In vitro and in vivo model systems used in prostate cancer research

David Cunningham[1], Zongbing You[1,2,3,4,5*]

[1]Department of Structural & Cellular Biology, [2]Department of Orthopaedic Surgery, [3]Tulane Cancer Center and Louisiana Cancer Research Consortium, [4]Tulane Center for Stem Cell Research and Regenerative Medicine, [5]Tulane Center for Aging, Tulane University Health Sciences Center, New Orleans, LA, USA

*Corresponding author: Zongbing You, MD, PhD, Department of Structural & Cellular Biology, Tulane University School of Medicine, 1430 Tulane Ave Mailbox 8649, New Orleans, LA 70112, USA. Tel.: 504-988-0467; Fax: 504-988-1687; E-mail: zyou@tulane.edu.

Competing interests: The authors have declared that no competing interests exist.

Abbreviations used: AR, androgen receptor; ARBS, androgen receptor binding sites; ASIR, age standardized incidence rate; BPH, benign prostatic hyperplasia; CBP, Creb binding protein; EGF, epidermal growth factor; FGF, fibroblast growth factor; HA, hemagluttinin; HECD, E-cadherin; HPE, human prostatic epithelium; HPV, human papilloma virus; IVIS, in vivo imaging system; kD, kilodalton; LAPC, Los Angeles Prostate Cancer; LKB1, liver kinase B1; LPB, long probasin; mCRPC, metastatic castration-resistant prostate cancer; MMP, matrix metalloproteinase; mPIN, mouse prostatic intraepithelial neoplasia; NE, neuroendocrine; NSE, neuron specific enolase; PAP, prostatic acid phosphatase; PB/rPB, probasin; PCa, prostate cancer; PEG, polyethylene glycol; PIN, prostatic intraepithelial neoplasia; PhIP, 2-amino-1-methyl-6-phenylimidazo(4,5-b)pyridine; PSA, prostate specific antigen; PTEN, phosphatase and tensin homologue; Rb, retinoblastoma; ROS, reactive oxygen species; SRC, subrenal capsule; RGD, arginine-glycine-aspartate; STK11, serine/threonine kinase 11; SV40, simian virus 40; TMPRSS2-ERG, transmembrane protease, serine 2, ETS regulated gene; TGF-α, transforming growth fact α; TGF-β, transforming growth factor β; TRAMP, transgenic adenocarcinoma of the mouse prostate; WHO, World Health Organization; WT, wild type

Received April 27, 2015; Revision received May 18, 2015; Accepted May 18, 2015; Published June 4, 2015

Abstract New incidence of prostate cancer is a major public health issue in the Western world, and has been rising in other areas of the globe in recent years. In an effort to understanding the molecular pathogenesis of this disease, numerous cell models have been developed, arising mostly from patient biopsies. The introduction of the genetically engineered mouse in biomedical research has allowed the development of murine models that allow for the investigation of tumorigenic and metastatic processes. Current challenges to the field include lack of an animal model that faithfully recapitulates bone metastasis of prostate cancer.

Keywords: prostate cancer, cell lines, intratibial injection, mouse models, xenograft

Introduction

The incidence of prostate cancer (PCa) is one of the most prevalent cancer diagnoses throughout the world, and is one of the most intensely studied problems in human disease. According to the American Cancer Society, in 2014 there were over 233,000 new cases of PCa diagnosed in the US, resulting in about 29,340 deaths [1]. As cancer is a disease of aging, prostate lesions occur infrequently before the age of 40, with the peak incidence occurring between the age of 70–74 [2]. According to the World Health Organization (WHO)'s International Agency for Research on Cancer, the cancer incidence on five continents indicates that the age standardized incidence rate (ASIR) of PCa among some Asian populations has increased from nearly three to over fifty fold in places such as Hong Kong and Shanghai [3].

The etiology of PCa has remained a puzzling issue. A strong correlation exists with age, with increased relative risk for individuals with a family history [4]. African-American men are at significantly higher incidence, with one recent study citing a 12% increase in the proportion of an African-American cohort with multiple positive biopsy cores compared to a White-American cohort [5]. Environmental risk factors, which have been quantified in adoption studies at 4.8%, include the consumption of long chain polyunsaturated fatty acids found in smoked or over-cooked fish, vitamin D deficiency in people with reduced tanning potential, and dietary factors such as intake of red meat [6-9]. Smoking may also put individuals at increased risk for PCa,

and sexually transmitted diseases have been identified as a risk factor [10,11]. Inflammatory factors have been implicated in the development of PCa, such as bacterial toxins and exogenous carcinogens such as 2-amino-1-methyl-6-phenylimidazo(4,5-b)pyridine or PhIP [12,13].

There is a discussion in the field regarding the specificity of the prostate specific antigen (PSA) test as a useful prognostic indicator of potential metastatic castration resistant prostate cancer (mCRPC), with some saying that widespread epidemiological screening does not justify the modest decrease in cancer death [14-16]. In the new paradigm of "predict, prevent, and personalize" medicine, understanding the molecular phenotype and pathogenesis of disease will allow for more accurate and precise treatment of PCa

In vitro model systems

By far the most useful *in vitro* model that we have of PCa is cell culture. The sheer number of cell lines that are available for study is expansive, due to the fact that PCa can arise from one of several cell sources in the prostate. In addition to the information contained in this review, a good cell line database is available from the British Columbia (BC) Cancer Agency [17]. A comprehensive and exhaustive two-part compendium of PCa cell lines is available from Sobel and Sadar [18,19]. All information is derived from the BC Cancer Agency Prostate Cancer Cell Line Database cited previously unless otherwise specified. A summary is available in **Table 1**.

DU-145

Along with LNCaP and PC3 cells, DU-145 cells were once considered part of the triad that constituted the gold standard of PCa cell culture lines. DU-145 cells were first isolated from a brain metastatic prostate tumor in 1975 [20]. The isolation of this line represented an answer to the criticism of then existing cell lines MA 160 and EB 33, both of which represented cell isolated from admixtures of benign tumor or moderately differentiated adenocarcinoma. It is hormone independent and does not express androgen receptor (AR) mRNA/protein or PSA mRNA/protein. This observation has brought this cell line into disfavor among investigators. The vast majority of human prostate tumors express AR, so some would contend that this is a model that does not faithfully mimic human disease [21]. This is an important consideration, as the selection of this cell line for experimentation such as studies that seek to test the effect of hormone status would be futile on this model. In addition to absent AR, this line demonstrates a heterozygous P223L/V274F p53 expression pattern, changing its transcriptional program [22]. Phosphatase and tensin homolog (PTEN) expression is heterozygous [23]. This line contains marker chromosomes M_1, M_2, and M_3. Its karyotype ranges from 46 to 143 (modal of 64) with a metacentric Yq+ chromosome. This cell line's doubling time is established at 34 hours [24].

An important consideration in the selection of a PCa cell model is growth rate and behavior as a xenograft. DU-145 cells maintain phenotype and genotype when injected into mice and metastasize to a variety of organs, including spleen, lung, and liver [25,26]. Tumor growth in SCID mice has a 7-day latency with a biphasic growth rate of 5.5 for doubling in days 7–14 and 8.5 days for doubling after day 14 when placed without sponge material [27].

The response to growth factors is another important consideration to make when choosing a PCa cell line as growth factor independence via autocrine signaling has been defined as one of the hallmarks of cancer [28]. A substantial amount of work has been done on the effect of growth factors on this cell line. Of particular note is the increased expression of TGF-α and IGF-1 [29,30]. More information on the growth factors and growth factor receptor expression profile is available in **Table 1**.

Investigators have recently started to interrogate the role of energy metabolism in the role of PCa carcinogenesis. One of the main factors in energy metabolism is liver kinase B1 (LKB1, also referred to as serine/threonine kinase 11; STK11), an upstream kinase of Wnt/β-catenin and Hedgehog signaling pathway targets [31,32]. Recent work done on glucose deprivation and activation of AMP-activated protein kinase by vascular endothelial growth factor detected an absence of LKB1 in this cell line [33].

As a final note, one of the obvious goals of cancer treatment is to induce cancer cells into apoptosis and thus decreased tumor bulk. Pro- and anti-apoptotic proteins are delicately balanced in non-cancerous cells but can become deranged in cancer settings. An important consideration to make using DU-145 cells as an *in vitro* model system is the presence of the pro-apoptotic protein Bax. The expression of Bax in this cell line has been debatable. An older report by Shirahama et al. indicates the expression of Bax in this line [34], but the bulk of subsequent literature seems to indicate its absence [35-37]. It is recommended that Western blot analysis on this line's Bax expression be conducted prior to utilization as a research model.

PC3

PC3 cells were isolated from a vertebral metastatic prostate tumor in 1979 in answer to the concern that few lines were available that were entirely composed of carcinoma cells [38]. This cell line is similar to DU-145 cells in that it is hormone insensitive and presents no AR or PSA mRNA/protein, bringing it into similarly marginal favor with some investigators. As a highly aneuploid line, its karyotype has a modal number of 58 with a doubling time of approximately 33 hours. Among the interesting observations made about this cell line is the expression of transferrin receptor and growth stimulation by treatment with bone marrow derived transferrin [39,40]. Autonomous growth can be attributed to high expression levels of TGF-α and EGF-R [41]. This has led some investigators to speculate that this is one factor that makes bone a hospitable metastatic site. It expresses aberrant p53 with a C deletion in codon 138 causing a nonsense codon at 169 (causing a loss of heterozygosity) and is PTEN deficient [42,43]. Little information is available on xenograft growth rates, but our experience with intratibial cancer cell inoculations suggests it is similar to DU-145 cells with latency of approximately one week and doubling times in the range of 4–5 days. Of important note to investigators working with PC3 cells is the recent observation that this line is more characteristic of neuroendocrine, or small cell, carcinoma rather than adenocarcinoma [44].

LNCaP

As the lynchpin for numerous subsequent derivatives, LNCaP cells were first isolated from a human metastatic prostate adenocarcinoma found in a lymph node [45]. The original LNCaP cell line is androgen responsive with AR and PSA mRNA/protein expression. Of particular note, this cell line contains a T877A mutation in the AR coding sequence that gives it promiscuous binding affinity to a range of steroid compounds [46]. This slow growing cell line doubles every 60–72 hours (depending on serum concentration) and has a karyotype of 33 to 91 chromosomes (modal of 76–91). They are responsive to TGF-α, EGF and IGF-1, and express EGF/TGF-α-R, FGF-R, and IGF-1-R [47,48]. Of note is the expression of cytokeratins (CK) 8, 18, and 20, wild type (WT) p53 and PTEN inactivation [49-51]. Xenografting demonstrates a modest 50% success rate with a tumor doubling time of 86 hours when combined with a Matrigel™ formulation [18].

C4-2B

These cells were isolated from a mouse vertebral metastasis in 1994 as a subline of LNCaP xenografts, a derivation of a previous cell line established by Wu et al [52]. The C4 line was generated using subcutaneous co-injection of LNCaP and human osteosarcoma MS cells. Mice were then castrated to drive the tumors into androgen independence, and subsequent tumor cells were cultured and termed C4. The C4 cells were then subcutaneously co-injected into a castrated mouse. Then, the tumor cells were cultured from the tumors formed and termed C4-2. These cells were then subcutaneously or orthotopically injected into castrated mice, with subsequent surveillance for metastasis. Several metastases were detected, with one metastasis spreading to the bone. These bone metastatic cancer cells were finally isolated and termed C4-2B [53]. The authors who initially isolated this line have noted that castrated mice have a higher incidence of bone metastasis than do intact mice. Karyotyping ranges from 61–90 chromosomes (modal of 83) in the C4 line with a doubling time of 48 hours for C4-2B specifically.

Table 1. Common cell lines used in Prostate Cancer Research.

Name	Source	Metastasis in mice	Doubling time	PSA RNA	PSA protein	AR RNA	AR protein	Cytokeratin expression	Other markers	Karyotype	Reference
Normal											
pRNS-1-1	radical prostatectomy	No	~68 hours	No	No	Yes	Yes	5, 8		49–52, Monosomy 5, 16	Lee, 1994 [84]
RWPE-1	HPE cells from the peripheral zone	No	120 hours	Yes	Yes	Yes	Yes	8, 18		45/51, Loss of 7q and 18	Rhim, 1994 [75], Bello, 1997 [74]
BPH 1	Epithelial cells from a transurethral specimen demonstrating BPH	No	~35 hours	No	No	No	No	8, 18	Increased p53, PTEN, Bax, p21	71–79, modal of 76	Hayward, 1994 [81]
PIN											
PIN Cells	Cultured from a human high grade PIN lesion	No (non-tumorigenic)	Not reported	Yes	Yes	Not reported	Not reported	18, 34βE12	IL10 receptor	Not reported	Stearns, 1999 [89]
Hormone Naïve											
RWPE-2	v-Ki-ras transfection in RWPE-1	Not metastatic; tumorigenic in mice	58 hours	Yes	Yes	Yes	Yes	8,18	p53, Rb	Hyperdiploid	Bello, 1997 [74]
LNCaP	Lymph node	Yes; osteoblastic phenotype in bone metastasis	28–60 hours	Yes	Yes	Yes	Yes	CK-8, 18, 20	Vimentin, HECD 1, a-, b-, g-catenin, PAP, CBP	33–91; highly aneuploid	Horoszewicz, 1980 [45]
LAPC-4	Lymph node of androgen insensitive patient	Yes; osteolytic phenotype in bone metastasis	~72 hours	Yes	Yes	Yes	Yes	5, 8, and 18	2 p53 mutations (P72R and R175H)	79–92, modal of 89	Klein, 1997 [55]
LAPC-9	Femoral metastasis in patient undergoing homone ablation therapy	Yes; osteoblastic phenotype in bone metastasis	Not reported	Yes	Yes	Yes	Yes	5	Ki-67 in the presence of androgens only	Not reported	Craft, 1999 [58]
VCaP	Vertebral metastasis	Yes; osteoblastic phenotype in bone metastasis	120–144 hours	Yes	Yes	Yes	Yes	8, 18	PAP, Rb, p53, one copy of TMPRSS2-ERG gene fusion	Hypodiploid to hypertriploid	Korenchuk, 2001 [61]
MDA PCa 2a/2b	Two different areas of patient bone metastasis	Yes (both); osteoblastic phenotype in bone metastasis	82–93 hours/42–73 hours	Yes	Yes	Yes	Yes	5, 8, and 18	WT p53, p21, Rb, and Bcl-2	49–94, modal of 63/44–92, modal of 47	Navone, 1997 [70]
LuCaP	Lymph node (23.1, 23.8) and liver (23.12) metastasis in human patient	Yes; osteoblastic phenotype in bone metastasis	11, 15, 21 days for 23.1, 23.8, 23.12	Not reported	Yes	Not reported	Not reported	Not reported	5-alpha reductase type 1 but not type 2	62–112 (modal of 78); adequate karyotype was difficult due to poor morphology	Ellis, 1996 [77]

Table 1 (continued). Common Cell Lines Used in Prostate Cancer Research.

Name	Source	Metastasis in mice	Doubling time	PSA RNA	PSA protein	AR RNA	AR protein	Cytokeratin expression	Other markers	Karyotype	Reference
Castration Resistant											
C4-2	s.c. LNCaP tumor in nude mouse	Yes; osteoblastic phenotype in bone metastasis	~48 hours	Yes	Yes	Yes	Yes	CK-8	marker chromosome m1	61–90, modal of 84	Wu, 1994 [52]
C4-2B	Bone met LNCaP tumor in nude mouse	Yes; osteoblastic phenotype in bone metastasis	48 hours	Yes	Yes	Yes	Yes	CK-8	ABCG2	72–90, modal of 87, markers m1=i(7q), m2=der(11), t(11p+?)	Thalmann, 1994 [53]
22Rv1	CWR22R xenograft line	Yes; osteolytic phenotype in bone metastasis	35–40 hours	Yes	Yes	Yes	Yes	8, 18	45–56 kD cytokeratins; AR splice variants	50	Sramkoski, 1999 [63]
ARCaP (aka MDA PCa 1)	Ascites aspirate from a patient with bone metastasis	Yes; osteolytic phenotype in bone metastasis	-	Yes	Yes	Yes	Yes	8, 18	EGF receptor, c-erb B2/neu, c-erb B3, bombesin, serotonin, NSE, c-met, neurophysin, substance P, gelatinase A and stromelysin	near tetraploid	Zhau, 1996 [69]
PC-3	Vertebral metastasis	Yes; osteolytic phenotype in bone metastasis	~33 hours	No	No	No	No	7, 8, 18, 19, HECD 1,	Transferrin receptor, TGF-α, EGF-R	aneuploid, modal of 58	Kaighn, 1979 [38]
DU-145	Brain metastasis	Yes; osteolytic phenotype in bone metastasis	~34 hours	No	No	No	No	CK-8, -18	TGF-α, IGF-1, EGF, bFGF IGF-I, TGF-β; EGF/ TGF-α-R, FGF-R, IGF-I-R, TGF-β-R	46 - 143, modal of 64; M1, M2, M3, Yq+	Stone, 1978 [20]

They express AR and PSA mRNA/protein. These cells express low levels of p53 and are PTEN null [54]. This line consistently grows in either intact or castrated mice

LAPC-4

This cell line was introduced in 1997 and was the result of a series of subcutaneous xenografting experiments into SCID mice [55]. Explants from 6 of 8 Los Angeles prostate cancer (LAPC) patients were found to sustain growth very well in mice, doubling or tripling in volume. After several passages, several of the explants did not demonstrate detectable levels of human β-globin, and thus were deemed overrun by murine cells. This left only two explants, LAPC-3 and LAPC-4, as the sole survivors of the initial experiment. Of the two, LAPC-4 was the only cell line that retained its androgen dependence. Cytogenetics shows a range of 79–92 chromosomes (modal of 89) with loss of the Y chromosome. Doubling rate was calculated at around 72 hours. They are positive for AR/PSA mRNA/protein with P72R and R175H mutations in the p53 gene and express WT PTEN [56]. A mutation in the tumor protein 53 (TP53) gene causes an A175H mutation in p53. They can grow subcutaneously, orthotopically, or intratibially in mice [57]. Orthotopic inoculation tends to metastasize more frequently, while intratibial injection demonstrates an osteoblastic phenotype, mimicking frequent osteoblastic lesions found in humans

LAPC 9

These cells were first isolated from a femoral metastasis that formed in a patient undergoing androgen ablation therapy in 1999 [58]. Developed by the same group that isolated LAPC-4, it is a response to the need for an androgen sensitive counterpart. It is WT AR/PSA positive and will form tumors through subcutaneous injection in intact mice with as few as 10 cells. However, the investigators report that only a fraction of such injections will form tumors in castrated mice. A result of serial passaging in SCID mice, tumor-volume doubling time is approximately three weeks. These cells undergo growth arrest upon the removal of androgen but will retain sensitivity for up to 6 months post-removal. A very small subpopulation of cells was found to express cytokeratin 5 [59].

VCaP

These cells were first isolated in 2001, as the result of a vertebral metastatic lesion utilizing a procurement team designed to implement "warm" autopsies [60,61]. The cell line is positive for androgen sensitivity with wild-type AR mRNA/protein, and expresses PSA mRNA/protein, in addition to expressing prostatic acid phosphatase (PAP), retinoblastoma (Rb) and p53 (with an A248W mutation due to a mutation in TP53). Doubling time is 5 to 6 days and cytogenetics demonstrates a range of hypodiploid to hypertriploid genomes. PTEN remains intact in this cell line. These cells grow well in intact mice (doubling time of 10 days) as well as castrated mice (doubling time of 13 days) [18]. An important consideration to make in early PCa invasion is the role of the transmembrane protease, serine 2–ETS regulated gene (TM-PRSS2-ERG) gene rearrangement. This translocation occurs when the 3' end of ERG (21q22.3), ETV1 (7p21.2), or ETV4 (17q21) is added to the 5' end of the androgen responsive TMPRSS2 gene, creating an androgen-responsive oncoprotein [62]. VCaP cell line is one of the few PCa cell lines to demonstrate at least one copy of this rearrangement.

22Rv1

22Rv1 is a cell line that is representative of prostate carcinoma introduced in 1999 [63]. This line was isolated from the xenograft CWR22R that was isolated from a patient with bone metastasis. 22Rv1 was developed by plating CWR22R on irradiated feeder cells, trypsinized, and isolated via CD44 staining. Two successive regrowths on feeder cells allowed for the isolation of this line. Doubling time ranges from 35–40 hours. Cytogenetic analysis reveals a hyperdiploid genome in early passages but will expand to a stable tetraploid state as passaging continues. It consistently demonstrates trisomy for chromosomes 7, 8, and 12. It is positive for AR mRNA/protein and PSA mRNA, but negative for PSA protein. It is responsive to EGF, but it is not inhibited by TGF-β. It expresses WT PTEN [64-66]. This model is of particular interest to investigators researching AR splice variants. Variants that activate in a ligand independent manner have been identified as one of the main players in hormone refractory tumor progression [67]. Two such variants have been identified as being expressed endogenously in 22Rv1, specifically a full length isoform with an exon 3 duplication and C terminal domain truncations with aberrant exon 2b expression [68].

ARCaP

ARCaP was first introduced by Zhau et al. in 1996 and is also referred to as MDA PCa 1 [69]. First isolated from the ascites fluid of a patient with metastatic disease, it is highly metastatic with hallmarks of adenocarcinoma expression patterns of low AR mRNA and PSA mRNA/protein, with additional markers suggesting selective neuroendocrine differentiation. It expresses a H847Y AR variant and a Q331R p53 variant. This line has a take (or engraftment) rate of 100% in intact or castrated nude mice. A surprising observation is that tumors grow three times faster in castrated mice than in intact mice. Treatment with steroid compounds *in vitro* inhibits growth as well. TP53 analysis reveals a loss of heterozygosity and an A196end mutation [18].

MDA PCa 2a/2b

These two cell lines were derived from a single patient with vertebral metastasis during late stage disease in 1997 [70]. These are one of the few cells lines that were isolated from an African-American patient. Coming from two different areas of the same lesion, they are both androgen sensitive and tumorigenic in mice, with the 2a form doubling in 82–93 hours and the 2b form doubling in 42–73 hours, depending on the passage number. Different growth rate indicates that these two lines are clones from different cells within the same lesion. The karyotypes are 49–92 (modal of 63) for 2a and 44–92 (modal of 47) for 2b. The 2a form has a higher incidence of chromosomal abnormalities with a tetraploid genome. The 2b form has unique genomic characteristics, demonstrating a near diploid karyotype during early passages but expands to near tetraploid at passage 36. This observable change indicates that these alterations are a product of *in vitro* passaging and not the result of a polyclonal population of cells derived from the patient sample. Both cell lines exhibit WT TP53, express AR mRNA/protein with 2 mutations in the ligand-binding domain of MDA PCa 2a (L701H and T877A), and express PSA mRNA/protein [71,72]. One dichotomizing factor between the 2a and 2b forms is the presence of Bax in only 2a. The take rate in mice is increased with the addition of Matrigel™. The 2b form grows faster than the 2a *in vivo*, although 2a is the only one of the two to form palpable intraprostatic tumors after 11 weeks. PTEN is intact [73]. When used in conjunction, this model is referred to as MDA

PCa 2 (two) a and 2b bone metastases model (TabBO).

RWPE-2

It is a genetically modified form of the human normal prostatic epithelium cell line RWPE-1 (discussed below). Ki-ras gene and human papilloma virus 18 (HPV) genome were introduced into the RWPE-1 line, making it tumorigenic [74,75]. It is positive for AR and PSA mRNA/protein, and is described as hormone sensitive but not hormone dependent. It will grow *in vitro* appreciably without androgens, but will increase proliferation in their presence. This line expresses cytokeratins 8 and 18 and has a 48–54 chromosome karyotype (modal of 51). A unique trait about this line is the nucleus stains very strongly for WT p53 and Rb. It responds very well to EGF treatment and is inhibited by TGF-β. An injection of one million cells subcutaneously will form undifferentiated tumors in nude mice with a latency of 3–4 weeks. Since it has been immortalized with HPV, it strongly expresses the viral protein E7. Of special consideration is the fact that HPV DNA is found in up to 90% of cervical, vulvar, penile, and perianal cancers [76]. This makes RWPE-2 an important model for studying the potential role of viral factors in the transformation of prostate epithelium into carcinomas.

LuCaP

Similar to MDA PCa 2a and 2b, LuCaP cells are the result of three successful xenografts from a single patient at autopsy. LuCaP 23.1 and 23.8 were isolated from lymph node metastases and 23.12 was isolated from a liver metastasis in response to the need for cell lines that faithfully model prostate carcinoma [77]. The patient was a 63 year old Caucasian and had undergone radiation, orchiectomy, and chemotherapy. The chromosome range is 62–112 (modal of 78). Retaining androgen sensitivity and responsiveness, these cells do not grow in culture but must be maintained in mouse hosts via serial transplantation [19]. Volume doubling time in mice is 11, 15, and 21 days for 23.1, 23.8, and 23.12, respectively.

Non-tumor prostatic epithelial lines

RWPE-1

In comparison to the cancer cell lines discussed previously, non-tumorigenic human prostatic epithelium (HPE) cell lines are used as a way to contrast PCa pathogenesis. The most prevalent of this type of cell lines is RWPE-1. These cells were immortalized with human papilloma virus (HPV) 18 with subsequent isolation and propagation over 6–7 weeks [78,79]. This cell line is positive for AR/PSA mRNA/protein and is androgen sensitive, which was specifically developed to address the problem of normal prostatic epithelial growth and development [74]. It responds normally to EGF and TGF-β treatment and does not form tumors in mice. Benign prostatic hyperplasia (BPH) is an inflammatory condition that generates reactive oxygen species (ROS) and recent studies have tried to make a link connecting BPH to neoplasia [80]. BPH is a common condition among men in their sixth decade, with about 50% of that population demonstrating symptoms. RWPE-1 is therefore a good model to investigate the molecular mechanisms underlying the proliferation of benign prostatic epithelial cells.

BPH1

Hayward et al. isolated this cell line from benign prostatic hypertrophy or hyperplasia (BPH) tissues obtained through transurethral resection from a 68 year old patient undergoing the procedure for urinary obstruction consistent with BPH in 1994 [81]. Histologically benign, these cells were cultured out of the surgical specimen and immortalized, but not transformed, with the SV40 large T antigen. Karyotype analysis shows an aneuploid genome, with a range of 71–79 (modal of 76). Cells were viable but non-tumorigenic in mice. They are EGF, TGF-α, and FGF1/7 responsive, and are inhibited by FGF2 and TGF-β1/2 with androgen insensitivity. They are AR/PSA negative and WT p53 positive. It expresses PTEN, p21Cip1/Waf1, and Bax [82,83]. Doubling time is approximately 35 hours.

pRNS-1-1

This is another type of HPE cell introduced into the literature in 1994 [84]. This line has been immortalized, to at least 50 passages, with the pRSV-T plasmid, which contains an origin-defective SV40 genome and the rous sarcoma virus long terminal repeat (LTR) along with a neomycin resistance gene. It expresses cytokeratins 5 and 8, responds normally to typical growth signals (EGF, IGF, pituitary extract) and inhibitory signals (retinoic acid, TGF-β). However, investigation by Lee and colleagues seemed to indicate that these cells lost responsiveness to growth inhibitory signals such as TNF-α and vitamin D3, indicating the ability of this line to transform. This cell line has a karyotype of modal chromosome number of 49–52 with a doubling time of approximately 72 hours. PSA was detected in patient and early passage samples, but lost expression during later passages. An interesting observation made by Brinkmann and colleagues found these cells form normal prostatic glandular structures from cellular aggregates when treated with hepatocyte growth factor/scatter factor (HGF/SF) [85]. These aggregates would usually collapse after about a week in culture.

An important footnote in the story of pRNS-1-1 cells is the development of WPMY-1, isolated and developed by the same group and from the same prostate that gave us pRNS-1-1 [79]. This is a non-neoplastic stromal cell line that allows for the investigation of stromal/epithelial interactions in the development of PCa. Significant investigation is being conducted in the potential role that stromal cells play in the transition from hormone sensitive to castration resistant PCa [86].

PIN

No review of PCa cell lines would be complete without at least a mention of prostatic intraepithelial neoplasia (PIN). This is a condition that has been identified as a premalignant lesion in the development of PCa [87,88]. PIN cells are thus an important cell line for the investigation of oncogenic processes and molecules in a premalignant context. Developed and introduced into the literature in 1999 by Stearns and colleagues, this is a line isolated from an African-American patient [89]. The same HPV-18 construct used to immortalize RWPE-1 cells was also used to immortalize this cell line. The authors described this line in detail at passages 5 and 15, demonstrating PSA production via stimulation with DHT and dihydroxyepiandosterone at passage 5 but moving on to androgen insensitivity at passage 15. They also note that early passage cells expressed CK18 and 34βE12, but later passages lost detection of 34βE12. They indicated this as basal cell origin with no contribution from nearby cancer tissue. This group also looked at the role of interleukin-10 (IL-10), activin A, and inhibin (both members of the TGF-β superfamily) in this line's proliferative ability. They noted the ability of activin to stimulate growth even as TGF-β was able to reduce growth. Also noted is the ability of activin A to inhibit growth when present in culture media with IL-10. They also postulated that activin A blocks growth of these high grade PIN lesion cells by binding follistatin, thus preventing IL-10 growth stimulation. Xenograft studies revealed an inability of these cells to grow in mice.

In vivo model systems

Since the initial use of the mouse model in biomedical research nearly a century ago, scientists have gone from techniques as simple as cross breeding in a desired trait to selectively manipulating the genome to induce a disease state. PCa understanding has benefitted tremendously from the use of these techniques, particularly through the use of the mouse models detailed below. The need for *in vivo* models was an outgrowth of the understanding that cancer cell lines in general (and prostate lines in particular) do not and cannot recapitulate the disease processes that occur in living tissues. Teasing apart molecular interactions and alterations in cancer cells is of incredible worth, but they do not take into account all of the cellular interactions that occur as a cell goes through the oncogenic and metastatic process. Before progressing to the main models of this review, it should be noted that rat and canine have been used for some important discoveries about spontaneous prostate lesions. They are not often favored by the PCa research community due either to inadequate genetic manipulation in case of rats or expensive price and pet affection in case of dogs. However, descriptions of these models can be found in the references [90,91]. Thus, mice have been the favored model organism in recapitulating various aspects of the disease process. Excellent reviews devoted solely to mouse models are available in the references [92,93]. Information provided in this section is summarized in **Table 2**.

Mouse surgery and xenograft models

The most widely utilized tool in PCa research is the use of human PCa cell lines transplanted into mice, referred to as xenograft or xeno-transplantation. There are three modes of xenograft employed today: subcutaneous, orthotopic, and under the subrenal capsule (SRC). Each has its own costs and benefits.

Subcutaneous xenograft models were developed first utilizing the insertion of patient prostatic tissue into the shoulder of nude mice in the late 1970s by Schröder and colleagues at Erasmus University Rotterdam. This gave us the first transplantable tumor cell line, PC-82 [94]. The advantages of this model system include easy accessibility and less demanding technical expertise, as well as the amount of tumor tissue that can be introduced. The take is reportedly very low, owing to poor vascularization of the dermal tissue. Take rates vary in the literature in recent years from as low as 3% to as high as 58% [95]. Additionally, low to moderately aggressive tumors do not seem to thrive very well in this type of environment, as only highly aggressive tumors seem to account for the majority of tumors recovered from nude mice [96].

Orthotopic xenograft models allow for the introduction of cancerous prostatic tissue into the mouse prostate. Stephenson et al. first introduced this model in 1992. In their initial study they very clearly concluded that the subcutaneous, or any ectopic, model does not reveal the metastatic potential of implanted human PCa tissue. Additionally, this work was expanded by An and colleagues to show a high degree of lung and lymph node metastasis, the first such demonstration published in the literature [97]. The obvious advantages of this model include the relevance of the interactions between the implanted tissue and the organ of origin, as bolstered by the evidence that metastatic potential is increased not only in orthotopically implanted prostatic tissue but also other organs as well [98]. The take rate for this procedure is in a much more favorable, reported at nearly 72% [96]. This model also offers the advantage of implanting a wider range of tumor aggression levels, allowing for the investigation of tumorigenic and metastatic processes. The main disadvantages to this type of procedure include the skill set that is required for conducting the surgery and the limited amount of tissue that can be introduced into the mouse prostate. Additionally, androgen ablation therapy prior to tissue introduction causes such a large amount of prostatic regression that it makes implementation of some experimental designs practically impossible.

Table 2 . Common Mouse Models Used in Prostate Cancer Research.

Model	Genetic Alteration	Tumorigenic Time Course	Metastasis	Purpose	Reference
Pten	Cre recombinase to excise the coding sequence of Pten between two loxP sites	6 weeks to PIN formation; 9–29 weeks for invasive carcinoma	Lymph nodes, lung	Defined time course through PIN lesions	Wang, 2003 [132]
TRAMP	SV40 large/small t antigen driven using a prostate specific promoter PB; can combine ARR2 with PB to drive SV40 or a host of other molecules	Mild hyperplasia in 8–12 weeks, metastatic disease starting at 18 weeks	Bone, lymph node, lung, adrenal gland, kidney	Fast time course to metastatic disease	Greenberg, 1995 [109], Gingrich, 1996 [110]
Hi Myc	ARR2-PB promoter to drive high expression of c-myc with androgen responsiveness	3 months to PIN formation; 6 months to invasive cancer	None noted [145]	Defined time course; androgen sensitivity of promoter	Ellwood-Yen, 2003 [128]
Lo Myc	PB promoter to drive low expression of c-myc	6–12 months for PIN; 12 months or more for invasive cancer	None noted [146]	Slower time course; allows for investigation of oncogenic/castration resistance drivers	Ellwood-Yen, 2003 [128]
LADY	LPB; -12kb/+28 region of the PB promoter used to solely drive expression of SV40 large T antigen	10–15 weeks for dysplasia, 15–22 weeks for carcinoma in the 12T-7 (fast and slow)	Regional lymph nodes, liver, lung	Tumors demonstrate neuroendocrine differentiation [147] considered a late event in tumorigenesis	Kasper, 1998 [126]
MPAKT	-421/+28bp of PB driving the myr-HA-Akt1 construct	PIN lesions early in life; no invasive carcinoma detected up to 78 weeks	None reported	Study the transformation of prostatic epithelial cells	Majumder, 2003 [138]

SRC xenografts are the most recent development in xenotransplantation. Initially introduced into the literature by Wang in 2005, they were able to successfully recover over 93% of tumors [96]. They note the caveat that there was a spread of recovery rates dependent on individual experience of the person performing the procedure, but this still illustrates that SRC grafting is a viable way to recover xenograft material. The authors postulate that this technique is so successful because of the high degree of vascularity to the tissue. It would be difficult to comment on the metastatic potential of this model, as searches on PubMed for the term "prostate cancer subrenal capsule" returns 31 hits. The majority of these publications are aimed at investigating the primary tumor itself and don't comment on metastatic potential, although some studies, which are described in the references, do conclude that the investigation of metastatic genes is possible with this technique [99-101]. Despite the disadvantage of not being the native tissue from which the implanted tumor arises, SRC xenografts offer some exciting possibilities when combined with using patient tissue as the graft material. Wang and colleagues have recently commented on the utility of this system, pointing to the fact that such a high degree of tumors can be recovered using the SRC grafts [102]. They suggested that this could impact basic research on PCa, identification of metastatic driver genes, and the ability to tailor clinical therapy to the patient's tumor biology [103-105]. Preclinical *in vivo* studies are difficult to translate to the bedside because of the homogeneity of cultured cell lines, which can be contrasted with the heterogeneity of different subpopulations of both cancer and stromal cells within a tumor mass. Next generation patient-derived PCa xenograft models allow the cancerous subpopulations of cells to express their dominance when transplanted into NOD/SCID mice, and therapy can be tested and tailored to the tumor biology depending on the tumor's growth response. One organization that has been developing this avenue of investigation is the Living Tumor Laboratory [106].

Relevant to the topic of xenograft procedures, the issue of bioengineered materials should be discussed. Substances such as Matrigel™, polyethylene glycol (PEG), collagen and sponge are often used in the creation of a cell suspension pre-procedure to allow a microenvironment hospitable for cancer cell growth. There is no question that these materials are useful in rendering a more faithful tumor microenvironment both in xenograft and 3-dimensional (3D) culture models. However, it is still being discussed in the field about the effect that these materials have on gene expression profiles and responsiveness to androgen. Work by Lang et al. seems to indicate that Matrigel™ induces morphological changes that may be indicative of gene expression profile changes [107]. More recent studies have indicated more finely tuned materials such as PEG hydrogels, containing arginine-glycine-aspartate (RGD) and matrix metalloproteinase (MMP) cleavage sites, provide a microenvironment that better recapitulates native tissue environment [108]. This consideration should be made particularly if hormone sensitivity or drug discovery studies are being conducted.

TRAMP

Norman Greenberg and his group first developed this mouse in 1995 with subsequent description of metastatic disease in these animals by 28 weeks of age [109,110]. Spontaneously occurring prostate lesions were observed in limited types of animals; however, rats seem to form spontaneous adenocarcinoma on a regular basis [111,112]. Subsequent investigation showed that rat prostate contained a prostate specific promoter probasin (PB or rPB) that directed an androgen sensitive transcriptional program [113]. When considering the responsiveness of a mouse model to the development of PCa, the consideration and choice

of promoter is absolutely essential and is the main distinguishing feature between different models. Greenberg and his group were able to drive the expression of the SV40 large T antigen using the upstream 426 bp 5' flanking region and 28 bp of the 5' untranslated region. Experimentation with chloramphenicol acetyl transferase and luciferase reporter assays demonstrated that the -426/+28 PB promoter contained two androgen receptor binding sites (ARBS) in the regions of -236 to -223 and -140 to -117, respectively, with the region containing both of these sites (-244 to -40) referred to as ARR [113]. The oncogenic effects of the SV40 protein are primarily interactions with the tumor suppressors p53 and Rb. The loss of both of these proteins has been implicated in the development and progression of prostate lesions [114-117]. Using the composite of two linked ARR regions, referred to as ARR2, the combination ARR2-PB construct has been used to investigate a wide range of molecules and their role in the initiation and progression of PCa. Such examples include the growth signaling molecule Ras, hepsin, the ubiquitin E3 ligase SCF (SKP2, an F box protein), and FGF8 [118-121]. Bigenic models have also been developed. Fibroblast growth factor 2 (FGF2) null mice have been crossed with TRAMP animals to investigate the role of that factor in tumor development, drawing upon observations of high expression of FGF2 in PC3 and DU-145 cells [122]. This model is generally regarded with very high utility, as the progression through PIN lesions to malignant disease is on a predictable time course that mimics human oncogenic milestones. However, the TRAMP model may not be the best suited for oncogenic studies. It is well purposed for studies of treatment and prevention. Recent studies of this model have shown that many tumors display neuroendocrine (NE) differentiation, suggesting that this model is likely a model of small cell carcinoma, rather than adenocarcinoma [123,124].

LADY

One of the more insidious aspects of PCa is the fact that several cell types can contribute to the initiation or sustenance of a tumor. The typical prostate tissue architecture is composed of stroma, basal and luminal cells. However, in the consideration of the molecular and cellular mechanisms to account for castration resistance, scientists and physicians made the discovery that the nervous system makes a contribution to prostate tissue architecture in the form of NE cells. NE cells are thought to be paracrine signaling cells that control the growth of prostatic epithelium [125], and due to their lack of androgen receptor are thought to be a driver in castration resistance. The work of two groups led to the development of a mouse model that faithfully recapitulated the progression of prostatic intraepithelial neoplasia all the way to malignant disease [126,127]. The genetic modifications of the LADY model are similar to the TRAMP model, with a larger 12 kb region of the rPB promoter (referred to as the LPB) situated upstream of the sole SV40 large T antigen. The LADY model also has the distinction of containing the d1 2005 deletion mutation in the small t antigen, eliminating its expression. It was for the purpose of investigating the role of neuroendocrine differentiation that the LADY model was developed, and Masumori et al. were able to successfully demonstrate the presence of neuroendocrine differentiation in metastatic lesions [127].

Hi/Lo-Myc mouse

Sawyers and colleagues published their work on using two constructs based on using the PB promoter to drive prostate specific expression of c-Myc oncogene. One construct, designated Lo-myc, uses the PB promoter alone to drive c-Myc expression. The other, designated Hi-myc, uses PB coupled with a sequence of the ARR2 promoter, both

of which lie upstream of the human c-Myc gene to drive progression from mouse prostatic intraepithelial neoplasia (mPIN) to invasive adenocarcinoma [128]. An important distinction between these two models is the androgen responsiveness of the Hi-myc model. The ARR2 is androgen responsive, which has the implication of reversing mPIN by silencing expression of the transgene in mice 3 months post-castration and causing regression, but not elimination, of prostate tumors in mice out to 5 months post-castration. The Lo-myc model displays no such responsiveness [128].
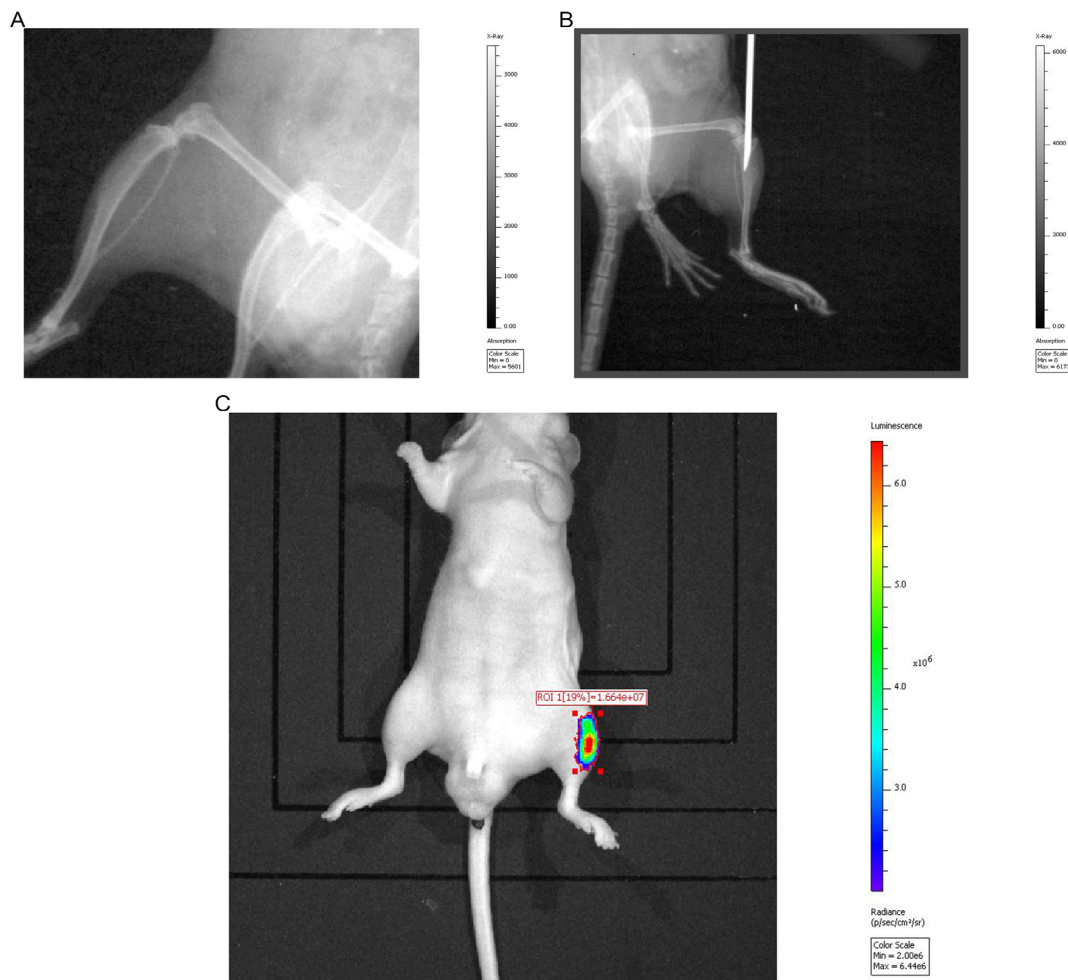
Figure 1. Intratibial cancer cell injection mouse model. A. X-ray image taken of a normal mouse tibia pre-procedure on the contralateral side with the radiolucent bone marrow cavity visible on the proximal end of the bone. **B.** X-ray image of a mouse tibia with a 21-gauge needle inserted into the proximal bone marrow cavity. **C.** Results of successful injection of PC3 cells expressing luciferase (and an injection of D-luciferin) using an IVIS (IVIS® Illumina XRMS Series III, PerkinElmer, Waltham, MA, USA).

Pten knockout

PTEN ablation has been shown to be an early event in PCa initiation and progression [129], and new technologies have allowed for this molecule to be knocked out in a mouse model. Briefly, it was discovered that Cre recombinase, a nuclease in the bacteriophage P1, recognized conserved sequences called loxP sites and excised any genetic information encoded between two of these sites [130]. Thus, mouse embryos can be engineered to have loxP sites flanking a gene of interest (a "floxed" gene). When Cre and a floxed gene are present in the same organism, the Cre will excise the floxed gene using loxP recognition, either in a constitutive manner or inducible manner through the use of estrogen compounds. A valuable tool in the investigation of PCa has been the Pten null mouse [131]. The value of this model is that the animal demonstrates the range of conditions seen in human subjects, progressing through low grade PIN to invasive carcinoma and metastasis in only 12 weeks [132]. There is also the benefit of Cre expression being predominantly tissue specific with highest expression seen in the lateral prostate [132]. The Pten knockout model has been successfully used to ascertain the contributions that IL-17 makes to the tumor microenvironment in a Pten and IL-17 receptor C double knockout model [133,134]. Additional bigenic models have also been developed using the Pten knockout background. Cross breeding between Pten heterozygotes and TRAMP, Nkx3.1, and p27Kip1 are all currently being implemented in experimentation [135-137].

MPAKT

The mouse prostate Akt (MPAKT) model was developed to study the role of protein kinase B (Akt) in the transformation of prostate epithelial cells [138]. The alteration to this animal's genome is the introduction of the coding sequence for Akt1 along with a myristolation sequence (myr) and a hemagluttinin (HA) epitope for proper targeting of the protein to the inner leaflet of the cell membrane. These elements are combined to create the PB-myr-HA-Akt1 construct [139]. PIN lesions were described via immunohistochemical (IHC) analysis early on in the mouse's life, but no invasive carcinoma was detected in any animals even after 78 weeks. The authors who originally developed this model think that there are more oncogenic factors at play in the transformation of prostate epithelium than just constitutive activation of the Akt

pathway. One interesting observation is the similarity of MPAKT and haploinsufficient Pten PIN lesions.

Metastatic models

Several important models for studying PCa metastasis are currently available. In an effort to model the bone metastatic tumor growth, the intratibial injection model was developed. PCa cells can be suspended in phosphate-buffered saline or Matrigel™ and directly injected into the tibias of genetically manipulated or WT mice. Imaging technologies can be applied to track changes in bone lesions and morphology of the tissue itself [140]. In vivo imaging system (IVIS) technologies have recently been implemented to track lesion growth, taking advantage of stable luciferase expression in PCa cell lines. An example of whole mouse *in vivo* imaging can be found in **Figure 1**.

Tail vein cancer cell injection is another method of assessing the role of different molecules in metastasis. The interactions between circulating tumor cells and the endothelium are extremely important factors in this process, and tail vein injections allow the delivery of a bolus of cancer cells into the bloodstream in a tolerable volume [141]. An excellent example of the utility of this model can be found in the recent publication by LeBeau and colleagues [142].

The other option for introducing cancer cells into the bloodstream is the use of intracardiac injection. Campbell and colleagues have introduced a good description of the protocol with accompanying visual documentation [140]. Jenkins et al. have combined the strategies of intracardiac inoculation of genetically altered PC-3M-luc-C6 cells to track propagation of metastasis [143].

Conclusions

When juxtaposed with the depth and breadth of characterization of breast cancer, the field of PCa research has been lagging behind in the understanding of how molecular phenotype affects clinical parameters and prognosis. Markers commonly used to diagnose this disease, such as PSA, have been met with opposition to its continued widespread use. Significant molecules in the initiation and progression of PCa, specifically AR, have yet to be fully elucidated in terms of their contribution to castration resistance. It is for this reason that investigation needs to continue, specifically to better understand the drivers behind oncogenesis, how this affects clinical course, and to provide therapeutic targets. The techniques and models described herein are essential tools for bolstering that understanding. Perhaps the biggest challenge in the field is developing an animal model that faithfully recapitulates bone metastasis. Due to the high degree of morbidity and mortality that is associated with this type of tumor progression, an animal model that reliably seeds tumor cells from prostate to bone would be of substantial value, both clinically and scientifically [144]. Such a model would allow for investigation of circulating tumor cell/bone matrix interactions and potential therapeutic targets for metastatic prevention. As a final note, a thorough investigation of cell lines reveals few *in vitro* models being isolated from African-American men that are widely available to the research community. Due to the increased incidence of PCa in this population, we would be remiss if not making a concerted effort to correct this oversight.

Acknowledgements

References

1. American C (2014) Prostate Cancer: Detailed Guide. Cited November 21. Available from http://www.cancer.org/cancer/prostatecancer/detailedguide/prostate-cancer-key-statistics 2. Lesko SM, Rosenberg L, Shapiro S (1996) Family history and prostate cancer risk. Am J Epidemiol 144: 1996-144.

2. Lesko SM, Rosenberg L, Shapiro S (1996) Family history and prostate cancer risk. Am J Epidemiol 144: 1041-1047. PMID: 8942435

3. Cullen J, Elsamanoudi S, Brassell SA, Chen Y, Colombo M, et al. (2012) The burden of prostate cancer in Asian nations. J Carcinog 11: 7. doi: 10.4103/1477-3163.94025. PMID: 22529743

4. Albright F, Stephenson RA, Agarwal N, Teerlink CC, Lowrance WT, et al. (2014) Prostate cancer risk prediction based on complete prostate cancer family history. Prostate 75: 390-398. doi: 10.1002/pros.22925. PMID: 25408531

5. Ha YS, Salmasi A, Karellas M, Singer EA, Kim JH, et al. (2013) Increased incidence of pathologically nonorgan confined prostate cancer in African-American men eligible for active surveillance. Urology 81: 831-5. doi: 10.1016/j.urology.2012.12.046. PMID: 23465143

6. Sundquist K, Sundquist J, Ji J (2015) Contribution of shared environmental factors to familial aggregation of common cancers: an adoption study in Sweden. Eur J Cancer Prev 24: 162-164. doi: 10.1097/CEJ.0000000000000101. PMID: 25415834

7. Dybkowska E, Swiderski F, Waszkiewicz-Robak B (2014) Fish intake and risk of prostate cancer. Postepy Hig Med Dosw 68: 1199-205.

8. Beyene D, Daremipouran M, Apprey V, Williams R, Ricks-Santi L, et al. (2014) Use of Tanning Potential as a Predictor for Prostate Cancer Risk in African-American Men. In Vivo 28: 1181-7. PMID: 25398820

9. Wright JL, Neuhouser ML, Lin DW, Kwon EM, Feng Z, et al. (2011) AMACR polymorphisms, dietary intake of red meat and dairy and prostate cancer risk. Prostate 71: 498-506. doi: 10.1002/pros.21267. PMID: 20945498

10. Shahabi A, Corral R, Catsburg C, Joshi AD, Kim A, et al. (2014) Tobacco smoking, polymorphisms in carcinogen metabolism enzyme genes, and risk of localized and advanced prostate cancer: results from the California Collaborative Prostate Cancer Study. Cancer Med 3: 1644-1655. doi: 10.1002/cam4.334. PMID: 25355624

11. Caini S, Gandini S, Dudas M, Bremer V, Severi E, et al. (2014) Sexually transmitted infections and prostate cancer risk: a systematic review and meta-analysis. Cancer Epidemiol 38: 329-338. doi: 10.1016/j.canep.2014.06.002. PMID: 24986642

12. Sfanos KS, De Marzo AM (2012) Prostate cancer and inflammation: the evidence. Histopathology 60: 199-215. doi: 10.1111/j.1365-2559.2011.04033.x. PMID: 22212087

13. Nakai Y, Nelson WG, De Marzo AM (2007) The dietary charred meat carcinogen 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine acts as both a tumor initiator and promoter in the rat ventral prostate. Cancer Res 67: 1378-1384. doi: 10.1158/0008-5472.CAN-06-1336. PMID: 17264317

14. Blanker MH, Noordzij MAA (2014) [Prostate cancer screening benefit very low, even after 13 years]. Ned Tijdschr Geneeskd 158: PMID: 25492737

15. Duffy MJ (2014) PSA in screening for prostate cancer: more good than harm or more harm than good? Adv Clin Chem. 2014;66:1-23. Adv Clin Chem 66: 1-23. PMID: 25344984

16. Thakur V, Talwar M, Singh PP (2014) Low free to total PSA ratio is not a good discriminator of chronic prostatitis and prostate cancer: An Indian experience. Indian J Cancer 51: 335-7.

17. Cell L, Cell L. BC Cancer Agency (2001) Cited November 21, 2014. Available from http://capcelllines.ca.

18. Sobel RE, Sadar MD (2005) Cell lines used in prostate cancer research: a compendium of old and new lines--part 1. J Urol 173: 342-359. doi: 10.1097/01.

ju.0000141580.30910.57. PMID: 15643172

19. Sobel RE, Sadar MD (2005) Cell lines used in prostate cancer research: a compendium of old and new lines--part 2. J Urol 173: 360-372. doi: 10.1097/01. ju.0000149989.01263.dc. PMID: 15643173

20. Stone KR, Mickey DD, Wunderli H, Mickey GH, Paulson DF (1978) Isolation of a human prostate carcinoma cell line (DU 145). Int J Cancer 21: 274-281. PMID: 631930

21. Heinlein CA, Chang C (2004) Androgen receptor in prostate cancer. Endocr Rev 25: 276-308. doi: 10.1210/er.2002-0032. PMID: 15082523

22. Bajgelman MC, Strauss BE (2006) The DU145 human prostate carcinoma cell line harbors a temperature-sensitive allele of p53. Prostate 66: 1455-1462. doi: 10.1002/pros.20462. PMID: 16741917

23. Fraser M, Zhao H, Luoto KR, Lundin C, Coackley C, et al. (2011) PTEN deletion in prostate cancer cells does not associate with loss of RAD51 function: implications for radiotherapy and chemotherapy. Clin Cancer Res 18: 1015-1027. doi: 10.1158/1078-0432.CCR-11-2189. PMID: 22114138

24. Webber MM, Bello D, Quader S (1997) Immortalized and tumorigenic adult human prostatic epithelial cell lines: characteristics and applications Part 2. Tumorigenic cell lines. Prostate 30: 58-64. PMID: 9018337

25. Mickey DD, Stone KR, Wunderli H, Mickey GH, Vollmer RT, et al. (1977) Heterotransplantation of a human prostatic adenocarcinoma cell line in nude mice. Cancer Res 37: 4049-4058. PMID: 908039

26. Bastide C, Bagnis C, Mannoni P, Hassoun J, Bladou F (2002) A Nod Scid mouse model to study human prostate cancer. Prostate Cancer Prostatic Dis 5: 311-315. doi: 10.1038/sj.pcan.4500606. PMID: 12627217

27. Billström A, Lecander I, Dagnaes-Hansen F, Dahllöf B, Stenram U, et al. (1995) Differential expression of uPA in an aggressive (DU 145) and a nonaggressive (1013L) human prostate cancer xenograft. Prostate 26: 94-104. PMID: 7531848

28. Hanahan D, Weinberg RA (2011) Hallmarks of cancer: the next generation. Cell 144: 646-74. doi: 10.1016/j.cell.2011.02.013. PMID: 21376230

29. Carruba G, Leake RE, Rinaldi F, Chalmers D, Comito L, et al. (1994) Steroid-growth factor interaction in human prostate cancer. 1. Short-term effects of transforming growth factors on growth of human prostate cancer cells. Steroids 59: 412-420. PMID: 7974525

30. Pietrzkowski Z, Mulholland G, Gomella L, Jameson BA, Wernicke D, et al. (1993) Inhibition of growth of prostatic cancer cell lines by peptide analogues of insulin-like growth factor 1. Cancer Res 53: 1102-1106. PMID: 8439954

31. Green JBA (2004) Lkb1 and GSK3-beta: kinases at the center and poles of the action. Cell Cycle 3: 12-14. PMID: 14657655

32. Xu P, Cai F, Liu X, Guo L (2014) LKB1 suppresses proliferation and invasion of prostate cancer through hedgehog signaling pathway. Int J Clin Exp Pathol 7: 8480-8488. PMID: 25674212

33. Yun H, Lee M, Kim SS, Ha J (2005) Glucose deprivation increases mRNA stability of vascular endothelial growth factor through activation of AMP-activated protein kinase in DU145 prostate carcinoma. J Biol Chem 280: 9963-72. doi: 10.1074/jbc.M412994200. PMID: 15640157

34. Shirahama T, Sakakura C, Sweeney EA, Ozawa M, Takemoto M, et al. (1997) Sphingosine induces apoptosis in androgen-independent human prostatic carcinoma DU-145 cells by suppression of bcl-X(L) gene expression. FEBS Lett 407: 97-100. PMID: 9141489

35. Nutt LK, Chandra J, Pataer A, Fang B, Roth JA, et al. (2002) Bax-mediated Ca2+ mobilization promotes cytochrome c release during apoptosis. J Biol Chem 277: 20301-20308. doi: 10.1074/jbc.M201604200. PMID: 11909872

36. von Haefen C, Wieder T, Gillissen B, Stärck L, Graupner V, et al. (2002) Ceramide induces mitochondrial activation and apoptosis via a Bax-dependent pathway in human carcinoma cells. Oncogene 21: 4009-4019. doi: 10.1038/sj.onc.1205497. PMID: 12037683

37. Marcelli M, Marani M, Li X, Sturgis L, Haidacher SJ, et al. (2000) Heterogeneous apoptotic responses of prostate cancer cell lines identify an association between sensitivity to staurosporine-induced apoptosis, expression of Bcl-2 family members, and caspase activation. Prostate 42: 260-273. PMID: 10679755

38. Kaighn ME, Narayan KS, Ohnuki Y, Lechner JF, Jones LW (1979) Establishment and characterization of a human prostatic carcinoma cell line (PC-3). Invest Urol 17: 16-23. PMID: 447482

39. Keer HN, Kozlowski JM, Tsai YC, Lee C, McEwan RN, et al. (1990) Elevated transferrin receptor content in human prostate cancer cell lines assessed in vitro and in vivo. J Urol 143: 381-385. PMID: 1688956

40. Rossi MC, Zetter BR (1992) Selective stimulation of prostatic carcinoma cell proliferation by transferrin. Proc Natl Acad Sci U S A 89: 6197-6201. PMID: 1631108

41. KZ. Ching (1993) Ramsey E, Pettigrew N, D'Cunha R, Jason M, Dodd JG (1993) Expression of mRNA for epidermal growth factor, transforming growth factor-alpha and their receptor in human prostate tissue and cell lines. Mol Cell Biochem. 1993;126(2):151-8. Mol Cell Biochem 126: 151-8. PMID: 7508078

42. van Bokhoven A, Varella-Garcia M, Korch C, Hessels D, Miller GJ (2001) Widely used prostate carcinoma cell lines share common origins. Prostate 47: 36-51. doi: 10.1002/pros.1045. PMID: 11304728

43. Barlaam B, Cosulich S, Degorce S, Fitzek M, Green S, et al. (2015) Discovery of (R)-8-(1-(3,5-difluorophenylamino)ethyl)-N,N-dimethyl-2-morpholino-4-oxo-4H-chromene-6-carboxamide (AZD8186): a potent and selective inhibitor of PI3Kβ and PI3Kδ for the treatment of PTEN-deficient cancers. J Med Chem 58: 943-962. doi: 10.1021/jm501629p. PMID: 25514658

44. Tai S, Sun Y, Squires JM, Zhang H, Oh WK, et al. (2011) PC3 is a cell line characteristic of prostatic small cell carcinoma. Prostate 71: 1668-1679. doi: 10.1002/pros.21383. PMID: 21432867

45. Horoszewicz JS, Leong SS, Chu TM, Wajsman ZL, Friedman M, et al. (1980) The LNCaP cell line--a new model for studies on human prostatic carcinoma. Prog Clin Biol Res 37: 115-132. PMID: 7384082

46. Veldscholte J, Ris-Stalpers C, Kuiper GG, Jenster G, Berrevoets C, et al. (1990) 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 Commun 173: 534-540. PMID: 2260966

47. Connolly JM, Rose DP (1990) Production of epidermal growth factor and transforming growth factor-alpha by the androgen-responsive LNCaP human prostate cancer cell line. Prostate 16: 209-218. PMID: 2330326

48. Nakamoto T, Chang CS, Li AK, Chodak GW (1992) Basic fibroblast growth factor in human prostate cancer cells. Cancer Res 52: 571-577. PMID: 1732045

49. Carroll AG, Voeller HJ, Sugars L, Gelmann EP (1993) p53 oncogene mutations in three human prostate cancer cell lines. Prostate 23: 123-134. PMID: 8104329

50. Isaacs WB, Carter BS, Ewing CM (1991) Wild-type p53 suppresses growth of human prostate cancer cells containing mutant p53 alleles. Cancer Res 51: 4716-4720. PMID: 1873816

51. Carson JP, Kulik G, Weber MJ (1999) Antiapoptotic signaling in LNCaP prostate cancer cells: a survival signaling pathway independent of phosphatidylinositol 3'-kinase and Akt/protein kinase B. Cancer Res 59: 1449-1453. PMID: 10197612

52. Wu HC, Hsieh JT, Gleave ME, Brown NM, Pathak S, et al. (1994) Derivation of androgen-independent human LNCaP prostatic cancer cell sublines: role of bone stromal cells. Int J Cancer 57: 406-412. PMID: 8169003

53. Thalmann GN, Anezinis PE, Chang SM, Zhau HE, Kim EE, et al. (1994) Androgen-independent cancer progression and bone metastasis in the LNCaP model of human prostate cancer. Cancer Res 54: 2577-2581. PMID: 8168083

54. Conley-LaComb MK, Saliganan A, Kandagatla P, Chen YQ, Cher ML, et al. (2013) PTEN loss mediated Akt activation promotes prostate tumor growth and metastasis via CXCL12/CXCR4 signaling. Mol Cancer 12: 85. doi: 10.1186/1476-4598-12-85. PMID: 23902739

55. Klein KA, Reiter RE, Redula J, Moradi H, Zhu XL, et al. (1997) Progression of metastatic human prostate cancer to androgen independence in immunodeficient SCID mice. Nat Med 3: 402-408. PMID: 9095173

56. Neshat MS, Mellinghoff IK, Tran C, Stiles B, Thomas G, et al. (2001) Enhanced sensitivity of PTEN-deficient tumors to inhibition of FRAP/mTOR. Proc Natl Acad Sci U S A 98: 10314-10319. doi: 10.1073/pnas.171076798. PMID: 11504908

57. Sawyers C, Sawyers C (2001) Xenograft Models and the Molecular Biology of Human Prostate Cancer. In: Xenograft Models and the Molecular Biology of Human Prostate Cancer. In, , Chung, LWK, Isaacs WB, Simons JW (editors). Prostate Cancer, , Biology, Genetics, and the New Therapeutics , editors. In: Chung, LWK, Isaacs WB, Simons JW (editors). Prostate Cancer: Biology, Genetics, and the New Therapeutics. New York City: Humana Press. pp 163-174; 2001. p. 163-174

58. Craft N, Chhor C, Tran C, Belldegrun A, DeKernion J, et al. (1999) Evidence for clonal outgrowth of androgen-independent prostate cancer cells from androgen-dependent tumors through a two-step process. Cancer Res 59: 5030-5036. PMID: 10519419

59. Silvers CR, Williams K, Salamone L, Huang J, Jordan CT, et al. (2010) A novel in vitro assay of tumor-initiating cells in xenograft prostate tumors. Prostate 70: 1379-1387. doi: 10.1002/pros.21171. PMID: 20687210

60. Rubin MA, Putzi M, Mucci N, Smith DC, Wojno K, et al. (2000) Rapid ("warm") autopsy study for procurement of metastatic prostate cancer. Clin Cancer Res 6: 1038-1045. PMID: 10741732

61. Korenchuk S, Lehr JE, MClean L, Lee YG, Whitney S, et al. (2001) VCaP, a cell-based model system of human prostate cancer. In Vivo 15: 163-8. PMID: 11317522

62. Mertz KD, Setlur SR, Dhanasekaran SM, Demichelis F, Perner S, et al. (2007) Molecular characterization of TMPRSS2-ERG gene fusion in the NCI-H660 prostate cancer cell line: a new perspective for an old model. Neoplasia 9: 200-6. PMID: 17401460

63. Sramkoski RM, Pretlow TG, Giaconia JM, Pretlow TP, Schwartz S, et al. (1999) A new human prostate carcinoma cell line, 22Rv1. In Vitro Cell Dev Biol Anim 35: 403-9. doi: 10.1007/s11626-999-0115-4. PMID: 10462204

64. Chlenski A, Nakashiro K, Ketels KV, Korovaitseva GI, Oyasu R (2001) Androgen receptor expression in androgen-independent prostate cancer cell lines. Prostate 47: 66-75. doi: 10.1002/pros.1048. PMID: 11304731

65. Tepper CG, Boucher DL, Ryan PE, Ma AH, Xia L, et al. (2002) Characterization of a novel androgen receptor mutation in a relapsed CWR22 prostate cancer xenograft and cell line. Cancer Res 62: 6606-14. PMID: 12438256

66. Tan J, Sharief Y, Hamil KG, Gregory CW, Zang DY, et al. (1997) Dehydroepiandrosterone activates mutant androgen receptors expressed in the androgen-dependent human prostate cancer xenograft CWR22 and LNCaP cells. Mol Endocrinol 11: 450-459. doi: 10.1210/mend.11.4.9906. PMID: 9092797

67. Li Y, Alsagabi M, Fan D, Bova GS, Tewfik AH, et al. (2011) Intragenic rearrangement and altered RNA splicing of the androgen receptor in a cell-based model of prostate cancer progression. Cancer Res 71: 2108-2117. doi: 10.1158/0008-5472.CAN-10-1998. PMID: 21248069

68. Dehm SM, Schmidt LJ, Heemers HV, Vessella RL, Tindall DJ (2008) Splicing of a novel androgen receptor exon generates a constitutively active androgen receptor that mediates prostate cancer therapy resistance. Cancer Res 68: 5469-5477. doi: 10.1158/0008-5472.CAN-08-0594. PMID: 18593950

69. Zhau HY, Chang SM, Chen BQ, Wang Y, Zhang H, et al. (1996) Androgen-repressed phenotype in human prostate cancer. Proc Natl Acad Sci U S A 93: 15152-15157. PMID: 8986779

70. Navone NM, Olive M, Ozen M, Davis R, Troncoso P, et al. (1997) Establishment of two human prostate cancer cell lines derived from a single bone metastasis. Clin Cancer Res. 1997;3(12 Pt 3: 1-2493. PMID: 9815652

71. Navone NM, Rodriquez-Vargas MC, Benedict WF, Troncoso P, McDonnell TJ, et al. (2000) TabBO: a model reflecting common molecular features of androgen-independent prostate cancer. Clin Cancer Res 6: 1190-1197. PMID: 10741751

72. Zhao XY, Boyle B, Krishnan AV, Navone NM, Peehl DM, et al. (1999) Two mutations identified in the androgen receptor of the new human prostate cancer cell line MDA PCa 2a. J Urol 162: 2192-2199. PMID: 10569618

73. Alimonti A, Nardella C, Chen Z, Clohessy JG, Carracedo A, et al. (2010) A novel type of cellular senescence that can be enhanced in mouse models and human tumor xenografts to suppress prostate tumorigenesis. J Clin Invest 120: 681-693. doi: 10.1172/JCI40535. PMID: 20197621

74. Bello D, Webber MM, Kleinman HK, Wartinger DD, Rhim JS (1997) Androgen responsive adult human prostatic epithelial cell lines immortalized by human papillomavirus 18. Carcinogenesis 18: 1215-1223. PMID: 9214605

75. Rhim JS, Webber MM, Bello D, Lee MS, Arnstein P, et al. (1994) Stepwise immortalization and transformation of adult human prostate epithelial cells by a combination of HPV-18 and v-Ki-ras. Proc Natl Acad Sci U S A 91: 11874-11878. PMID: 7991549

76. Woodworth CD, Waggoner S, Barnes W, Stoler MH, DiPaolo JA (1990) Human cervical and foreskin epithelial cells immortalized by human papillomavirus DNAs exhibit dysplastic differentiation *in vivo*. Cancer Res 50: 3709-3715. PMID: 1692766

77. Ellis WJ, Vessella RL, Buhler KR, Bladou F, True LD, et al. (1996) Characterization of a novel androgen-sensitive, prostate-specific antigen-producing prostatic carcinoma xenograft: LuCaP 23. Clin Cancer Res 2: 1039-1048. PMID: 9816265

78. Webber MM (1979) Normal and benign human prostatic epithelium in culture. I. Isolation. In Vitro 15: 967-982. PMID: 94033

79. Webber MM, Trakul N, Thraves PS, Bello-DeOcampo D, Chu WW, et al. (1999) A human prostatic stromal myofibroblast cell line WPMY-1: a model for stromal-epithelial interactions in prostatic neoplasia. Carcinogenesis 20: 1185-92. PMID: 10383888

80. Kosova F, Temeltaş G, Arı Z, Lekili M (2013) Possible relations between oxidative damage and apoptosis in benign prostate hyperplasia and prostate cancer patients. Tumour Biol 35: 4295-4299. doi: 10.1007/s13277-013-1560-y. PMID: 24375255

81. Hayward SW, Dahiya R, Cunha GR, Bartek J, Deshpande N, et al. (1995) Establishment and characterization of an immortalized but non-transformed human prostate epithelial cell line: BPH-1. In Vitro Cell Dev Biol Anim 31: 14-24. doi: 10.1007/BF02631333. PMID: 7535634

82. Jerde TJ, Wu Z, Theodorescu D, Bushman W (2011) Regulation of phosphatase homologue of tensin protein expression by bone morphogenetic proteins in prostate epithelial cells. Prostate 71: 791-800. doi: 10.1002/pros.21295. PMID: 21456062

83. Myzak MC, Hardin K, Wang R, Dashwood RH, Ho E (2005) Sulforaphane inhibits histone deacetylase activity in BPH-1, LnCaP and PC-3 prostate epithelial cells. Carcinogenesis 27: 811-819. doi: 10.1093/carcin/bgi265. PMID: 16280330

84. Lee M, Garkovenko E, Yun J, Weijerman P, Peehl D, et al. (1994) Characterization of adult human prostatic epithelial-cells immortalized by polybrene-induced DNA transfection with a plasmid containing an origin-defective sv40-genome. Int J Oncol 4: 821-830. PMID: 21566988

85. Brinkmann V, Foroutan H, Sachs M, Weidner KM, Birchmeier W (1995) Hepatocyte growth factor/scatter factor induces a variety of tissue-specific morphogenic programs in epithelial cells. J Cell Biol. 1995;131(6 Pt 131: 1-1573. PMID: 8522613

86. Chung LW, Huang WC, Sung SY, Wu D, Odero-Marah V, et al. (2006) Stromal-epithelial interaction in prostate cancer progression. Clin Genitourin Cancer 5: 162-70. doi: 10.3816/CGC.2006.n.034. PMID: 17026806

87. Abate-Shen C, Shen MM (2000) Molecular genetics of prostate cancer. Genes Dev 14: 2410-2434. PMID: 11018010

88. Weinberg DS, Weidner N (1993) Concordance of DNA content between prostatic intraepithelial neoplasia and concomitant invasive carcinoma. Evidence that prostatic intraepithelial neoplasia is a precursor of invasive prostatic carcinoma. Arch Pathol Lab Med 117: 1132-1137. PMID: 8239935

89. Wang M, Liu A, Garcia FU, Rhim JS, Stearns ME (1999) Growth of HPV-18 immortalized human prostatic intraepithelial neoplasia cell lines. Influence of IL-10, follistatin, activin-A, and DHT. Int J Oncol 14: 1185-1195. PMID: 10339677

90. Dunning WF (1963) Prostate Cancer in the Rat. Natl Cancer Inst Monogr 12: 351-69. PMID: 14073009

91. Bigio A, Bigio A (2012) Canine prostatic carcinoma. In: . Compend Contin Educ Vet. 2012;34(10):E1-5; 2012 PMID: 23532757

92. Kasper S (2005) Survey of genetically engineered mouse models for prostate cancer: analyzing the molecular basis of prostate cancer development, progression, and metastasis. J Cell Biochem 94: 279-297. doi: 10.1002/jcb.20339. PMID: 15565647

93. Parisotto M, Metzger D (2013) Genetically engineered mouse models of prostate cancer. Mol Oncol 7: 190-205. doi: 10.1016/j.molonc.2013.02.005. PMID: 23481269

94. Hoehn W, Schroeder FH, Reimann JF, Joebsis AC, Hermanek P (1980) Human prostatic adenocarcinoma: some characteristics of a serially transplantable line in nude mice (PC 82). Prostate 1: 95-104. PMID: 7279800

95. van Weerden WM, Romijn JC (2000) Use of nude mouse xenograft models in prostate cancer research. Prostate 43: 263-271. PMID: 10861745

96. Wang Y, Revelo MP, Sudilovsky D, Cao M, Chen WG, et al. (2005) Development and characterization of efficient xenograft models for benign and malignant human prostate tissue. Prostate 64: 149-159. doi: 10.1002/pros.20225. PMID: 15678503

97. Stephenson RA, Dinney CP, Gohji K, Ordóñez NG, Killion JJ, et al. (1992) Metastatic model for human prostate cancer using orthotopic implantation in nude mice. J Natl Cancer Inst 84: 951-957. PMID: 1378502

98. An Z, Wang X, Geller J, Moossa AR, Hoffman RM (1998) Surgical orthotopic implantation allows high lung and lymph node metastatic expression of human prostate carcinoma cell line PC-3 in nude mice. Prostate 34: 169-174. PMID: 9492844

99. Giavazzi R, Campbell DE, Jessup JM, Cleary K, Fidler IJ (1986) Metastatic behavior of tumor cells isolated from primary and metastatic human colorectal carcinomas implanted into different sites in nude mice. Cancer Res. 1986;46(4 Pt 46: 2-1928. PMID: 3948174

100. Gohji K, Nakajima M, Dinney C, Fan D, Pathak S, et al. (1993) The importance of orthotopic implantation to the isolation and biological characterization of a metastatic human clear cell renal-carcinoma in nude-mice. Int J Oncol 2: 23-32. PMID: 21573511

101. Naito S, von Eschenbach AC, Giavazzi R, Fidler IJ (1986) Growth and metastasis of tumor cells isolated from a human renal cell carcinoma implanted into different organs of nude mice. Cancer Res 46: 4109-4115. PMID: 3731078

102. Watahiki A, Wang Y, Morris J, Dennis K, O'Dwyer HM, et al. (2011) MicroRNAs associated with metastatic prostate cancer. PLoS One 6: doi: 10.1371/journal.pone.0024950. PMID: 21980368

103. Xie N, Cheng H, Lin D, Liu L, Yang O, et al. (2015) The expression of glucocorticoid receptor is negatively regulated by active androgen receptor signaling in prostate tumors. Int J Cancer 136: 2015-136. doi: 10.1002/ijc.29147. PMID: 25138562

104. Lin D, Watahiki A, Bayani J, Zhang F, Liu L, et al. (2008) ASAP1, a gene at 8q24, is associated with prostate cancer metastasis. Cancer Res 68: 4352-4359. doi: 10.1158/0008-5472.CAN-07-5237. PMID: 18519696

105. Lin D, Xue H, Wang Y, Wu R, Watahiki A, et al. (2014) Next generation patient-derived prostate cancer xenograft models. Asian J Androl 16: 407-12. doi: 10.4103/1008-682X.125394. PMID: 24589467

106. Living Tumour Laboratory. (2009) The Prostate Center at Vancouver General Hospital, British Columbia Cancer Research Center. Cited November 21, 2014. Available from http://www.livingtumorlab.com/index.html.

107. Lang SH, Sharrard RM, Stark M, Villette JM, Maitland NJ (2001) Prostate epithelial cell lines form spheroids with evidence of glandular differentiation in three-dimensional Matrigel cultures. Br J Cancer 85: 590-9. doi: 10.1054/bjoc.2001.1967. PMID: 11506501

108. Sieh S, Taubenberger AV, Rizzi SC, Sadowski M, Lehman ML, et al. (2012) Phenotypic characterization of prostate cancer LNCaP cells cultured within a bioengineered microenvironment. PLoS One 7: doi: 10.1371/journal.pone.0040217. PMID: 22957009

109. Greenberg NM, DeMayo F, Finegold MJ, Medina D, Tilley WD, et al. (1995) Prostate cancer in a transgenic mouse. Proc Natl Acad Sci U S A 92: 3439-3443. PMID: 7724580

110. Gingrich JR, Barrios RJ, Morton RA, Boyce BF, DeMayo FJ, et al. (1996) Metastatic prostate cancer in a transgenic mouse. Cancer Res 56: 4096-4102. PMID: 8797572

111. Pollard M (1973) Spontaneous prostate adenocarcinomas in aged germfree Wistar rats. J Natl Cancer Inst 51: 1235-1241. PMID: 4745857

112. Wilson EM, Viskochil DH, Bartlett RJ, Lea OA, Noyes CM, et al. (1981) Model systems for studies on androgen-dependent gene expression in the rat prostate. Prog Clin Biol Res 75A: 351-380. PMID: 6175980

113. Rennie PS, Bruchovsky N, Leco KJ, Sheppard PC, McQueen SA, et al. (1993) Characterization of two cis-acting DNA elements involved in the androgen regulation of the probasin gene. Mol Endocrinol 7: 23-36. doi: 10.1210/mend.7.1.8446105. PMID: 8446105

114. DeCaprio JA, Ludlow JW, Figge J, Shew JY, Huang CM, et al. (1988) SV40 large tumor antigen forms a specific complex with the product of the retinoblastoma susceptibility gene. Cell 54: 275-283. PMID: 2839300

115. Gannon JV, Lane DP (1987) p53 and DNA polymerase alpha compete for binding to SV40 T antigen. Nature 329: 456-8. doi: 10.1038/329456a0. PMID: 3309672

116. Bookstein R, Rio P, Madreperla SA, Hong F, Allred C, et al. (1990) Promoter deletion and loss of retinoblastoma gene expression in human prostate carcinoma. Proc Natl Acad Sci U S A 87: 7762-7766. PMID: 2217208

117. Fan K, Dao DD, Schutz M, Fink LM (1994) Loss of heterozygosity and overexpression of p53 gene in human primary prostatic adenocarcinoma. Diagn Mol Pathol 3: 265-270. PMID: 7866637

118. Scherl A, Li J, Cardiff RD, Schreiber-Agus N (2004) Prostatic intraepithelial neoplasia and intestinal metaplasia in prostates of probasin-RAS transgenic mice. Prostate 59: 448-459. doi: 10.1002/pros.20020. PMID: 15065094

119. Song Z, Wu X, Powell WC, Cardiff RD, Cohen MB, et al. (2002) Fibroblast growth factor 8 isoform B overexpression in prostate epithelium: a new mouse model for prostatic intraepithelial neoplasia. Cancer Res 62: 5096-5105. PMID: 12208767

120. Shim EH, Johnson L, Noh HL, Kim YJ, Sun H, et al. (2003) Expression of the F-box protein SKP2 induces hyperplasia, dysplasia, and low-grade carcinoma in the mouse prostate. Cancer Res 63: 1583-8. PMID: 12670908

121. Klezovitch O, Chevillet J, Mirosevich J, Roberts RL, Matusik RJ, et al. (2004) Hepsin promotes prostate cancer progression and metastasis. Cancer Cell 6: 185-95. doi: 10.1016/j.ccr.2004.07.008. PMID: 15324701

122. Polnaszek N, Kwabi-Addo B, Peterson LE, Ozen M, Greenberg NM, et al. (2003) Fibroblast growth factor 2 promotes tumor progression in an autochthonous mouse model of prostate cancer. Cancer Res 63: 5754-5760. PMID: 14522896

123. Chiaverotti T, Couto SS, Donjacour A, Mao JH, Nagase H, et al. (2008) Dissociation of epithelial and neuroendocrine carcinoma lineages in the transgenic adenocarcinoma of mouse prostate model of prostate cancer. Am J Pathol 172: 236-46. doi: 10.2353/ajpath.2008.070602. PMID: 18156212

124. di Sant'Agnese PA (1992) Neuroendocrine differentiation in carcinoma of the prostate. Diagnostic, prognostic, and therapeutic implications. Cancer 70: 254-268. PMID: 1350941

125. Terry S, Beltran H (2014) The many faces of neuroendocrine differentiation in prostate cancer progression. Front Oncol 4: 60. doi: 10.3389/fonc.2014.00060. PMID: 24724054

126. Kasper S, Sheppard PC, Yan Y, Pettigrew N, Borowsky AD, et al. (1998) Development, progression, and androgen-dependence of prostate tumors in probasin-large T antigen transgenic mice: a model for prostate cancer. Lab Invest 78: PMID: 9645768

127. Masumori N, Thomas TZ, Chaurand P, Case T, Paul M, et al. (2001) A probasin-large T antigen transgenic mouse line develops prostate adenocarcinoma and neuroendocrine carcinoma with metastatic potential. Cancer Res 61: 2239-2249. PMID: 11280793

128. Ellwood-Yen K, Graeber TG, Wongvipat J, Iruela-Arispe ML, Zhang J, et al. (2003) Myc-driven murine prostate cancer shares molecular features with human prostate tumors. Cancer Cell 4: 223-238. PMID: 14522256

129. Ciuffreda L, Falcone I, Incani UC, Del Curatolo A, Conciatori F, et al. (2014) PTEN expression and function in adult cancer stem cells and prospects for therapeutic targeting. Adv Biol Regul 56: 66-80. doi: 10.1016/j.jbior.2014.07.002. PMID: 25088603

130. Abremski K, Hoess R (1984) Bacteriophage P1 site-specific recombination. Purification and properties of the Cre recombinase protein. J Biol Chem 259: 1509-1514. PMID: 6319400

131. Kwak MK, Johnson DT, Zhu C, Lee SH, Ye D, et al. (2013) Conditional deletion of the Pten gene in the mouse prostate induces prostatic intraepithelial neoplasms at early ages but a slow progression to prostate tumors. PLoS One 8: doi: 10.1371/journal.pone.0053476. PMID: 23308230

132. Wang S, Gao J, Lei Q, Rozengurt N, Pritchard C, et al. (2003) Prostate-specific deletion of the murine Pten tumor suppressor gene leads to metastatic prostate cancer. Cancer Cell 4: 209-221. PMID: 14522255

133. Li Q, Liu L, Zhang Q, Liu S, Ge D, et al. (2014) Interleukin-17 Indirectly Promotes M2 Macrophage Differentiation through Stimulation of COX-2/PGE2 Pathway in the Cancer Cells. Cancer Res Treat 46: 297-306. doi: 10.4143/crt.2014.46.3.297. PMID: 25038765

134. Zhang Q, Liu S, Xiong Z, Wang AR, Myers L, et al. (2014) Interleukin-17 promotes development of castration-resistant prostate cancer potentially through creating an immunotolerant and pro-angiogenic tumor microenvironment. Prostate 74: 869-79. doi: 10.1002/pros.22805. PMID: 24691769

135. Kwabi-Addo B, Giri D, Schmidt K, Podsypanina K, Parsons R, et al. (2001) Haploinsufficiency of the Pten tumor suppressor gene promotes prostate cancer progression. Proc Natl Acad Sci U S A 98: 11563-11568. doi: 10.1073/pnas.201167798. PMID: 11553783

136. Kim MJ, Cardiff RD, Desai N, Banach-Petrosky WA, Parsons R, et al. (2002) Cooperativity of Nkx3.1 and Pten loss of function in a mouse model of prostate carcinogenesis. Proc Natl Acad Sci U S A 99: 2884-2889. doi: 10.1073/pnas.042688999. PMID: 11854455

137. Di Cristofano A, De Acetis M, Koff A, Cordon-Cardo C, Pandolfi PP (2001) Pten and p27KIP1 cooperate in prostate cancer tumor suppression in the mouse. Nat Genet 27: 222-224. doi: 10.1038/84879. PMID: 11175795

138. Majumder PK, Yeh JJ, George DJ, Febbo PG, Kum J, et al. (2003) Prostate intraepithelial neoplasia induced by prostate restricted Akt activation: the MPAKT model. Proc Natl Acad Sci U S A 100: 7841-7846. doi: 10.1073/pnas.1232229100. PMID: 12799464

139. Ramaswamy S, Nakamura N, Vazquez F, Batt DB, Perera S, et al. (1999) 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 U S A 96: 2110-2115. PMID: 10051603

140. Campbell JP, Merkel AR, Masood-Campbell SK, Elefteriou F, Sterling JA (2012) Models of bone metastasis. J Vis Exp : 2012-67. doi: 10.3791/4260. PMID: 22972196

141. Vlodavsky I, Vlodavsky I (2001) Tail vein assay of cancer metastasis. In: Curr Protoc Cell Biol. Curr Protoc Cell Biol. 2001;Chapter 19:Unit 19.2; 2001

142. LeBeau AM, Sevillano N, Markham K, Winter MB, Murphy ST, et al. (2015) Imaging active urokinase plasminogen activator in prostate cancer. Cancer Res 75: 1225-1235. doi: 10.1158/0008-5472.CAN-14-2185. PMID: 25672980

143. Jenkins DE, Yu S, Hornig YS, Purchio T, Contag PR (2003) In vivo monitoring of tumor relapse and metastasis using bioluminescent PC-3M-luc-C6 cells in murine models of human prostate cancer. Clin Exp Metastasis 20: 745-756.

PMID: 14713108

144. Rucci N, Sanità P, Delle Monache S, Alesse E, Angelucci A (2014) Molecular pathogenesis of bone metastases in breast cancer: Proven and emerging therapeutic targets. World J Clin Oncol 5: 335-347. doi: 10.5306/wjco.v5.i3.335. PMID: 25114849

145. Nandana S, Ellwood-Yen K, Sawyers C, Wills M, Weidow B, et al. (2010) Hepsin cooperates with MYC in the progression of adenocarcinoma in a prostate cancer mouse model. Prostate 70: 591-600. doi: 10.1002/pros.21093. PMID: 19938013

146. Valkenburg KC, Williams BO (2011) Mouse models of prostate cancer. Prostate Cancer 2011: 895238. doi: 10.1155/2011/895238. PMID: 22111002

147. Abate-Shen C, Shen MM (2002) Mouse models of prostate carcinogenesis. Trends Genet 18: PMID: 120479