins, cyclin-dependent kinases, and cyclin-dependent kinase inhibitors in androgen-induced prostate proliferation in castrated rats. *Cell Growth and Diff* 7, 1571-1578 (1996)

99. Sherr C.J., J.M. Roberts: Inhibitors of mammalian G1 cyclin-dependent kinases. *Genes Dev* 9, 1149-1163 (1995)

100. Sherr C.J.: Tumor surveillance via the ARF-p53 pathway. *Genes Dev* 12, 2984-2991 (1998)

101. Cheng M., P. Olivier, J.A. Diehl, M. Fero, M.F. Roussel, J.M. Roberts, C.J. Sherr: The $p21^{Cip1}$ and $p27^{Kip1}$ CDK 'inhibitors' are essential activators of cyclin D-dependent kinases in murine fibroblasts. *EMBO J* 18, 1571-1583 (1999)

102. Sherr C.J., J.M. Roberts: CDK inhibitors: positive and negative regulators of G1-phase progression. *Genes Dev* 13, 1501-1512 (1999)

103. LaBaer J., M.D. Garrett, L.F. Stevenson, J.M. Slingerland, C. Sandhu, H.S. Chou, A. Fattaey, E. Harlow: New functional activities for the p21 family of CDK inhibitors. *Genes Dev* 11, 847-862 (1997)

104. De Marzo AL. Dawson, X. Li, S.J. Maygarde D.S. Coffey: Expression of the cell cycle inhibitor $p27^{Kip1}$ in normal, hyperplastic, and neoplastic cells. *Am J Path* 153, 911-919 (1998)

105. Yang R.M., J. Naitoh, M. Murphy, H.J. Wang, J. Phillipson, J.B. deKernion, M. Loda, R.E. Reiter: Low p27 expression predicts poor disease-free survival in patients with prostate cancer. *J Urol* 159, 941-945 (1998)

106. Tsihlias J., L.R. Kapusta, G. DeBoer, I. Morava-protzner, I. Zbieranowski, N. Bhattacharya, G.C. Catzavelos, L.H. Klotz, J.M. Slingerland: Loss of cyclin-dependent kinase inhibitor p27Kip1 is a novel prognostic factor in localized human prostate adenocarcinoma. *Cancer Res* 58, 542-548 (1998)

107. Cordon-Cardo C., A. Koff, M. Drobnjak, P. Capodieci, I. Osman, S.S. Millard, P.B. Gaudin, M. Fazzari, Z. Zhang, J. Massague, H.I. Scher: Distinct altered patterns of p27KIP1 gene expression in benign prostatic hyperplasia and prostatic carcinoma. *J Natl Cancer Inst* 90, 1284-1291 (1998)

108. Lloyd R.V., L.A. Erickson, L. Jin, E. Kulig, X. Qian, J.C. Cheville, B.W. Scheithauer: p27kip1: A multifunctional cyclin-dependent kinase inhibitor with prognostic significance in human cancers. *Am J Pathol* 154, 313-323 (1999)

109. Sporn M.B., A.B. Roberts: The transforming growth factor βs. In: Peptide Growth Factors and Their Receptors. Eds: Sporn, M.B., Roberts, A.B., Springer-Verlag, NY 419 (1990)

110. Wang Q.F., K.I. Tilly, J.L. Tilly, F. Preffer, A.L. Schneyer, W.F. Crowley, P.M. Sluss: Activin inhibits basal and androgen-stimulated proliferation and induces apoptosis in the human prostatic cancer cell line, LNCaP. *Endocrinology* 137, 5476-5483 (1996)

111. Dalkin A.C., J.T. Gilrain, D. Bradshaw, C.E. Meyers: Activin inhibition of prostate cancer cell growth: Selective action on androgen-responsive LNCaP cells. *Endocrinology* 137, 5230-5235 (1996)

112. Ropiquet F., S. Huguenin, J.M. Villette, V. Ronfle, G. Le Brun, N.J. Maitland, O. Cussenot, J. Fiet, P. Berthon: FGF7/KGF triggers cell transformation and invasion on immortalised human prostatic epithelial PNT1A cells. *Int J Cancer* 82, 237-243 (1999)

113. Ropiquet F., D. Giri, D.J. Lamb, M. Ittmann: FGF7 and FGF2 are increased in benign prostatic hyperplasia and are associated with increased proliferation. *J Urol* 162, 595-599 (1999)

114. Feng S., F. Wang, A. Matsubara, M. Kan, W.L. McKeehan: Fibroblast growth factor receptor 2 limits and receptor 1 accelerates tumorigenicity of prostate epithelial cells. *Cancer Res* 57, 5369-5378 (1997)

115. Thompson T.C., S. Egawa, D. Kadmon, G.J. Miller, T.L. Timme, P.T. Scardino, S.H. Park: Androgen sensitivity and gene expression in ras + myc-induced mouse prostate carcinomas. *J Steroid Biochem Molec Biol* 43, 79-85 (1992)

116. Srivastava S., D. Katayose, Y.A. Tong, C.R. Craig D.G. McLeod, J.W. Moul, K.H. Cowan, P. Seth: Recombinant adenovirus vector expressing wild-type p53 is a potent inhibitor of prostate cancer cell proliferation. *Urology* 46, 843-8 (1995)

117. Furuya Y., X.S. Lin, J.C. Walsh, W.G. Nelson, J.T. Isaacs: Androgen ablation-induced programmed death of prostatic glandular cells does not involve recruitment into a defective cell cycle or p53 induction. *Endocrinology* 136, 1898-1906 (1995)

118. Gumerlock P.H., S.G. Chi, X.B. Shi, H.J. Voeller, J.W. Jacobson, E.P. Geerera, R.W. deVere White: p53 abnormalities in primary prostate cancer: single-strand conformation polymorphism analysis of complementary DNA in comparison with genomic DNA. The Cooperative Prostate Network. *J Natl Cancer Inst* 89, 66-71 (1997)

119. Hall M.C., N.M. Navone, P. Troncoso, A. Pollack, G.K. Zagars, A.C. von Eschenbach, C.J. Conti, L.W. Chung: Frequency and characterization of p53 mutations in clinically localized prostate cancer. *Urology* 45, 470-475 (1995)

120. Chi S.G., R.W. deVere White, F.J. Meyers, D.B. Siders, F. Lee, P.H. Gumerlock: p53 in prostate cancer: frequent expressed transition mutations. *J Natl Cancer Inst* 86, 926-933 (1994)

121. Ittmann M., R. Wieczorek, P. Heller, A. Dave, J. Provet, J. Krolewski: Alterations in the p53 and MDM-2 genes are infrequent in clinically localized, stage B prostate adenocarcinomas. *Am J Pathol* 145, 287-293 (1994)

122. Mirchandani D., J. Zheng, G.J. Miller, A.K. Ghosh, D.K. Shibata, R.J. Cote, P. Roy-Burman: Heterogeneity in intratumor distribution of p53 mutations in human prostate cancer. *Am J Pathol* 147, 92-101 (1995)

123. Meyers F.J., P.H. Gumerlock, S.G. Chi, H. Borchers, A.D. Deitch, R.W. deVere White: Very frequent p53 mutations in metastatic prostate carcinoma and in matched primary tumors. *Cancer* 83, 2534-2539 (1998)

124. Ljung G., L. Egevad, M. Norberg, L. Holmberg, S. Nilsson, C. Busch: Expression of p21 and mutant p53 gene products in residual prostatic tumor cells after radical radiotherapy. *Prostate* 32, 99-105 (1997)

125. Colombel M., F. Symmans, S. Gil, K.M. O'Toole, D. Chopin, M. Benson, C.A. Olsson, S. Korsmeyer, R. Buttyan: Detection of the apoptosis-suppressing oncoprotein bc1-2 in hormone-refractory human prostate cancers. *Am J Pathol* 143, 390-400 (1993)

126. Raffo A.J., H. Perlman, M.W. Chen, M.L. Day, J.S. Streitman, R. Buttyan: Overexpression of bcl-2 protects prostate cancer cells from apoptosis in vitro and confers resistance to androgen depletion in vivo. *Cancer Res* 55, 4438-4445 (1995)

127. Dorai T., H. Perlman, K. Walsh, A. Shabsigh, E.T. Goluboff, C.A. Olsson, R. Buttyan: A recombinant defective adenoviral agent expressing anti-bcl-2 ribozyme promotes apoptosis of bcl-2-expressing human prostate cancer cells. *Int J Cancer* 82, 846-852 (1999)

128. Miyake H., A. Tolcher, M.E. Gleave: Antisense Bcl-2 oligodeoxynucleotides inhibit progression to androgen-independence after castration in the Shionogi tumor model. *Cancer Res* 59, 4030-4034 (1999)

129. Holt S.E., J.W. Shay: Role of telomerase in cellular proliferation and cancer. *J Cell Physiol* 180, 10-18 (1999)

130. Paradis V., D. Dargere, I. Laurendeau, G. Benot, V. M, A. Jardin, P. Bedossa: Expression of the RNA component of human telomerase (hTR) in prostate cancer, prostatic intraepithelial neoplasia, and normal prostate tissue. *J Pathol* 189, 213-218 (1999)

131. Lin Y., H. Uemura, K. Fujinami, M. Hosaka, M. Harada, Y. Kubota: Telomerase activity in primary prostate cancer. *J Urol* 157, 1161-1165 (1997)

132. Zhang W., L.R. Kapusta, J.M. Slingerland, L.H. Klotz: Telomerase activity in prostate cancer, prostatic intraepithelial neoplasia, and benign prostatic epithelium. *Cancer Res* 58, 619-621 (1998)

133. Wullich B., V. Rohde, B. Oehlenschlager, H. Bonkhoff, R. Ketter, T. Zwergel, H.P. Sattler: Focal intratumoral heterogeneity for telomerase activity in human prostate cancer. *J Urol* 161, 1997-2001 (1999)

134. Chin L., S.E. Artandi, Q. Shen, A. Tam, S.L. Leel J.A., M. Cheng, M.F. Roussel, R.A. DePinho: p53 deficiency rescues the adverse effects of telomere loss and cooperates with telomere dysfunction to accelerate carcinogenesis. *Cell* 97, 527-538 (1999)

135. Greenberg R.A., L. Chin, A. Femino, K.H. Lee, G.J. Gottlieb, R.H. Singer, C.W. Greider, R.A. DePinho: Short dysfunctional telomeres impair tumorigenesis in the INK4a (delta2/3) cancer-prone mouse. *Cell* 97, 515-525 (1999)

136. de Lange T., T. Jacks: For better or worse? Telomerase inhibition and cancer. *Cell* 98, 273-275 (1999)

137. Feldman D., R.J. Skowronski, D.M. Peehl: Vitamin D and prostate cancer. *Adv Exp Med Biol* 375, 53-63 (1995)

138. Blutt S.E., E.A. Allegretto, J.W. Pike, N.L. Weigel: 1,25-dihydroxyvitamin D3 and 9-cis-retinoic acid act synergistically to inhibit the growth of LNCaP prostate cells and cause accumulation of cells in G1. *Endocrinology* 138, 1491-7 (1997)

139. Hedlund T.E., K.A. Moffatt, M.R. Uskokovic, G.J. Miller: Three synthetic vitamin D analogues induce prostate-specific acid phosphatase and prostate-specific antigen while inhibiting the growth of human prostate cancer cells in a vitamin D receptor-dependent fashion. *Clin Cancer Res* 3, 1331-1338 (1997)

140. Zhuang S.H., K.L. Burnstein: Antiproliferative effect of 1alpha, 25-dihydroxyvitamin D3 in human prostate cancer cell line LNCaP involves reduction of cyclin-dependent kinase 2 activity and persistent G1 accumulation. *Endocrinology* 139, 1197-1207 (1998)

141. Blutt A.E., T.J. McDonnell, T.C. Polek, N.L. Weigel: Calcitriol-induced apoptosis in LNCaP cells is blocked by overexpression of Bcl-2. *Endocrinology* 141, 10-17

142. Hisatake J., T. Kubota, Y. Hisatake, M. Uskokovic, S. Tomoyasu, H.P. Koeffler: 5,6-trans-16-ene-vitamin D3: a new class of potent inhibitors of proliferation of prostate, breast, and myeloid leukemic cells. *Cancer Res* 4023-4029 (1999)

143. Aaltonen L.A., P. Peltomaki, F.S. Leach, P. Sistonen, L. Pylkkanen, J.P. Mecklin, H. Jarvinen, S.M. Powell, J. Jen, S.R. Hamilton: Clues to the pathogenesis of familial colorectal cancer. *Science* 260, 812-816 (1993)

144. Dahiya R., C. Lee, J. McCarville, W. Hu, G. Kaur, G. Deng: High frequency of genetic instability of microsatellites in human prostatic adenocarcinoma. *Int J Cancer* 72, 762-767 (1997)

145. Rohrbach H., C.J. Haas, G.B. Baretton, A. Hirschmann, J. Diebold, R.P. Behrendt, U. Lohrs: Microsatellite instability and loss of heterozygosity in prostatic carcinomas: comparison of primary tumors, and of corresponding recurrences after androgen-deprivation therapy and lymph-node metastases. *Prostate* 40, 20-27 (1999)

146. Crundwell M.C., D.G. Morton, D.G. Arkell, S.M. Phillips: Genetic instability in incidentally discovered and advanced prostate cancer. *Br J Urol Int* 84, 123-127 (1999)

147. Fishel R.: Signaling mismatch repair in cancer. *Nat Med* 5, 1239-1241 (1999)

148. Boyer J.C., A. Umar, J.I. Risinger, J.R. Lipford, M. Kane, S. Yin, J.C. Barrett, R.D. Kolodner, T.A. Kunkel: Microsatellite instability, mismatch repair deficiency, and genetic defects in human cancer cell lines. *Cancer Res* 55, 6063-6070 (1995)

149. Hsiao P.W., D.L. Lin, R. Nakao, C. Chang: The linkage of Kennedy's neuron disease to ARA24, the first identified androgen receptor polyglutamine region-associated coactivator. *J Biol Chem* 274, 20229-20234 (1999)

150. Ingles S.A., G.A. Coetzee, R.K. Ross, B.E. Henderson, L.N. Kolonel, L. Crocitto, W. Wang, R.W. Haile: Association of prostate cancer with vitamin D receptor haplotypes in African-Americans. *Cancer Res* 58, 1620-1623 (1998)

151. Taylor J.A., A. Hirvonen, M. Watson, G. Pittman, J.L. Mohler, D.A. Bell: Association of prostate cancer with vitamin D receptor gene polymorphism. *Cancer Res* 56, 4108-4110 (1996)

152. Kibel A.S., S.D. Isaacs, W.B. Isaacs, G.S. Bova: Vitamin D receptor polymorphisms and lethal prostate cancer. *J Urol* 160, 1405-1409 (1998)

153. Ma J., M.J. Stampfer, P.H. Gann, H.L. Hough, E. Giovannucci, K.T. Kelsey, C.H. Hennekens, D.J. Hunter: Vitamin D receptor polymorphisms, circulating vitamin D metabolites, and risk of prostate cancer in United States physicians. *Cancer Epidemiol Biomarkers Prev* 7, 385-390 (1998)

154. Lara P.N.J., F.J. Meyers: Treatment options in androgen-independent prostate cancer. *Cancer Investigation* 17, 137-723 (1999)

155. De Marzo A.M., D. Coffey, W.G. Nelson: New concepts in tissue specificity for prostate cancer and benign prostatic hyperplasia. *Urology* 53, 29-42 (1999)

156. Robson M., N. Dawson: How is androgen-dependent metastatic prostate cancer best treated? *Hematol Oncol Clin North Am* 10, 727-747 (1996)

157. Bubley G.J., S.P. Balk: Treatment of metastatic prostate cancer. Lessons from the androgen receptor. *Hematol Oncol Clin North Am* 10, 713-725 (1996)

158. Smith D.C., K.J. Pienta: Paclitaxel in the Treatment of Hormone-Refractory Prostate Cancer. *Semin Oncol* 26, 109-111 (1999)

159. DiPaola R.S., J. Aisner: Overcoming *bcl2*- and *p53*-mediated resistance in prostate cancer. *Semin Oncol* 26, 112-116 (1999)

160. Zi X., R. Agarwal: Silbinin decreases prostate-specific antigen with cell growth inhibition via G1 arrest, leading to differentiation of prostate carcinoma cells: Implications for prostate cancer intervention. *Proc Natl Acad Sci* 96, 7490-7495 (1999)

161. Hodge J.W., J. Schlom, S.J. Donohue, J.E. Toaszewski, C.W. Wheeler, B.S. Levine, L. Gritz, D. Panicali, J.A. Kantor: A recombinant vaccinia virus expressing human prostate-specific antigen (PSA): safety and immunogenicity in a non-human primate. *Int J Cancer* 63, 231-237 (1995)

162. Sharma P., N. Schreiber-Agus: Mouse models of prostate cancer. *Oncogene* 18, 5349-5355 (1999)

163. Maini A., C. Archer, Y. Wang, G.P. Haas: Comparative pathology of benign prostatic hyperplasia and prostate cancer. *In Vivo* 11, 293-299 (1997)

164. Waters D.J., W.A. Sar, D.W. Hayden, C.M. Lang, L. McKinney, G.P. Murphy, R. Radinsky, R. Ramoner, R.C. Richardson, J. Tindall: Workgroup 4: spontaneous prostate carcinoma in dogs and nonhuman primates. *Prostate* 36, 64-67 (1998)

165. Pylkkanen L., S. Makela, R. Santti: Animal models for the preneoplastic lesions of the prostate. *Eur Urol* 30, 243-248 (1996)

166. Royai R., P.H. Lange, R. Vessela: Preclinical models of prostate cancer. *Semin Oncol* 23, 35-40 (1996)

167. Stearns M.E., J.L. Ware, D.B. Agus, C.J. Chang, I.J. Fidler, R.S. Fife, R. Goode, E. Holmes, M.S. Kinch, D.M. Peehl, I.T. Pretlow, G.M. Thalmann: Workgroup 2: human xenograft models of prostate cancer. *Prostate* 36, 56-58 (1998)

168. Schreiber-Agus N., Y. Meng, T. Hoang, H.J. Hou, K. Chen, R. Greenberg, C. Cordon-Cardo, H.W. Lee, R.A. DePinho: Role of Mxi1 in ageing organ systems and the regulation of normal and neoplastic growth. *Nature* 393, 483-487 (1998)

169. Krege J.H., J.B. Hodgin, J.F. Couse, E. Enmark, M. Warner, J.F. Mahler, M. Sar, K.S. Korach, J.A. Gustafsson, O. Smithies: Generation and reproductive phenotypes of mice lacking estrogen receptor beta. *Proc Natl Acad Sci USA* 95, 15677-15682 (1998)

170. Podsypanina K., L.H. Ellenson, A. Nemes, J. Gu, M. Tamura, K.M. Yamada, C. Cordon-Cardo, G. Catoretti, P.E. Fisher, R. Parsons: Mutation of Pten/Mmac1 in mice causes neoplasia in multiple organ systems. *Proc Natl Acad Sci USA* 96, 1563-1568 (1999)

171. Di Cristofano A., B. Pesce, C. Cordon-Cardo, P.P. Pandolfi: PTEN is essential for embryonic development and tumour suppression. *Nat Genet* 19, 348-355 (1998)

172. Cordon-Cardo C., A. Koff, M. Drobnjak, P. Capodieci, I. Osman, S.S. Millard, P.B. Gaudin, M. Fazzari, Z.F. Zhang, J. Massague, H.I. Scher: Distinct altered patterns of p27KIP1 gene expression in benign prostatic hyperplasia and prostatic carcinoma. *J Natl Cancer Inst* 90, 1284-1291 (1998)

173. Bhatia-Gaur R., A.A. Donjacour, P.J. Sciavolino, M. Kim, N. Desai, P. Young, C.R. Norton, T. Gridley, R.D. Cardiff, G.R. Cunha, C. Abate-Shen, M.M. Shen: Roles for Nkx3.1 in prostate development and cancer. *Genes Dev* 13, 966-977 (1999)

174. Green J.E., N.M. Greenberg, C.L. Ashendel, J.C. Barrett, C. Boone, R.H. Getzenberg, J. Henkin, R. Matusik, T.J. Janus, H.I. Scher: Workgroup 3: transgenic and reconstitution models of prostate cancer. *Prostate* 36, 59-63 (1998)

175. Greenberg N.M., F.J. DeMayo, P.C. Sheppard, R. Barrios, R. Lebovitz, M. Finegold, R. Angelopoulou, J.G. Dodd, M.L. Duckworth, J.M. Rosen, R.J. Matusik: 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 (1994)

176. Yan Y., P.C. Sheppard, S. Kasper, L. Lin, S. Hoare, A. Kapoor, J.G. Dodd, M.L. Duckworth, R.J. Matusik: Large fragment of the probasin promoter targets high levels of transgene expression to the prostate of transgenic mice. *Prostate* 32, 129-139 (1997)

177. Maroulakou I.G., M. Anver, L. Garrett, J.E. Green: Prostate and mammary adenocarcinoma in transgenic mice carrying a rat C3 (1) simian virus 40 large tumor antigen fusion gene. *Proc Natl Acad Sci USA* 91, 11236-11240 (1994)

178. Kitsberg D.I., P. Leder: Keratinocyte growth factor induces mammary and prostatic hyperplasia and mammary adenocarcinoma in transgenic mice. *Oncogene* 13, 2507-2515 (1996)

179. Muller W.J., F.S. Lee, C. Dickson, G. Peters, P. Pattengale, P. Leder: The int-2 gene product acts as an epithelial growth factor in transgenic mice. *EMBO J* 9, 907-913 (1990)

180. Garabedian E.M., P.A. Humphrey, J.I. Gordon: A transgenic mouse model of metastatic prostate cancer originating from neuroendocrine cells. *Proc Natl Acad Sci USA* 95, 15382-15387 (1998)

181. Perez-Stable C., N.H. Altman, J. Brown, M. Harbison, C. Cray, B.A. Roos: Prostate, adrenocortical, and brown adipose tumors in fetal globin/T antigen transgenic mice. *Lab Invest* 74, 363-373 (1996)

182. Skalnik D.G., D.M. Dorfman, D.A. Williams, S.H. Orkin: Restriction of neuroblastoma to the prostate gland in transgenic mice. *Mol Cell Biol* 11, 4518-4527 (1991)

183. Greenberg N.M., F. DeMayo, M.J. Finegold, D. Medina, W.D. Tilley, J.O. Aspinall, G.R. Cunha, A.A. Donjacour, R.J. Matusik, J.M. Rosen: Prostate cancer in a transgenic mouse. *Proc Natl Acad Sci USA* 92, 3439-3443 (1995)

184. Kasper S., P.C. Sheppard, Y. Yan, N. Pettigrew, A.D. Borowsky, G.S. Prins, J.G. Dodd, M.L. Duckworth, R.J. Matusik: 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 (1998)

185. Choi Y.W., D. Henrard, I. Lee, S.R. Ross: The mouse mammary tumor virus long terminal repeat directs expression in epithelial and lymphoid cells of different tissues in transgenic mice. *J Virol* 61, 3013-3019 (1987)

186. Landis S.H., T. Murray, S. Bolden, P.A. Wingo: Cancer Statistics, 1999. *CA Cancer J Clin* 49, 8-31 (1999)

Key Words: Prostate Cancer, Cell Cycle, Cyclin D1, Transgenic Model, Review

Send Correspondence to: Dr Richard G. Pestell, The Albert Einstein Cancer Center, Departments of Medicine, and Developmental and Molecular Biology, Albert Einstein College of Medicine, Chanin 302, 1300 Morris Park Ave, Bronx, New York, 10461, Tel: 718-430-8662, Fax: 718-430-8674, E-mail: pestell@aecom.yu.edu

Footnote: Mr. Derek F. Amanatullah and Dr. Anne T. Reutens contributed equally to this manuscript.