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

David Cunningham¹, Zongbing You¹²³⁴⁵*

¹Department of Structural & Cellular Biology, ²Department of Orthopaedic Surgery, ³Tulane Cancer Center and Louisiana Cancer Research Consortium, ⁴Tulane Center for Stem Cell Research and Regenerative Medicine, ⁵Tulane 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.

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 (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.

Received April 27, 2015; Revision received May 18, 2015; Accepted May 18, 2015; Published June 4, 2015
**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 M1, M2, and M3. 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-apoptotic 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, and 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 |
|------|
| **Normal** |
| **pRNS-1-1** | radical prostatectomy | No | ~68 hours | No | No | Yes | Yes | 5, 8 |
| | | | | | | | | [84] |
| **RWPE-1** | HPE cells from the peripheral zone | No | 120 hours | Yes | Yes | Yes | Yes | 8, 18 |
| | | | | | | | | [75], [74] |
| **BPH 1** | Epithelial cells from a transurethral specimen demonstrating BPH | No | ~35 hours | No | No | No | No | 8, 18 |
| | | | | | | | | [81] |
| **PIN** |
| **PIN Cells** | Cultured from a human high grade PIN lesion | No (non-tumorigenic) | Not reported | Yes | Yes | Not reported | Not reported | 18, 34bE12 IL10 receptor | Not reported |
| | | | | | | | | | [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 |
| | | | | | | | | | [74] |
| **LNCaP** | Lymph node | Yes; osteoblastic phenotype in bone metastasis | 28–60 hours | Yes | Yes | Yes | Yes | CK-8, 18, 20 Vimentin, HEC18, a-, b-, g-catenin, PAP, CBP | 33–91; highly aneuploid |
| | | | | | | | | | [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 2p53 mutations (P72R and R175H) | 79–92, modal of 89 |
| | | | | | | | | | [55] |
| **LAPC-9** | Femoral metastasis in patient undergoing hormone 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 |
| | | | | | | | | | [58] |
| **VCaP** | Vertebra 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 |
| | | | | | | | | | [81] |
| **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 |
| | | | | | | | | | [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 | 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 |
| | | | | | | | | | [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, neurophisin, 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, HECDO 1, | Transferrin receptor, TGF-α, EGFR-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, EGFR, bFGF, IGF-I, TGF-β1; EGF/ TGF-αR, 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 intratrabically in mice [57]. Orthotopic inoculation tends to metastasize more frequently, while intratrabial 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 hyperdiploid to hyptriploid 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 (TMPRSS2-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 Zha 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 8 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, orchietomy, 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 inhibitin (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                           | 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                           | 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]. Biclonal 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 hemaglutinin (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 contributions 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

The authors thank the members of the You Laboratory for their support in generating animal data, particularly Drs. Qiuyang Zhang, Keshab R. Parajuli, Sen Liu, and Jiandong Mei. D. C. would like to acknowledge Keith Pickett at the Matas Library for training in Endnote. Z.Y. was supported in whole or in part by Department of Defense Health Program (W81XWH-14-1-0050, W81XWH-14-1-0149, W81XWH-14-1-0458, and OR140380; the U.S. Army Medical Research Acquisition Activity, 820 Chandler Street, Fort Detrick MD 21702-5014 is the awarding and administering acquisition office) and by National Institutes of Health (R01CA174714 and P20GM103518). The content of this article is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health or the Department of Defense.

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.
3. Cullen J, Elsamamouni S, Brassell SA, Chen Y, Colombo M, et al. (2012) The burden of prostate cancer in Asian nations. J Carcinog 11: 7. doi: 10.4137/1477-3163.94025. PMID: 22529743
4. Albright F, Stephenson EA, 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, Karella M, Singer EA, Kim JH, et al. (2013) Increased incidence of pathologically nonanterior confined prostate cancer in African-American men eligible for active surveillance. Urology 81:3-15. 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, Darampou Mar 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.21217. PMID: 20945498
10. Shahabi A, Corral R, Carsburg 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. Caining S, Gadini 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.jepidem.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. Blankler MH, Noordzij MAA (2014) [Prostate cancer screening benefit very low, even after 13 years]. Ned Tijdschr Geneeskd 158: PMID: 25429277
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.
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.0000149899.01263.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. Endocer 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, Coacoley C, et al. (2011) PTEN deletion in prostate cancer cells does not associate with loss of RAD51 function: implications for radiotherapy and chemotheraphy. Clin Cancer Res 18: 1015-1027. doi: 10.1158/1078-0432.CCR-11-2189. PMID: 22114138

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

25. Mickey DD, Stone KR, Wunderli H, Mickey GH, Vollner 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 I, Bladou F (2002) A Nod Scid mouse model to study human prostate cancer. Prostate Cancer Prostatic Dis 5: 311-315. doi: 10.1093/sj.pea.4500606. PMID: 12627217

27. Billström A, Lecander I, Dagnaes-Hansen F, Dahlbøf L, 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: 21876230

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 pathway in human carcinoma cells. Oncogene 21: 4009-4019. doi: 10.1038/sj.onc.1205497. PMID: 15036590

32. West M, Hahn MW, Miller RA, Zetter BR (2005) Sphingosine induces apoptosis in androgen-independent human prostatic carcinoma DU-145 cells by suppression of bel-2(L) gene expression. FEMS Lett 470: 97-100. PMID: 9141489

33. Nottw JK, 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

34. von Haefen C, Wieder T, Gillissen B, Stärck L, Graupner V, et al. (2002) PTEN loss mediated Akt activation promotes prostate tumor growth and metastasis via CXCL12/CXCR4 signaling. Mol Cell 12: 85. doi: 10.1016/S1476-4598(12)-85. PMID: 23902739

35. 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: 9051973

36. Neshat MS, Mellighoff 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.104677998. PMID: 11504908

37. Sawyer C, Sawyer 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, 2nd ed. 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.

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

39. 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

40. 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
60. 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

61. Mertz KD, Settur SR, Dhanasekaran SM, Demichelis F, Perner S, et al. (2007) Molecular characterization of TMRPSS2-ERG gene fusion in the NCI-H660 prostate cancer cell line: a new perspective for an old model. Neoplasia 9: 200-6. PMID: 17401460

62. 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

63. 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/CGCC.2006.a.034. PMID: 17026806

64. 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

65. Dhern 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

66. Zhao XY, 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: 8967797

67. 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: 9186552

68. Navone NM, Rodriguez-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

69. Dunning WF (1963) Prostate Cancer in the Rat. Natl Cancer Inst Monogr 12: 681-693. doi: 10.1172/JCI40535. PMID: 9092797

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

71. Li Y, Alasagabi 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

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 genetically engineered mouse model of 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: 15566407

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

75. Ellis WJ, Vessella RL, Buhler KR, Bladou F, True LD, et al. (1996) Characterization of a novel androgen-sensitive, prostate-specific antigen-negative human tumor xenograft: LuCaP 23. Clin Cancer Res 2: 681-693. doi: 10.1002/pros.1048. PMID: 8522613

76. Tepper CG, Boucher DL, Ryan PE, Ma AH, Xia L, et al. (2002) Characterization of a novel prostate cancer xenograft in the nude mouse: xenograft 22R2 prostate cancer xenograft and cell line. Cancer Res 62: 6606-14. PMID: 12438256

77. 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/CGCC.2006.a.034. PMID: 17026806

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

79. Alimonti A, Nardella C, Chen Z, Clohessy JG, Carracedo A, et al. (2010) A genetically engineered mouse model of 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: 15566407

80. Stephenson RA, Dinney CP, Golghi K, Ordoñez 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

81. Alimonti A, Nardella C, Chen Z, Clohessy JG, Carracedo A, et al. (2010) A genetically engineered mouse model of 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: 15566407
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