Preface

Prostate cancer is the second leading cancer in men in Western society. A major concern, and an area of intensive research, involves understanding why certain prostate cancers remain localized or indolent, whereas others become aggressive and metastasize. The differences between these cancer types have profound implications for patients and physicians. Indolent disease, which grows very slowly, generally does not cause any problems to the patient, whereas aggressive disease requires immediate treatment, the earlier the better. At present, there are no markers that discriminate between these two entities, thus causing a dilemma for the management of patients who have recently been diagnosed. The aim of *Prostate Cancer Methods and Protocols* is to explore cutting-edge molecular methods that may have the potential to reveal markers of disease for use in more accurate diagnoses of prostate cancer and, consequently, to lead to new treatment strategies. This book provides a comprehensive collection of both in vitro and in vivo step-by-step protocols currently used by leaders in prostate cancer research, advice on approaches that can be used in the study of prostate cancer, as well as reviews covering areas less amenable to laboratory research, such as environmental factors in prostate cancer, to provide the reader with an overview of the prostate cancer research field as it currently stands.

Prostate Cancer Methods and Protocols is divided into sections covering in vitro techniques for the study of normal prostate epithelial cells and stem cells, their immortalization and growth in three-dimensional culture as spheroids, as well as in traditional monolayer culture, and in vivo models of prostate cancer for study, including new transgenic lines, and models for studying the mechanisms by which prostate cancer cells metastasize to the bone. One section covers new methods for accurate diagnosis of prostate cancer, including histological assessment, studies of cells in semen, and methylation analysis of the GSTpi (the pi isozyme of glutathione-S-transferase), a potential prostate cancer-specific marker. Another section examines extensive molecular, biological, and biochemical approaches—such as studies of enzymes secreted by prostate cancer cells, some of which may be cancer-specific—and changes in the androgen receptor in cancer that can influence

the outcome of hormonal treatments, as well as proteome and microarray analyses. The final section addresses new strategies for the treatment of refractory disease, including the possible role of flavonoids, targeted alpha therapy, methods for implementing immune therapy, and prostate-specific gene therapy.

Prostate Cancer Methods and Protocols provides helpful tools for all scientists engaged in prostate cancer research, students needing the basis of protocols and reviews, and clinicians wanting to know the latest methods in use for diagnosing, studying, and treating prostate cancer.

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Human Prostate Cancer Cell Lines

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1. Introduction

Prostate cancer affects many men in the West but rarely occurs in Japan or China. Some epidemiological factors that may be important in this are described elsewhere in this volume. Prostate cancer has become the most common malignancy and the second highest cause of cancer death in Western society. The disease is very heterogeneous in terms of grade, genetics, ploidy, and oncogene/tumor suppressor gene expression, and its biological, hormonal, and molecular characteristics are extremely complex. Growth of early prostate cancer requires 5α-dihydrotestosterone produced from testosterone by the 5α-reductase enzyme system; such prostate cells are described as androgen dependent (AD). Subsequently, the prostate cancer cells may respond to androgen but do not require it for growth; these cells are androgen sensitive (AS). Because of the requirement for androgen for growth of prostate cancer, patients whose tumors are not suitable for surgical intervention or radiotherapy may be treated by hormonal intervention, either continuous or intermittent, to prevent prostate cancer cell growth ***(1–3)***. This leads to periods of remission from disease, but almost invariably, the prostate cancer recurs, by which time the prostate cancer cells have become androgen-independent (AI) ***(4,5)***. This may be accompanied by changes in the androgen receptor (AR), which may undergo mutation ***(6,7)***, amplification ***(8)***, or loss ***(9)***. Prostate cancer cells metastasize to various organs but particularly to local lymph nodes and to skeletal bone. Important antigens expressed by prostate cancer cells include prostate-specific antigen (PSA), which has been used both for screening for prostate cancer and for management of patients with the disease ***(10,11)***. Prostate-specific membrane antigen (PSMA) is produced in two forms that differ in the normal prostate, benign hyperplasia of the prostate, and prostate cancer ***(12)***. PSMA is upregulated in prostate cancer compared with normal cells

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and is found in cells in increased concentration once they become AI ***(13,14)***. Interactions between epithelial cells and stroma appear to be very important in allowing prostate cells to grow and form tumors, partly because of paracrine pathways that exist in this tissue ***(15,16)***. Prostate cancer rarely arises spontaneously in animals, and the human cancer cells are particularly difficult to grow in culture as long-term cell lines ***(17)***. Elsewhere in this book, methods for growing primary cultures of the prostate, for immortalizing prostate cells, and for isolating prostate stem cells are described. This chapter describes the commonly used prostate cancer cell lines, their preferred media for growth, and some of their important uses, including inoculation into mice to produce bony metastases.

2. Lines Derived from Human Tumors

A listing of the major human prostate cell lines and their media requirements may be found in **Tables 1** and **2;** more specialized media for the establishment and growth of prostate cells are described elsewhere in this volume. The characteristics of a number of prostate cell lines are summarized in **Table 3**.

Most human prostate cancer cell lines have been established from metastatic deposits with the exception of PC-93 ***(18)***, which is grown from an AD primary tumor. However, PC-93 and other widely used lines, including PC-3 ***(19)***, DU-145 ***(20)***, and TSU-Pr1 ***(21)***, are all AI; all lack androgen receptors (with the possible exception of PC-93), PSA, and 5α-reductase; and all produce poorly differentiated tumors if inoculated into nude mice. Until very recently, the paucity of AD cell lines has made studies of the early progression of prostate cancer using human materials very difficult. However, metastatic sublines of PC-3 have been developed by injecting cells into nude mice via different routes, especially orthotopically ***(22)***, and this process can be readily followed by using PC-3 cells expressing luciferase ***(23)***.

Until recently, the LNCaP cell line, established from a metastatic deposit in a lymph node ***(24)***, was the only human prostate cancer cell line to demonstrate androgen sensitivity. After its initial characterization, several laboratories found LNCaP cells to be poorly tumorigenic in nude mice unless coinoculated with tissue-specific mesenchymal or stromal cells ***(25,26)*** or Matrigel™ ***(27)***, emphasizing the importance of extracellular matrix and paracrine-mediated growth factors in prostate cancer growth and site-specific metastasis ***(28)***. New lines were obtained by culturing LNCaP cells that had been grown in castrated mice ***(29)***. The C-4 LNCaP line is AI, produces PSA and a factor that stimulates PSA production, and the C4-2 and C4-2B lines metastasize to lymph nodes and bone after subcutaneous or orthotopic inoculation ***(29,30)***. Others have also selected more highly metastatic cells ***(22)*** by serial reinjection into the prostate of prostate cancer cell lines or by growing

Table 1
Profile of Established Human Prostate Cancer and Immortalized Cell Lines

Cell line	Source	Media requirements[a]	References
PC-93	AD primary prostate cancer	A	***18, 86***
PC-3	Lumbar metastasis	B or D (ATCC[b] recommendation)	***19***
DU-145	Central nervous system metastasis	B or E (ATCC recommendation)	***20***
TSU-Pr1[c]	Cervival lymph node metastasis in Japanese male	B or F	***21***
LNCaP	Lymph node metastasis in Caucasian male	G or B	***24, 87, 88, 89***
LNCaP-FGC[d]	Clonal derivative of LNCaP	B	***24, 87***
LNCaP-LN-3	Metastatic subline of LNCaP cells derived by orthotopic implantation	H or I	***22***
LNCaP-C4	Metastatic subline of LNCaP, derived after coinoculation of LNCaP and fibroblasts	G	***29, 30***
LNCaP-C4B	Metastatic subline derived from LNCaP-C4 after reinoculation into castrated mice	G	***29, 30***
MDA PCa 2a	AI bone metastasis from African-American male	J or K	***34, 35***
MDA PCa 2b	AI bone metastasis from African-American male	J or K	***34, 35***
ALVA-101	Bone metastasis	L	***36***
ALVA-31[e]	Well-differentiated adenocarcinoma	M	***38***
ALVA-41[e]	Bone metastasis	L	***42***
22Rv1	Derived from CWR22R, an androgen-dependent prostate cancer xenograft line	B	***43***
ARCaP	Derived from ascitic fluid from a patient with metastatic disease	G	***44***
PPC-1[e]	Poorly differentiated adenocarcinoma	B	***45***

(Table continues)

Table 1
(Continued)

Cell line	Source	Media requirements[a]	References
LAPC3	Derived from xenograft established from specimen obtained via transurethral resection of the prostate	N	***47***
LAPC4	Derived from xenograft established from a lymph node metastasis	N	***47***
P69SV40T	Immortalized cell line derived by transfection of adult prostate epithelial cells with the SV40 large T antigen gene	O	***58***
RWPE-2	Immortalized cell line initially derived by transfection of adult (Caucasian) prostatic epithelial cells with human papillomavirus 18, then made tumorigenic by infection with v-K-ras	P	***59***
CA-HPV-10	Immortalized cell line derived by human papilloma virus 18 transfection of prostatic epithelia cells from a high-grade adenocarcinoma	Q	***60***
PZ-HPV-7	Immortalized cell line derived by human papilloma virus 18 transfection of normal prostatic peripheral zone epithelial cells	Q	***60***

[a]*See* **Table 2** for details of cell line media requirements.
[b]ATCC: American Type Culture Collection (http://www.atcc.org).
[c]The nature of this cell line has recently been questioned; *see* **ref. *90***.
[d]LNCaP-FGC: LNCaP clone, Fast Growing Colony; available from the ATTC.
[e]The nature of this cell line has recently been questioned; *see* **ref. *91***.

Table 2
Media Requirements of Human Prostate Cancer and Immortalized Cell Lines

Designation	Components
A	Eagle's minimum essential medium (MEM) supplemented with 10% FBS and 2 m*M* L-glutamine
B	Roswell Park Memorial Institute (RPMI) 1640 medium, supplemented with 10% fetal bovine serum (FBS)
C	Dulbecco's modified Eagle medium (DMEM), supplemented with 10% FBS
D	Kaighn's modification of Ham's F-12 medium (F-12K), supplemented with 10% FBS and 2 m*M* L-glutamine, and adjusted to contain 1.5 g/L sodium bicarbonate
E	MEM supplemented with 10% FBS, 2 m*M* L-glutamine and Earle's balanced salt solution (BSS) adjusted to contain 1.5 g/L sodium bicarbonate, 0.1 m*M* nonessential amino acids and 1.0 m*M* sodium pyruvate
F	RPMI supplemented with 5% FBS
G	"T-medium": DMEM:F-12K, 4:1, supplemented with 5% FBS, 3 g/L sodium bicarbonate, 5 µg/mL insulin, 13.6 pg/mL triiodothyronine, 5 µg/mL transferrin, 0.25 µg/mL biotin, 25 µg/mL adenine ***(88,89)***
H	Medium "B" supplemented with sodium pyruvate, nonessential amino acids and vitamins
I	RPMI:F-12K, 1:1, supplemented with 10% FBS
J	BRFF HPC1 medium (Biological Research Faculty and Facility, Inc., Jamesville, MD) supplemented with 15% FBS
K	F-12K supplemented with 20% FBS, 10 mg/mL epidermal growth factor, 100 ng/mL hydrocortisone, 5 µg/mL insulin, 25 ng/mL cholera toxin, 5×10^{-6} *M* phosphoethanolamine, 3×10^{-8} *M* sodium selenite
L	RPMI supplemented with 5% FBS, 2 m*M* L-glutamine, and 1 m*M* sodium pyruvate
M	RPMI supplemented with 10% FBS, 1% L-glutamine, 1% sodium pyruvate, buffered to pH 7.4 with 7.5% (w/v) sodium bicarbonate
N	Iscove's modified Dulbecco's medium (IMDM) supplemented with 10% FBS and 10 n*M* R1881 synthetic steroid
O	Serum-free RPMI supplemented with 10 ng/mL epidermal growth factor, 5 µg/mL insulin, 5 µg/mL transferrin, 5 ng/mL selenium, and 0.1 µ*M* dexamethasone
P	Keratinocyte serum-free medium (KSFM) supplemented with 50 µg/mL bovine pituitary extract and 5 ng/mL epidermal growth factor
Q	Keratinocyte serum-free medium (KSFM) supplemented with 50 µg/mL bovine pituitary extract

Table 3
Characteristics of Human Prostate Cancer and Immortalized Cell Lines

Cell line	Androgen receptor	Androgen sensitivity	5α-Reductase
PC-93	?	AI	–
PC-3	–	AI	–
DU-145	–	AI	–
TSU-Pr1	–	AI	NR
LNCaP	+ Mutated	AS	–
Sublines:			
C4		AI	++
C4-2	AI + mets	+++	+
C4-2B	AI + mets	+++	+
P104-R2	Stimulated by finasteride		
LN3		Less sensitive than LNCaP	+++
MDA PCa 2a	+	AS	
MDA PCa 2b	+	AS	
ALVA101	+	AS	+
22Rv1	Grown from an AI xenograft	AS	NR
ARCaP	+	Androgen repressed	NR
LACP-4	+	AS	NR
P69SV40T	NR	NR	NR
RWPE-2	+	+	NR
CA-HPV-10	NR	NR	NR

[a]NR, not reported.

PSA	PAMA	p53/other features	References
–	NR[a]	NR	***18***
–	–	Deletion mutation of *p53*	***19,92***
–	–	Mutated *p53*, pro to leu codon 223; val to phe codon 274	***20,93***
		Has mutated p16 codon 84	***50***
–	NR	Mutated *p53* (has mutated H-ras)	***21,92***
+	+	Silent mutation of *p53*	***24,92***
+			***29***
		Low levels of functional *p53*	***30***
			30
			32
++			***22***
+	NR	NR	***34,35***
+	NR	NR	***34,35***
+	NR	NR	***36***
+	NR	–	***43***
+	NR	NR	***44***
+	NR	AI sublines express high levels of HER/2-neu	***47,48***
NR	NR	Express EGFR and TGFα	***58***
+	NR	Express HPV-18 E7 protein; mutated *Ki-ras*	***59***
NR	NR	Cytogenetic abnormalities	***61***

the cells in the prostate of SCID mice *(31)*. The LN3 cell line derived from the LNCaP line by this method is more metastatic to liver, less sensitive to androgen, and its cells produce high levels of both PSA and PSMA *(22)*. The LNCaP line expresses a mutated AR. Some mutations of the AR are associated with stimulation of the cells by antiandrogens, causing concern over the use of drugs, such as finasteride for the treatment of late-stage prostate cancer. The subline LNCaP 104-R2 manifests this phenomenon *(32)*. Using a subline, LNCaP-abl, produced by growing LNCaP cells in androgen-depleted medium for 87 passages, bicalutamide was shown to acquire agonistic properties that were not related to changes in AR activity or with amplification of the AR gene in these cells *(33)*. Recently, new androgen responsive lines have been established. The MDA PCa 2a and MDA PCa 2b lines were both isolated from a single bone metastasis from an African American male who had AI prostate cancer *(34)*. They both express PSA (MDA PCa 2a produces 0.43 and MDA PCa 2b produces 0.67 ng/mL of PSA/g of tumor), ARs, and are AS and both grow in nude mice; they differ in their morphology in vitro and in their karyotypes and are thought to represent distinct clones from the same tumor *(34,35)*. Despite their androgen sensitivity, these cells show intact p53 and Bcl-2, Rb, and p16, reflecting a common subset of human AI prostate cancer *(35)*. The ALVA-101 *(36)* line is also AS; these cells respond to 5α-DHT by upregulating an autocrine loop involving epidermal growth factor receptors and their ligand, transforming growth factor-α (TGFα) *(37)*. The tumor induction and take rates of the ALVA-31 cell line are similar in female and castrated male nude mice; however, an increased growth rate in intact male mice suggests a degree of androgen responsiveness *(38)*. The line is positive for both PSA and PAP, and expresses the highest level of vitamin D receptor of all established prostate cancer cell lines *(39)*. However, it lacks α-catenin *(40)*, and expresses only low levels of AR, p21, and p27 *(41)* The ALVA-41 line expresses AR with a binding capacity similar to that of LNCaP, and the line is androgen responsive *(42)*; it expresses PAP but not PSA.

A new prostate cancer cell line, 22Rv1, has been derived from the xenograft line, CWR22R: it expresses PSA, is slightly stimulated by DHT, and expresses an AR. Its growth is stimulated by epidermal growth factor but is not inhibited by transforming growth factor-beta 1 *(43)*.

An unusual cell line, ARCaP *(44)*, was derived from prostate cancer cells in ascites fluid of a man with metastatic disease and exhibits androgen- and estrogen-repressed growth and tumor formation in hormone-deficient or castrated mice. These cells express low levels of AR and PSA and are highly metastatic when inoculated orthotopically. Androgen-repressed prostate cancers are thought to occur only very late in the progression of the disease.

The PPC-1 cell line was established from a primary prostatic tumor site, a poorly differentiated adenocarcinoma ***(45)***. It is hormone insensitive ***(45)***, tumorigenic, and spontaneously metastasizes to the lungs and lymph nodes after sc inoculation in nude mice ***(46)***.

Two cell lines established from xenografted prostate cancer tissue, LAPC-3 and LAPC-4, showed chromosomal abnormalities and expressed wild-type ARs ***(47)***. LAPC-3 is AI, whereas LAPC-4 is AS. The LAPC-4 xenograft has been propagated as a continuous cell line that retains its hormone-responsive characteristics, but the xenografted line can progress to AI when grown in female or castrated male mice. In this model, the AI sublines express higher levels of HER-2/neu than the AS cells. Forced overexpression of HER-2/neu in AD cells allowed ligand-independent growth. HER-2/neu activated the AR pathway in the absence of ligand and synergized with low levels of androgen to superactivate the pathway ***(48)***.

Molecular analyses have shown that many of the cell lines contain *p53* mutations (*see* **Table 3**), consistent with the finding that *p53* mutations commonly occur in late-stage prostate cancer. When *p53* mutation occurs in early stage disease, however, it predisposes to cancer progression ***(49)***. In some cases, alterations of other oncogenes/suppressor genes have been observed. The DU-145 line shows a mutation in the *p16* gene, involved in cell cycle control ***(50)***. Relatively few of the lines express PSA or PSMA, which has been shown to be overexpressed in late-stage prostate cancers in man ***(51)***. Loss of the tumor suppressor gene, *PTEN/MMAC1*, which maps to 10q23.3, occurs commonly in prostate cancer. Several cell lines and xenograft lines show homozygous deletions of the *PTEN* gene or parts thereof (PC-3, PC133, PCEW, PC295, and PC324), and others contain nonsense mutations (PC82 and PC346) or frameshift mutations (LNCaP and PC374) ***(52)***. *PTEN/MMAC1* acts as a negative regulator of the phosphoinositide 3-kinase (P13-kinase)/Akt pathway and inactivation of the *PTEN/MMAC1* gene leads to constitutive activation of either P13-kinase or Akt, which can induce cellular transformation ***(53)***.

Cell–cell adhesion may be mediated through calcium-dependent, homotypic cadherin-catenin interactions ***(54)***; α-catenin, in turn, bridges the cadherin–catenin complex to the actin filaments of the cytoskeleton. Dysfunction of the cadherin pathway by gene deletions, gene promoter hypermethylation, and loss of heterozygosity (LOH) ***(54)*** is involved in tumor invasiveness and disease progression. E-cadherin is a prognostic marker for prostate cancer, based on a correlation of grade and aberrant E-cadherin staining, whereas P-cadherin is lost in all prostatic cancers, possibly because it is only expressed in basal cells. Expression of α-catenin, which binds to E-cadherin at the cytoplasmic domain, may also be reduced in prostatic tumors; α-catenin is not

expressed in PC-3 cells (that are E-cadherin positive) because of a homozygous deletion on chromosome 5q ***(55)***. Microcell transfer of chromosome 5 into PC-3 cells resulted in cell–cell adhesion and loss of tumorigenicity when the cells were implanted in nude mice. Similarly, the ALVA-31 line lacks α-catenin but is E-cadherin positive ***(40)***, whereas TSU-Pr1 cells express α-catenin but are E-cadherin negative ***(56)***. Expression of different cadherins and catenins in seven prostate cancer cell lines is described elsewhere ***(56)***. Expression of novel cadherins, such as N-cadherin and cadherin-11 (also called OB-cadherin) ***(56)***, may also play an important role in prostate cancer progression. A splice variant of cadherin-11 may act as a dominant-negative regulator of cell adhesion ***(57)***.

3. Immortalized Cell Lines

Several new immortalized nontumorigenic as well as tumorigenic adult human prostatic epithelial cell lines, which express functional characteristics of prostatic epithelial cells, provide additional in vitro cell models for studies on prostatic neoplasia. (These are described in more detail elsewhere in this volume.) Researchers have immortalized cells (*see* **Tables 1** and **3**) by transfection with an SV40 construct containing the SV40 large-T antigen gene ***(58)*** or by transfection with plasmids containing a single copy of the human papillomavirus (HPV) 18 genome ***(59–61)***. In each case, the viral proteins used interact with *p53,* indicating that loss of p53 function may be extremely important for the growth of prostate cancer cells. The use of HPVs for immortalization is based on observations that around 40% of prostate cancers contain DNA from either HPV-16, -18, or -33 ***(62,63)***, suggesting a possible role for HPV in prostate cancer. Further transformation of immortalized cells with the *Ki-ras,* based on observations of *Ki-ras* mutations in prostate cancer ***(63)***, was performed to make the cells tumorigenic ***(59)***, providing models for the study of genes involved in progression of prostate cancer, for example by comparative genomic hybridization ***(64)***. In addition, a stromal myofibroblast line has been established for studies of epithelial–stromal interactions. This line, WPMY-1, immortalized with SV40 large T antigen, expresses smooth muscle alpha-actin and vimentin, is positive for AR and large-T Ag, heterogeneous for p53 and pRb, and grows in serum-free medium ***(65)***. Conditioned medium from WPMY-1 cells causes marked inhibition of growth of WPE1-10 epithelial cells, immortalized from the same prostate. Other lines immortalized using HPV-18 include PZ-HPV-7 (normal prostate) and CA-HPV-10 (primary prostate cancer) ***(61)***, which show multiple cytogenetic changes. E6 and E7 transforming proteins of HPV-16 have been used to establish 14 immortal benign or malignant prostate epithelial cell cultures from primary adenocarcinomas ***(66)***, and these lines have been used to study allelic LOH. LOH at chro-

mosome 8p was seen in tumor-derived lines but not those from autologous benign prostatic epithelium.

In a similar fashion, normal rat prostate epithelial cells immortalized with SV-40 large T antigen have been used to study the progression to AI and malignancy in Copenhagen rats ***(67)***. The immortalized cells were transfected with *v*-H-*ras* and *c-myc* to create invasive cancer lines.

4. Primary Cultures

Stromal–epithelial interactions are pivotal in many aspects of prostatic biology. The investigation of factors that regulate these interactions and the growth and differentiation of human prostatic cells has been performed using defined and experimental culture systems for both epithelial and stromal cells from primary prostate cancers ***(68,69)***. Using such systems, fibroblastic or smooth muscle cells can be promoted, maintained, and investigated in a defined manner ***(70)***. The methods for developing primary cultures are described in Chapter 3.

5. Models for Bony Metastases

Prostate cancer is unique in that it is osteogenic, resulting in the formation of dense sclerotic bone with high levels of osteoblastic activity. A potential regulator of the tropism of prostate cancer to bone is a family of proteins that belong to the transforming growth factor β (TGF-β) family called bone morphogenetic protein (BMP), which are involved in stimulating bone formation in vivo. Some BMPs and their receptors are expressed on prostate cancer cells. These receptors are regulated by androgen and can differentially modulate prostate cancer cell growth in response to BMP under different hormonal conditions ***(71)***. Because xenografts grown subcutaneously in nude mice rarely metastasize, special methods have been developed to study bony metastases from human prostate cancers in experimental models. As mentioned previously, the C4-2 and C4-2B sublines that were developed from LNCaP cells by coinoculation with tissue-specific or bone-derived mesenchymal or stromal cells in castrated mice metastasize to lymph nodes and bone after subcutaneous or orthotopic inoculation ***(29,30)***. Intrafemoral injection has been used to establish osteoblastic bone lesions of PC-3, LNCaP, C4-2, and C4-2B4 in athymic ***(72)*** and SCID/bg mice. In the latter, osteoblastic tumors occurred in the bone marrow space within 3 to 5 wk, and serum PSA showed a stepwise elevation with tumor growth ***(73)***. The growth of PC-3 in the femur of nude mice was significantly inhibited by treatment with systemic interleukin-2, which caused vascular damage and infiltration of polymorphonuclear cells and lymphocytes in the tumor as well as in necrotic areas with apoptotic cells ***(74)***.

In a related model system, humanized SCID mice have been used to test the ability of prostate cancer cells to “home” to bone ***(75)***. C57.17 SCID-hu mice

were implanted with macroscopic fragments of human fetal bone, lung, or intestine or mouse bone subcutaneously and injected 4 wk later with human prostate cancer cells given via the tail vein or implanted transdermally. PC-3, DU-145, and LNCaP cells colonized implanted human bone fragments with osteolytic lesions in the case of PC-3 and DU-145 and osteoblastic and osteolytic lesions from LNCaP cells. Each cell line formed tumors in implanted human lung tissue, but these were very small. Similar studies were performed using humanized nonobese diabetic/severe combined immunodeficient (NOD/SCID-hu) mice engrafted with human adult bone or lung ***(76)***. In this study, a much higher take rate was shown by the LNCaP cells, which formed osteoblastic metastases (65% of LNCaP) than PC-3 cells that caused oesteolytic lesions (3% of PC-3) in the human bone but not in mouse bone.

6. Hormone Therapy

Prostate cancer cell lines have been used to study various treatment strategies. One of the mainstays of treatment for prostate cancer is androgen ablation, which can inhibit tumor growth when the cancer is AD or AS. However, prostate tumors can adapt to an environment with low androgen supply by using a hyperactive AR; the mechanisms involve mutations of the AR, generating receptors with broadened activation spectra, increased receptor expression, and activation by interaction with other signaling pathways ***(6,77)***. For these reasons, prostate cancer models have been widely used to study a variety of experimental hormonal manipulations, including those possibly suitable for AI disease. Intermittent use of hormone ablation in the LNCaP model prolonged the time until AI PSA production began ***(78)***. In LNCaP cells, an interaction occurs between the DNA- and ligand-binding domains of AR and the leucine zipper region of *c-Jun*. This association provides a link between the transcription factor, AP-1, and AR signal transduction pathways in the regulation of the *PSA* gene ***(78)***. Both luteinizing hormone-releasing hormone (LH-RH) and growth hormone-releasing hormone analogs have proved useful for treating PC-3 or DU-145 AI xenografts and both appear to invoke increased expression of mRNA for insulin-like growth factor II (IGF-II) in the tumors ***(79–82)***. LH-RH vaccines have also been used to induce atrophy of the prostate in rat models ***(83)*** and may provide an inexpensive alternative to the use of LH-RH analogs. Photodynamic therapy given in vitro was more effective in LNCaP cells when they were pretreated with 5α-dihydrotestosterone, suggesting an androgen-modulated effect on both uptake and phototoxicity ***(84)***. This effect was not observed in AI PC-3 cells in vitro. However, subsequent studies in vivo using R3327-MatLyLu Dunning cells grown orthotopically in the ventral prostate indicated that benzoporphyrin derivative monoacid ring A, combined with surgery, could inhibit both local primary tumor growth as well as reduce distant metastases ***(85)***.

Other modalities, including antibody-based therapy, gene therapy, and new chemotherapeutic drugs *(86)*, have also been extensively tested at a preclinical level using human prostate cancer cell lines, either in vitro or grown as xenografts in various locations (orthotopic, in the femur) of nude or SCID mice. Such treatments are outside the scope of this chapter.

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