# Chapter 1
# Cell Biology of Prostate Cancer and Molecular Targets

Martin E. Gleave, Michael E. Cox, and Yuzhuo Wang

**Abstract** While not appreciated at the time, the Nobel Prize-winning work of Huggins and Hodges in the 1940s illustrated the androgen dependence of prostate cancer and credentialized the first "targeted" (in this case, the androgen receptor) anticancer therapy. Androgen deprivation therapy induces long-term remission in most patients, but development of castration-resistant prostate cancer (CRPC) is inevitable. Most treatments for CRPC have been approved for symptomatic benefit, with only docetaxel shown to improve overall survival. Mechanisms underlying shift to castrate resistance have been attributed to a complex interplay of clonal selection, reactivation of AR axis despite castrate levels of serum T, adaptive upregulation of antiapoptotic and survival gene networks, stress-induced cytoprotective chaperones, and alternative growth factor pathways. CRPC tumors develop compensatory mechanisms during androgen deprivation, tailored to the synthesis of intratumoral androgens, which along with ligand-independent mechanisms involving cofactors or growth factor pathways, cooperatively trigger AR activation and thus disease progression. Over the last few years, numerous gene targets involved with CRPC that regulate apoptosis, proliferation, angiogenesis, cell signaling, and tumor-bone stromal interactions have been identified, and many novel compounds have entered clinical trials either as single agents or in combination with cytotoxic chemotherapy. In this review, several genes and pathways involved in CRPC progression will be reviewed, with particular emphasis on preclinically credentialized genes and pathways that are currently the targets of novel inhibitors in later stages of clinical development. These include the AR axis, molecular chaperones, tumor vasculature, bone stroma, and signal transduction pathways such as those triggered by IGF-1 and IL-6.

**Keywords** Castration-resistant prostate cancer • Androgen receptor • Clusterin • Hsp27 • IGF-1

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## Introduction

Prostate cancer (CaP) cell proliferation and survival are regulated through complex interactions between cell surface receptor-mediated cell signaling and transcription factor regulation of gene expression. Androgens are principal factors in CaP carcinogenesis and progression, regulating gene and signaling networks that promote cell survival through binding with the androgen receptor (AR), a ligand-responsive transcription factor. Testicular synthesis of testosterone (T) accounts for 90% of the dihydrotestosterone (DHT) formed in the prostate, with the remainder derived from less potent adrenal androgens. Once intracellular, T is converted to DHT by 5α-reductase, binding to and activating the AR that subsequently dimerizes, translocates to the nucleus, and interacts with promoter regions of specific genes to regulate transcription and hence protein synthesis, cell proliferation, survival, and differentiation.

Though not appreciated at the time, the Nobel Prize-winning work of Huggins and Hodges [1] in the 1940s credentialized the first "targeted" (in this case, the AR) anticancer therapy by confirming the androgen

W.D. Figg et al. (eds.), *Drug Management of Prostate Cancer*,
DOI 10.1007/978-1-60327-829-4_1, © Springer Science+Business Media, LLC 2010

dependence of CaP. Following androgen deprivation therapy (ADT), benign and malignant prostate epithelial cells undergo apoptotic regression leading to >80% objective response and prolonging median overall survival from ~18 to ~36 months in men with metastatic disease [2]. Serum PSA, an AR-regulated gene, remains the most useful marker of response and prognosis to ADT; PSA nadir levels above 4 μg/L after 6 months of ADT are associated with a median survival of 18 months compared with 40 months when nadirs below 4 μg/L are seen [3]. Despite high initial response rates, remissions are temporary because surviving tumor cells usually recur with castration-resistant prostate cancer (CRPC) phenotype. The earliest signal of CRPC is a rising PSA while on ADT, predating clinical progression by 6–12 months and death by 18-24 months [2, 4]. Thus, one of the main obstacles to the cure of advanced CaP by androgen ablation is progression to CRPC, a complex process involving variable combinations of clonal selection [5, 6], adaptive upregulation of antiapoptotic survival genes [6–11], AR transactivation from low levels of androgen, mutations or increased levels of coactivators [12–14], and alternative growth factor pathways [15–20] (Fig. 1.1). If we are to have a significant impact on survival, new therapeutic strategies designed to inhibit the emergence of this acquire treatment-resistant phenotype must be developed.

Improved understanding of the molecular basis underlying bone-specific metastases and resistance to ADT or chemotherapy will facilitate the rational design of targeted therapeutics. In addition to castrate-resistant disease, a second unique characteristic of CaP progression is bone-predominant metastatic progression. Bone provides a rich microenvironment for establishment of CaP metastasis, at least in part, because of its dense reservoir of growth regulatory factors, extracellular matrix proteins, and hydroxyapatite scaffolds to support tumor growth. Over the last few years, numerous gene targets that regulate apoptosis, proliferation, angiogenesis, cell signaling, and tumorbone stromal interactions have been identified, and many novel compounds have entered clinical trials either as single agents or in combination with cytotoxic chemotherapy. Because of rapid progress of this field, it is beyond the scope of this chapter to review all compounds under investigation. This review will focus on molecular and cellular mechanisms involved in CaP progression, metastases, and treatment resistance, with particular emphasis on preclinically credentialized genes and pathways that are currently the targets of novel inhibitors. These include the AR axis, molecular chaperones, tumor vasculature, bone stroma, and signal transduction pathways such as IGF-1 and IL-6.

## AR Axis

The AR is a ligand-dependent transcription factor and member of the class I subgroup of the nuclear receptor superfamily that plays a key role in prostate carcinogenesis and progression [21, 22]. The classical model of androgen-regulated AR transcriptional activity has not fully defined the many diverse effects of androgens on CaP cell survival and growth. In response to androgen, cytoplasmic AR rapidly translocates to the nucleus and interacts with sequence-specific androgen response elements (ARE) in the transcriptional regulatory regions of target genes [22, 23]. In addition to this transcriptional genomic action, androgens and other steroid hormones such as progesterone and estrogen can exert rapid nongenomic effects that are not mediated through nuclear receptors but rather initiated at the plasma membrane, presumably through surface receptors [24–26].

Androgens and AR are essential for CaP progression, and in many cases CRPC maintains many aspects of AR function by increased AR expression and/or mutagenesis resulting in increased sensitivity to androgens, permissive activation by nonandrogenic steroids, de novo steroid synthesis, and/or ligand-independent activation [6, 12–15]. Moreover, AR activation controls CaP proliferation and survival by upregulating responsiveness to autocrine and paracrine growth factor and cognate receptor loops [20, 27–30] discussed further below.

Almost uniformly, CRPC involves the reactivation of the AR, as illustrated by sentinel upregulation of PSA, a discretely androgen-regulated gene. Experimental models and molecular profiles of human CaP indicate that the AR becomes reactivated in most CRPC [31–35]. Several groups [12–14, 31, 32] reported that androgen-regulated genes become constitutively reexpressed in the absence of testicular androgens during "AI" progression. Moreover, downregulation of AR using siRNA can suppress "AI" tumor growth [14, 36], and many enzymes and gene networks implicated in

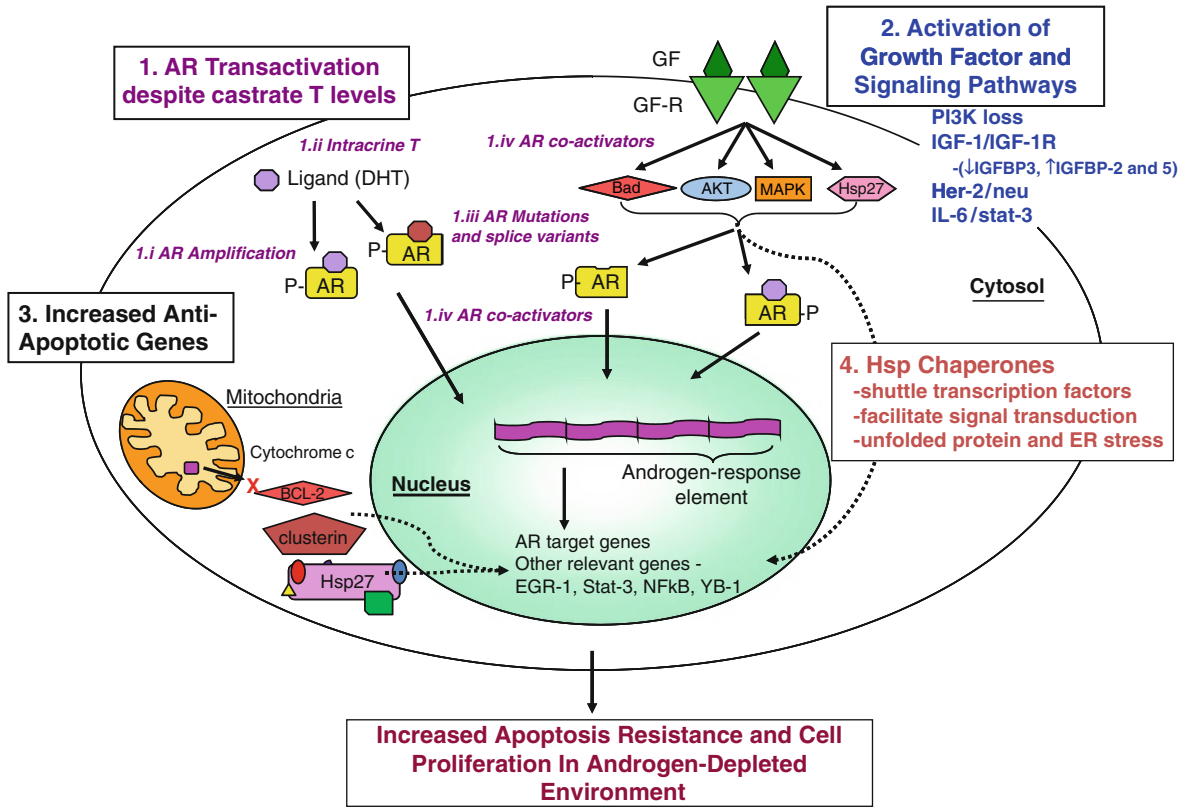


**Fig. 1.1** Schematic of molecular mechanisms contributing to castration-resistant disease. (1) Increased androgen receptor (AR) transcriptional activity in the presence of castrate levels of serum testosterone via (a) overamplification and increased hypersensitivity of AR; (b) de novo intracrine synthesis of DHT and other androgens via the backdoor pathways; (c) mutations in ligand-binding domain of AR leading to promiscuous activation by other ligands or splice variants lacking ligand-binding domain leading to ligand-independent AR transactivation; (d) increased coactivators (e.g., SRC, TIF-2, Ack1) that enhance AR activity. (2) Activation of proliferative growth factor and signaling pathways, notably insulin-like growth factor-1 (IGF-1) and interleukin-6 (IL-6). (3) Upregulation of cell survival genes that inhibit apoptotic pathway activation, including Bcl-2, clusterin, Hsp27, YB-1, and NF-kB. (4) Molecular chaperones (e.g., clusterin, Hsp27, Hsp90) facilitate protein interactions to shuttle transcriptional factors (e.g., AR), phosphorylation of signaling events, and suppress stress-induced cytochrome c release through interactions with proapoptotic Bcl family genes. Another mechanism includes selective outgrowth of subpopulations of preexisting androgen-independent CaP cells (clonal selection)

steroidogenesis are upregulated, leading to reactivation of AR [12, 37]. These data suggest that CRPC progression may not be entirely independent of androgen-driven activity of the AR, but in fact other sources of androgens are being capitalized upon for AR activation. Recent data suggests that at least two hypotheses may account for these observations: that the AR is activated independent of ligand (by mutations, overamplification, signaling pathways, or increased AR coactivators) or that androgen-regulated pathways within CaP cells are activated by alternative sources of androgenic steroids. These mechanisms are not mutually exclusive and expose the clinical problem of developing therapies that can account for the complex adaptive capacity of CRPC.

Persistent or reactivated AR signaling under ligand-deprived (or- independent) conditions may result from (a) amplified or elevated AR expression [38, 39]; (b) AR mutations in the ligand-binding domain that enhance AR promiscuity [40–43]; (c) expression of AR splice variants that lack a ligand-binding domain and are constitutively active in a ligand-independent manner [44, 45] (d) altered expression or activity of AR coactivator [46, 47] or chaperone [48] proteins, and (e) AR activation by certain kinases or signal transduction pathways that enhance AR activation in response

to low levels of androgen [49–54]. Previous studies established that the AR is phosphorylated at multiple serine/threonine sites [52, 55–57] and at several tyrosine residues. Tyrosine phosphorylation is mediated by at least two tyrosine kinases, Src and Ack1, and enhances AR responses to low androgen levels [58–60].

An important factor contributing to CRPC via the AR axis also includes suboptimal reduction of natural AR ligands by traditional ADT. Early studies by Geller and colleagues [61] indicated that concentrations of androgens sufficient to activate the AR remained in the prostate gland despite surgical or medical castration, and more recently, these were confirmed and extended using LC-MS by Mohler et al. [33, 34] and others [13, 31, 35]. Adrenal androgens were initially believed to be the sole source of androgens utilized by CaP tumors [33, 34, 37]. An alternative hypothesis is that cholesterol and its derivatives can be converted to androgens in prostate tumor cells through a series of well-characterized stepwise enzymatic events. Androgen synthesis is often described in terms of the classical steroidogenic pathway through DHEA and testosterone (T) (Fig. 1.2). A recently described "backdoor pathway" may serve as an alternative synthesis pathway, which utilizes progesterone as the primary steroidal precursor of DHT, thereby bypassing T as an intermediate [63]. Using the

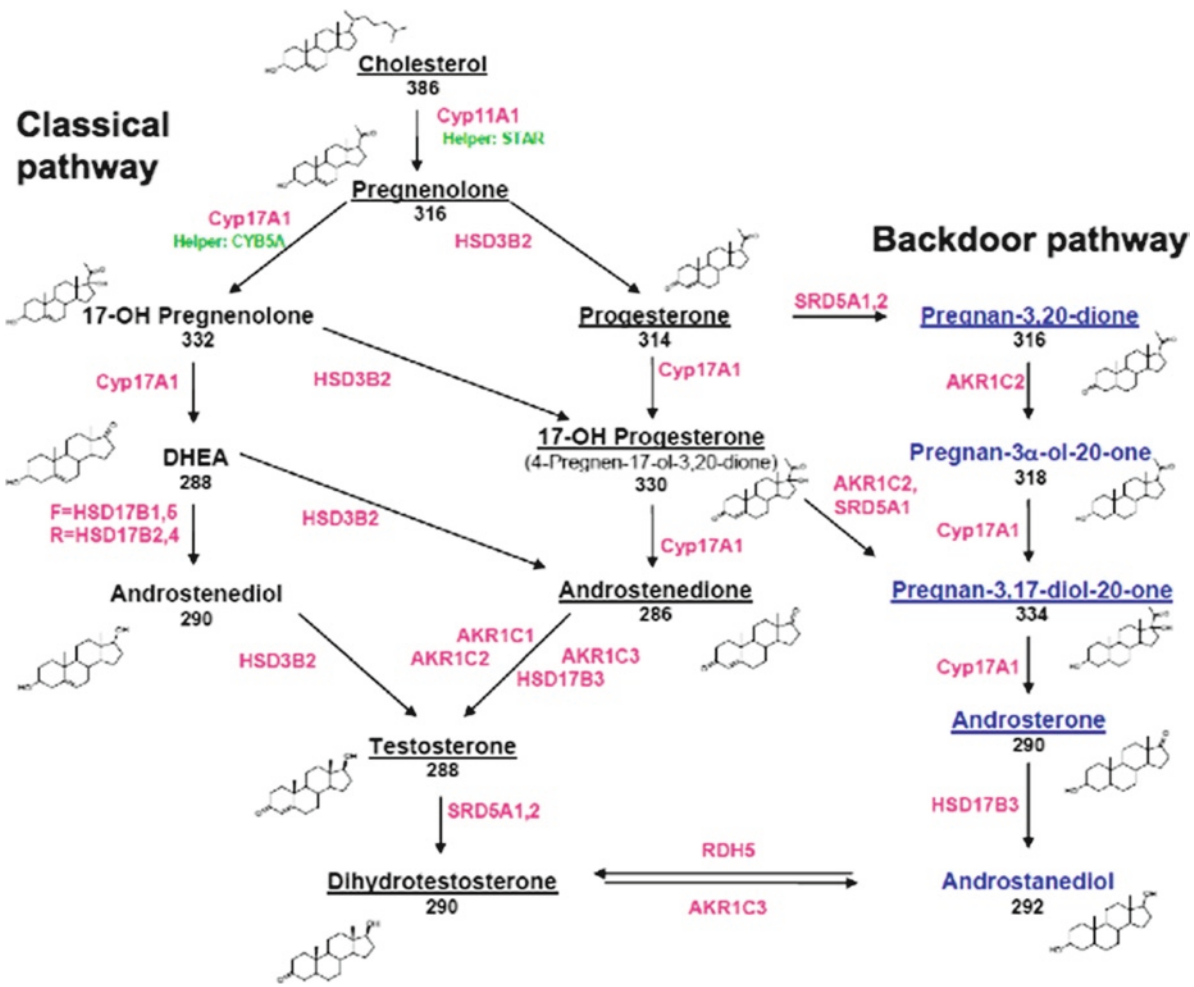


**Fig. 1.2** Intracrine de novo synthesis of testosterone. Steroidogenesis pathway converts cholesterol to DHT via the pathways involving the steroidal intermediates and interlinked enzymatic reactions. Steroids are portrayed in black (classical steroidogenesis pathway) and blue (backdoor steroidogenesis pathway), and enzymes are portrayed in pink and green. Some of the pathways are reversible while others are irreversible as indicated by the direction of the arrows

LNCaP xenograft model, Locke et al. [13] reported that tumor androgens, like PSA, increase during castrate-resistant progression. As mice do not synthesize adrenal androgens, LNCaP tumors themselves were investigated as the source of increased androgens. All enzymes necessary for androgen synthesis were expressed in castrate-resistant tumors, which were capable of de novo conversion of [$^{14}$C]-acetic acid to DHT and [$^{3}$H]-progesterone to six other steroids upstream of DHT. This evidence suggests that de novo androgen synthesis may be one of the mechanisms leading to CaP progression following castration.

Collectively, these studies suggest that CRPC tumors develop compensatory mechanisms during androgen starvation, tailored to the synthesis of intratumoral androgens, which along with ligand-independent or AR-sensitizing mechanisms outlined above, cooperatively trigger AR activation to facilitate disease progression. Hence, despite the failure of maximal androgen blockage trials using nonsteroid antiandrogens such as flutamide or bicalutamide, CRPC tumors are not uniformly hormone refractory and may remain sensitive to therapies directed against the AR axis. Several new classes of AR-targeting agents are now in clinical development, including more potent AR antagonists (e.g., MDV3100), inhibitors of steroidogenesis (abiraterone), and AR-disrupting agents that target AR chaperones such as Hsp90 (17-AAG analogs) or Hsp27 (OGX-427).

## AR Antagonists

First generation nonsteroidal antiandrogens (flutamide and bicalutamide) compete with T and DHT in binding to AR's steroid binding domain. However, these antiandrogens do not sufficiently inhibit AR transactivation in CRPC. Second generation antagonists have been identified that more potently block AR activity in CRPC. For example, MDV3100 is a novel AR antagonist [14, 64] that demonstrates antitumor activity in models with *AR* amplification and resistance to bicalutamide. Clinical activity has been observed in a phase 1 trial of MDV3100 in patients with both castration-resistant and docetaxel-refractory disease. This drug is currently in Phase II trials with PSA response rates exceeding 40% in CRPC and will move into Phase III registration trials in 2010 [64].

## Inhibitors of Androgen Synthesis

Historical attempts to suppress adrenal (as well as intracrine) androgen production have met with limited success. Ketoconazole inhibits several adrenal enzymes involved with adrenal androgen synthesis, but only modest therapeutic activities in CRPC were observed [65]. Abiraterone acetate is a potent steroidal irreversible inhibitor of CYP17 [17α hydroxylase/C17,20-lyase], blocking two important enzymatic activities in the synthesis of testosterone [66–68]. Pharmacodynamic studies demonstrated that its effects on adrenal steroid synthesis were consistent with its mechanism of action. In Phase II studies of chemotherapy-naïve men with CRPC, declines in PSA ≥ 30%, ≥50%, and ≥90% were observed in 80, 70, and 24% of patients, respectively, reflecting decreases in ligand-dependent AR transactivation. Consistent with abiraterone's mechanism of action, hypertension (HTN), hypokalemia, and lower extremity edema were the most commonly observed drug-related adverse events. Phase III trials of abiraterone in CRPC began in 2008 and data should be available by early 2011.

## AR Chaperone Inhibitors

Molecular chaperones are involved in processes of folding, activation, trafficking, and transcriptional activity of most steroid receptors, including AR. In the absence of ligand, AR is predominately cytoplasmic, maintained in an inactive, but highly responsive state by a large dynamic heterocomplex composed of heat-shock proteins (Hsp), cochaperones, and tetratricopeptide repeat (TPR)-containing proteins. Ligand binding leads to a conformational change in the AR and dissociation from the large Hsp complex [69–74]. Subsequently, the AR translocates to the nucleus, interacts with coactivators, dimerizes, and binds to ARE to transactivate target gene expression. Dissociation of the AR–chaperone complex after ligand binding is viewed as a general regulatory mechanism of AR signaling.

Several agents targeting AR-associated chaperones are in development. For example, Hsp90 inhibitors such as geldanamycin induce steroid receptor degradation by directly binding to the ATP-binding pocket of Hsp90 to inhibit its function [70, 71]. Several Hsp90

inhibitors are in Phase I-II trials in CRPC. Hsp27 is a cytoprotective chaperone expressed in response to many stress signals to regulate key effectors of the apoptotic machinery including the apoptosome, the caspase activation complex [75, 76], and proteasome-mediated degradation of apoptosis-regulatory proteins [77, 78]. Recently, a feed-forward loop was reported whereby androgen-bound AR induces rapid Hsp27 phosphorylation that in turn cooperatively facilitates genomic activity of the AR, thereby enhancing CaP cell survival. Antisense knockdown of Hsp27 (OGX-427) delays CRPC xenograft progression [10, 11], in part, by destabilizing the AR through ubiquitin-proteasome-mediated AR degradation [48] (Fig. 1.3). Interestingly, OGX-427 induces degradation of Hsp27, AR, and Hsp90, while geldanamycin inhibition of Hsp90 induces degradation of client proteins [71], but is accompanied by stress-activated increases in Hsp70 and Hsp27 [79]. A dose escalation Phase I trial of single agent OGX-427 in Hsp27-positive cancers was completed in 2008 and showed that OGX-427 was well tolerated. Decreases in PSA and CA-125, as well as CTC counts, suggest single-agent activity in CRPC and ovarian cancer, respectively. OGX-427 will move into Phase II trials in CRPC in 2010 [80].

## Regulation of Apoptosis

In mammals, programmed cell death can be initiated by extrinsic or intrinsic death pathways. The extrinsic pathway is triggered by extracellular ligands that induce oligomerization of death receptors such as Fas or other members of the TNF receptor superfamily, resulting in activation of a caspase cascade leading to apoptosis. The instrinsic pathway is triggered in response to a variety of apoptotic stimuli that induce damage within the cell including anticancer agents, oxidative damage, UV irradiation, and growth factor withdrawal and is mediated through the mitochondria. These stimuli induce the loss of mitochondrial membrane integrity and result in the release of proapoptotic molecules, including cytochrome c (cyt c), which associates with Apaf-1 and caspase-9 to promote caspase activation, and SMAC/Diablo and Omi/HtrA2 that promote caspase activation by eliminating inhibition by IAPs (inhibitors of apoptosis proteins) [81–85].

Fas-induced death is the best understood extrinsic apoptotic pathway both in terms of mechanism and its physiological importance in vivo [86]. Multivalent cross-linking of the Fas receptor as a result of FasL binding to preassociated Fas receptor trimers triggers the recruitment of a set of effector proteins to the receptor, resulting in the formation of the death-inducing signaling complex (DISC). The DISC is composed of intracellular signaling proteins including FADD/MORT1, a death domain-containing adaptor protein, and Caspase-8 (also known as FLICE/MACH). Upon recruitment to the DISC, caspase-8 is autoproteolytically cleaved and activated, which then directly activates caspase-3 leading to execution of apoptosis. Caspase-8 also leads to activation of the mitochondrial amplification loop by proteolytic cleavage of the proapoptotic Bcl-2 member, Bid. The truncated Bid then translocates to the mitochondria and promotes cytochrome c release into the cytosol. In association with APAF-1 and pro-caspase-9, cytochrome c forms the apoptosome complex leading to the activation of caspase-9 that subsequently cleaves and activates effector caspases.

The propensity of tumor cells to undergo stress-induced apoptosis determines their susceptibility to biologic and cytotoxic therapies [85]. Adaptations achieved by progressively accumulating genetic mutations increase tumor heterogeneity and decrease susceptibility to treatment. Many of these adaptations involve changes in intrinsic and extrinsic apoptotic machinery, including Bcl family members, inhibitors of apoptosis, cytoprotective molecular chaperones, and/or activation of growth factor-mediated and convergent downstream prosurvival signaling cascades.

### *Bcl-2*

The *bcl-2* gene, initially identified in follicular B-cell lymphoma due to a characteristic t14;18 translocation [87], is a mitochondrial membrane protein that heterodimerizes with Bax and other proapoptotic regulators to prevent cytochrome c release from the mitochondria and subsequent activation of the intrinsic apoptotic cascade [88]. Competitive dimerization between pairs of pro and antiapoptotic *bcl-2* family members (and other chaperones such as clusterin) determines how a cell responds to an apoptotic signal.

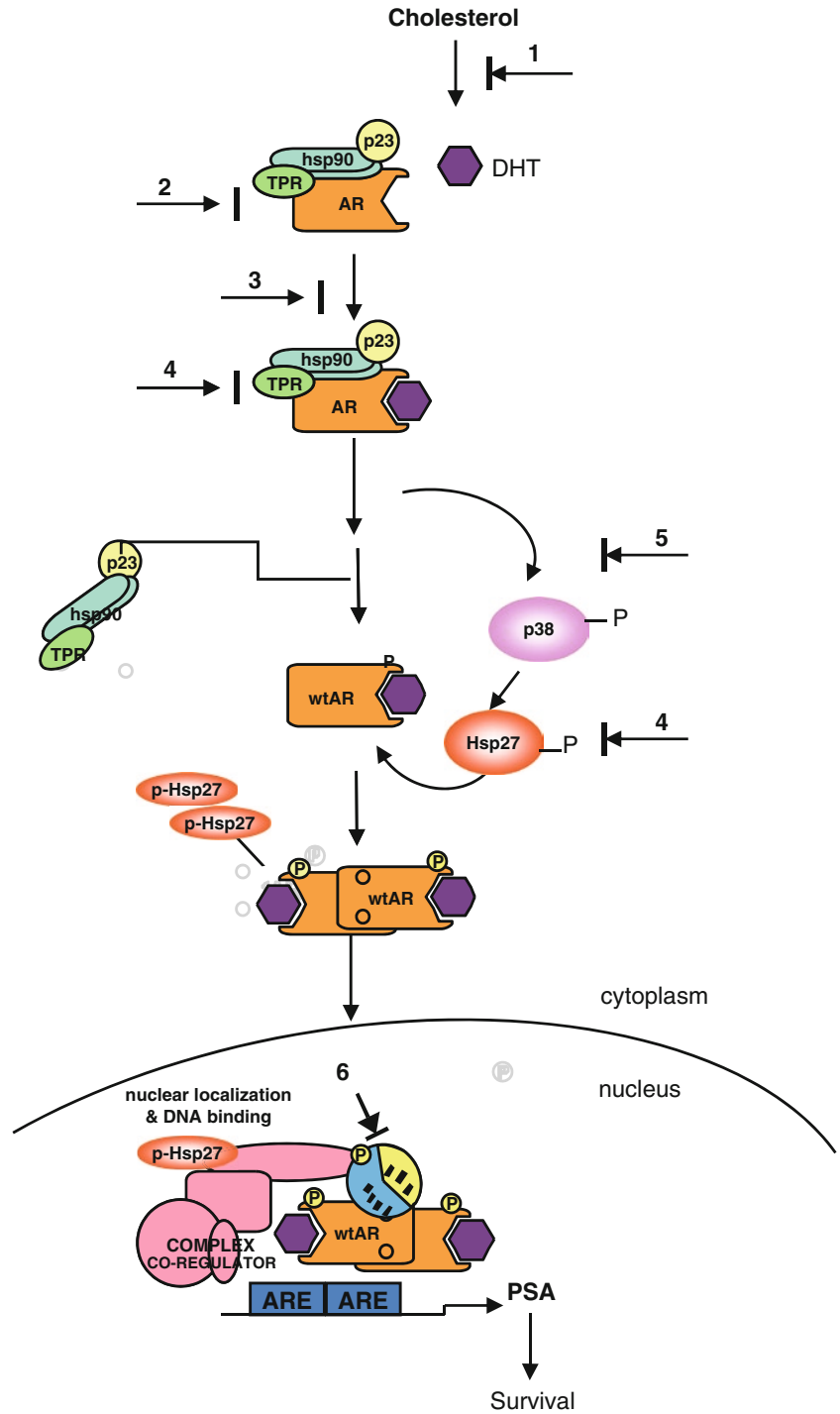


**Fig. 1.3** AR transactivation in castration-resistant prostate cancer and potential points of therapeutic intervention. Ligand-binding to the steroid-binding domain of the AR leads to dissociation of heat-shock proteins, p38 kinase-mediated phosphorylation of Hsp27 that replaces Hsp90 as the predominant AR chaperone to shuttle the dimerized and phosphorylated AR into the nucleus. Several mechanisms converge to support AR signaling in a castrate environment and are potential targets of therapeutic intervention. (1) Inhibitors of de novo androgen synthesis using abiraterone or 5 alpha reductase inhibitors to block enzymes involved in the synthesis and metabolism of androgens. (2) Target AR synthesis (antisense oligonucleotides or siRNA) or maturation [histone deacetylase (HDAC) inhibitors, e.g., SAHA]. (3) Potent second generation AR antagonists that block ligand-binding domain and prevent dimerization and nuclear translocation (e.g., MDV3100). (4) Target AR chaperones to destabilize and increase AR ubiquitination and degradation rates using inhibitors against Hsp90 (e.g., 17-allylaminogeldanamycin) or Hsp27 (OGX-427). (5) Inhibitors of nonnuclear AR signaling (e.g., SRC). (6) Coactivator inhibition

Many studies link overexpression of bcl-2 with treatment resistance [88–92], highlighting bcl-2 as the target to enhance chemotherapy-induced apoptosis. Targeted inhibition of bcl-2 was initially accomplished using antisense oligonucleotides (ASOs) with many reporting good hormone or chemosensitization activity in preclinical models [8, 93–96]. G3139, also referred to as oblimersen sodium or Genasense (Genta Inc.), is a first generation 18-mer phosphorothioate ASO evaluated in many clinical trials based on promising activity in preclinical models of many cancers [97–101]. Unfortunately, randomized Phase II or III trials in CRPC [101] and melanoma [102] or myeloma [103] did not show clear evidence of anticancer efficacy. These negative results have put future trials with this agent on hold. Issues persist about the dosing and regimen of this first generation ASO, and whether 6 days of 7 mg/kg/day treatment are enough to suppress target sufficiently.

*Bcl-xL* is another antiapoptotic bcl-2 family member. In tumors where bcl-2 and Bcl-xL are coexpressed, it is difficult to predict which of the two proteins is more critical for survival, and some tumor cells have been reported to switch expression from Bcl-2 to Bcl-xL [104]. Bcl-xL ASOs have been reported to sensitize various tumor cells, including prostate, to chemotherapy [105–109].

BH3 mimetics are a novel class of anticancer agents moving forward in clinical development that induce apoptosis in tumor cells, regardless of their p53 or Bcl-2 status by enhancing the proapoptotic potential of BH3-only proteins or bypassing the need for BH3-only proteins by directly blocking interactions of Bcl-2-like prosurvival molecules with Bax and/or Bak [110, 111].

## *CLU*

Human clusterin gene is located in chromosome 8p21-p12, where it is organized into nine exons [3] and encodes for two transcriptional isoforms in humans (Isoform 1, NM_001831 [GenBank]; Isoform 2, NM_203339 [GenBank]). These isoforms result from different transcriptional initiation sites and are produced only in humans and primates. In humans, clusterin exists as both an intracellular truncated 55-kDa nuclear splice variant (nCLU) and a 80-kDa secreted heterodimer disulfide-linked glycoprotein, making clusterin the only known secreted chaperone [112–114]. Clusterin isoform 2 (sCLU-2) is the predominant isoform and is highly conserved across species, while sCLU-1 is expressed only in primate species. sCLU is a multifunctional stress-activated molecular chaperone possessing chaperone-like properties similar to small heat-shock proteins that stabilize and/or scaffold multimeric protein conformations during times of cell stress. A low abundant proapoptotic nuclear (nCLU) splice variant with properties that can regulate DNA repair has also been described [115–117]. Hsp and CLU facilitate degradation of terminally misfolded proteins by the ubiquitin-proteasomal degradation or aggresome-autophagy systems [118]. The 60 kD cytoplasmic CLU interacts with and inhibits conformationally altered Bax in response to cytotoxic stress, impeding Bax oligomerization and intrinsic pathway activation [119, 120]. Cytoplasmic CLU also regulates NF-κB activation, a stress-regulated transcription factor that controls inflammatory and innate immune responses, as well as many aspects of oncogenesis. NF-κB is activated in cancer cells by chemo- and radiation therapy and associated with acquired anticancer treatment resistance, including CRPC [121–123]. In its inactive form, NF-κB is sequestered in the cytoplasm by members of the IκB family. In the canonical pathway, IKK complex phosphorylates IκB, which is then ubiquitinated and degraded in the 26S proteosome, exposing nuclear localization signals on NF-κB subunits with subsequent NF-κB dimer translocation to the nucleus and transactivation of NF-κB-regulated genes. CLU functions as a ubiquitin binding protein that enhances COMMD1 and I-κB proteasomal degradation through its interaction with members of the SCF-bTrCP E3 ligase family, which leads to increased NF-κB nuclear translocation and transcriptional activity.

Many mechanisms in heterogeneous cancers contribute to acquired resistance including stress-activated prosurvival genes transcriptionally activated by heat-shock factor 1 (HSF1). HSF1 is the key regulator of the heat-shock response, a highly conserved protective mechanism for eukaryotic cells under stress, and has been associated with oncogenic transformation, proliferation, and survival [124]. Targeting HSF1 [125] or multifunctional genes regulated by HSF1 that are associated with cancer progression and treatment resistance is a rational therapeutic strategy. CLU is transcriptionally activated by HSF1 [126, 127], IGF-1 signaling [128], and androgen [129] and is antiapoptotic in

response to hormone-, radiation-, and chemotherapy [9, 130–132]. Knockdown of CLU in CaP cells increases activated Bax levels with increased cytochrome c release from the mitochondria and subsequent activation of the intrinsic apoptotic cascade, as well as stabilization of I-κB with cytoplasmic NF-κB sequestration and decreased NF-κB activity. These data link stress-induced CLU expression with several antiapoptotic pathways relevant to acquired anticancer treatment resistance and mark CLU as an anticancer target.

Clusterin is overexpressed in a variety of human cancers, including those of the breast, lung, bladder, kidney, colon/rectum, and prostate [133–138]. Antisense- or siRNA-induced CLU knockdown enhances treatment-induced apoptosis and delays progression in many cancer models [9, 130, 139–141]. OGX-011 is a second-generation ASO that incorporates the 2′ MOE modification with four 2′ MOE-modified nucleosides at the 3′ and 5′ ends of the oligomer [141, 142] that decrease CLU levels >90% [143]. A randomized phase II study in chemo-naïve CRPC reported that OGX-011 + docetaxel prolonged overall survival by 7 months (16.9–23.8 months) and reduced death rates by 39%, compared with docetaxel alone [144]. Phase III trials are set to begin in 2010.

### *Hsp27*

Heat-shock protein 27 (Hsp27) is a 27-kDa molecular chaperone induced and phosphoactivated in response to a variety of biological, chemical, and physical stressors including heat-shock, oxidative stress, cytokines, and hormone- or chemotherapy [145]. Increased expression of Hsp27 during stress suppresses apoptosis, in part, from its role as a molecular chaperone to prevent protein aggregation or facilitate elimination of misfolded proteins. In addition, Hsp27 can act as a scaffolding protein to facilitate protein interactions and phosphorylation of signaling events [146]. Hsp27 is a multifunctional suppressor of apoptosis through interactions with Bid [75], procaspase-3 [147], cytochrome c [75], Smac/Diablo [148], and Daxx [149]. In addition, Hsp27 modulates the actin cytoskeleton [150] and intracellular levels of reactive oxygen species [151], interacts with several key client proteins involved in cell survival signals including IκBα [152], IKKβ [153], STAT-3 [11], AR [48], and Akt [154–156]. Akt is a key serine–threonine kinase that enhances the survival and proliferation of cells by regulating the function of proapoptotic proteins such as BAD and caspase-9, cell cycle regulators such as p27kip1, and mediators that control apoptosis and/or proliferation, such as MDM2, FOXO, GSK3, TSC2, and PRAS40 [156].

Hsp27 is frequently overexpressed in numerous malignancies, including prostate, [10, 157] and associated with poor clinical prognosis and therapeutic resistance [10, 158, 159]. Not only is Hsp-27 a powerful biomarker of aggressive CaP, but it is also a potential target for novel therapeutic intervention. Knockdown of Hsp27 suppresses tumor growth and sensitizes cancer cells to hormone-, chemo-, and radiotherapy [10, 11, 159]. The biphenyl isoxasole KRIBB3 inhibits protein kinase C-dependent phosphorylation of Hsp27 to induce mitotic arrest and enhances apoptosis [160]. Recently, pyrrolo-pyrimidones, a novel class of p38 MAPK/MAPK-activated protein kinase 2 (MK2) inhibitors, have been shown to inhibit phosphorylation of Hsp-27 at Ser78 and Ser82 by the MAPKAP kinase MK5 [161, 162]. Not only is the MAPKAPK2/Hsp-27 pathway a promising potential target for therapeutic intervention but the isoflavone genistein, an estrogen analog and candidate chemotherapeutic agent, inhibits cell migration by blocking activation of this pathway [163]. Recently, OGX-427, a selective, second-generation ASO inhibitor of Hsp27 has recently advanced into phase I/II clinical trials for treatment of a variety of cancers [80]. OGX-427 was well tolerated as a monotherapy and demonstrated declines in circulating tumor cells as well as reduction in PSA levels in three patients with CRPC. Reductions in both circulating tumor cells and tumor markers suggest single-agent activity warranting further clinical investigation.

## Signal Transduction Pathways

### *IGF and IGF-1R in CaP Progression*

The IGF axis is an important regulator of growth, survival, and metastatic potential in a variety of malignancies and is strongly implicated in CaP etiology [164–167]. This endocrine system consists of the ligands IGF-I and IGF-II, the receptor tyrosine kinase (IGF-1R) and the mannose-6-phosphate receptor (IGF-IIR), and a family of high-affinity IGF-binding proteins

(IGFBPs) and IGFBP-related proteins, which modulate IGF/IGF receptor biological activities, any of which change in many disease states [168–170]. IGF-1R overexpression has been found in a range of tumor types and is a predictor of poor prognosis in many cancers. IGF-1R signaling plays critical roles in the development and progression of cancer by allowing cells to overcome the propensity to die via apoptosis, necrosis, or autophagy in response to uncontrolled replication, loss of substrate adhesion, hypoxia, and therapeutic stress (Fig. 1.4).

Ligand activation of IGF-1R results in phosphorylation and membrane recruitment of insulin receptor substrate proteins (IRSs) and activation of intracellular signaling pathways including Ras/mitogen-activated protein kinase (MAPK) and phosphatidylinositol-3 kinase (PI3K)/AKT/mTOR that in turn control the various IGF-mediated biological effects [171]. IGFs are potent mitogens and antiapoptotic factors for many normal and malignant tissues [172]. Both receptor activation and these downstream signaling cascades are therapeutic target candidates.

Perturbations in intrinsic expression of IGF axis components are implicated in susceptibility and progression of CaP [173–181]. IGF-1R expression is elevated in metastatic [177] and CRPC [17, 20]. Furthermore, maintaining IGF-I responsiveness facilitates CaP survival and growth and is achieved

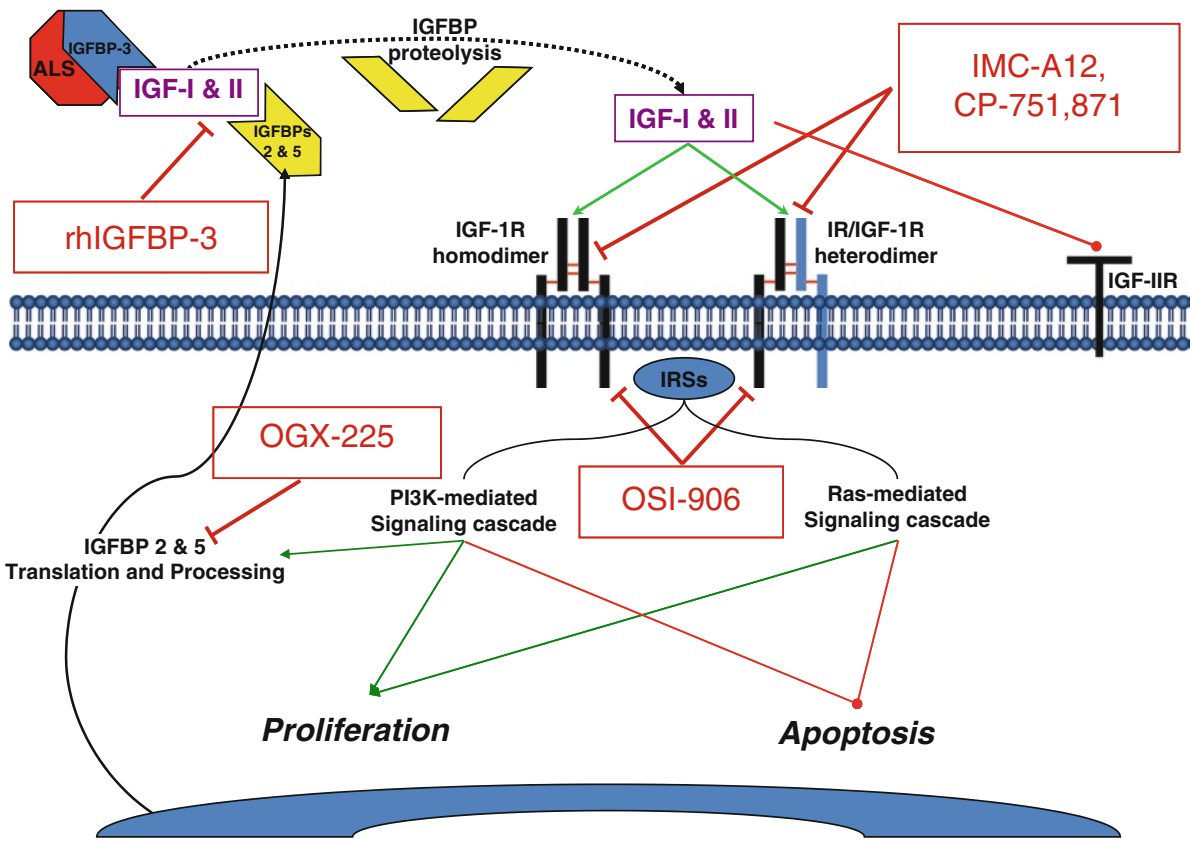


**Fig. 1.4** Rational Therapeutic Targeting of Insulin-like Growth Factor Axis. Insulin-like growth factors I and II (IGF-I & II) are sequestered in circulation by IGF-binding protein (IGFBP)-3/acid-labile subunit (ALS). IGFBPs -2 and -5 produced by tumor cells extract IGFs from IGFBP-3/ALS complex and release IGFs into the pericellular space upon proteolysis to facilitate IGF receptor binding and activation of proliferative and survival signaling via PI3K and Ras cascades. Retention of IGFs in the pericellular space can be competitively suppressed by administration of recombinant human IGFBP-3 (rhIGFBP-3) and by suppression of IGFBP-2 & -5 expression by OGX-225 antisense oligonucleotide. IGF-1R activation can be blocked by small molecule tyrosine kinase inhibitors such as OSI-906 and by induction of internalization and degradation by humanized anti-IGF-1R antibodies such as IMC-A12 and CP-751,871

through androgen-modulated IGF-1R expression [20, 182, 183]. While CaP cells can adapt to enhance IGF responsiveness, accumulating evidence indicates that paracrine sources of IGF-I and IGFBPs are also important mediators of CaP progression [184–187]. Such observations directly implicate the IGF axis as a mediator of CRPC progression and mark IGF-related signaling an attractive therapeutic target [188–192]. The clinical potential of a number of immunologic, antisense, and small molecules is now being investigated. As previously reviewed, these approaches convincingly demonstrate that perturbing IGF-1R availability significantly impacts growth and survival of in vitro and xenograft model systems.

The long list of TKIs and antibodies targeting IGF-1R highlights the high level of enthusiasm for this target in prostate and many other cancers. Many humanized antibodies targeting the IGF-1R are in early clinical development in CRPC and include IMC-A12 and CP-751,871 [193, 194]. IGF1R is highly homologous to insulin receptors (IRs) with 100% homology in the ATP-binding cleft commonly targeted for small molecule inhibitors. Because of their structural similarities, TKIs and Abs directed at IGF-1R often also affect signaling of IR. Small molecule IGF-1R kinase inhibitors, such as NVP-AEW541 [195, 196], initially showed great promise in preferentially targeting IGF-1R from its close homologue, the IR; however, the clinical use of such agents is hampered by off-target toxicity. Preclinical data of newly emerging agents, such as OSI-906 that showed strong antitumor activity and reduced incidence of IR-mediated side effects, and this TKI that is in Phase 1 trials are forthcoming [197].

## *IGFBPs and CRPC*

IGFBPs are a family of six circulating proteins that bind IGF-I and -II with equal or greater affinity than that of the IGF receptors and regulate IGF distribution, function, and activity [198, 199]. IGFBPs-2, 3, 4, 5, and 6 are expressed in prostatic tissues and cell lines [200–204]. IGFBP-2, 4, and 5 levels are correlated, while IGFBP-3 levels are inversely associated, with poor prognosis [200, 204]. The correlation between changes in IGFBP levels and concomitant changes in IGF-1R and IGF levels, disease state, and androgen ablation therapy implicates these adaptive responses in influencing disease progression.

Although it is clear that increased IGFBP-3 and 4 levels antagonize IGF signaling and increase sensitivity to apoptotic stress [205–207], other IGFBPs have been suggested to both inhibit and enhance IGF-1R-mediated signaling [208–211]. IGFBP-2 is one such factor whose expression is elevated in patients undergoing androgen ablation therapy [19]. Inhibiting IGFBP-2 expression in LNCaP cells increased androgen withdrawal-induced apoptosis and suppressed xenograft growth in castrated hosts [19]. Additionally, overexpressing IGFBP-5 accelerated AI progression of LNCaP tumors [18], while inhibiting IGFBP-5 expression decreased AI progression and IGF-I-dependent growth [212]. However, while elevated IGFBP-2 and 5 levels appear to contribute to disease progression at least in part by enhancing IGF responsiveness, IGFBPs have also been attributed with IGF-1R-independent activities that may contribute to prostatic oncogenesis [18, 208, 213–215] suggesting that binding and modulation of integrin signaling may also be critical to both IGF-1R-dependent and -independent IGFBP activities.

The primary IGF-binding protein, IGFBP-3, has also been attributed with IGF-dependent and -independent antiproliferative and proapoptotic activities on human cancer cells. In preclinical cancer models, recombinant human IGFBP-3 (rhIGFBP-3) is able to suppress growth of Herceptin-resistant breast, as well as lung and colon cancer xenografts as a single agent and on the latter xenograft model, augmented antitumor activity of irinotecan in combination [216, 217]. Consistent with the role of IGFBPs in modulating IGF signaling, these antitumor activities are correlated with suppression of AKT signaling in these models. In the CaP xenograft model, LAPC-4, rhIGFBP-3 synergized with the retinoid X receptor-alpha ligand VTP194204, to dramatically inhibit tumor growth by induction of apoptosis [218].

Also targeting IGFBPs is OGX-225, an ASO that effectively suppresses expression of IGFs -2, -3, and -5. Since IGFBP-2 and -5 are reproducibly upregulated in breast and CaPs, targeting their expression can selectively disrupt IGF signaling in tumor cells. Preclinical studies in human prostate, bladder, glioma, and breast cancer models indicate that reducing IGFBP-2 and IGFBP-5 production with OGX-225 promotes apoptosis and sensitize all of these tumor types to chemotherapy [219]. OGX-225 has completed preclinical pharmacology and is being evaluated for clinical trials.

### *Phosphatidylinositol 3-Kinase-Mediated Survival Signaling in CaP*

A key oncogenic feature of IGF signaling is protection against cytotoxic stress mediated by PI3K/AKT/PTEN signal transduction-triggered intracellular signaling cascades [190, 220]. The serine/threonine kinase, AKT, is a prominent node in the convergence of various growth and survival-promoting intracellular signaling cascades. Its activation is triggered by PI3K and generation of phosphatidylinositol 3-, 4-, 5-triphosphate (PIP-3), which serves to recruit pleckstrin homology (PH) domain-containing proteins to the plasma membrane, including the S/T kinases, PDK-1 and -2, or ILK and AKT [221, 222].

A signature event impacting PI3K signaling in ~50% of advanced CaP is homozygous loss of the tumor-suppressor gene, *PTEN* [223] and among those patients who are not *PTEN* null, many exhibit loss of one *PTEN* allele [224]. Recently, hemizygous *PTEN* loss combined with the presence of TMPRSS2:ERG gene rearrangements were reported to increase the risk of biochemical progression [225]. PTEN is a tumor suppressor that functions as a 3′ phosphatase of PIP3. It acts as a negative regulator of cell migration, cell survival, and cell cycle progression [226] and is associated with increased resistance to chemotherapy and increased angiogenesis [227, 228]. Its loss results in aberrant accumulation of PIP3 and subsequent survival signals [224, 229, 230]. Demonstration that prostate-specific PTEN knock-out mice develop metastatic CaP [231] and that ectopic expression of PTEN reduces CaP cell growth and induces apoptosis [232–234] underscores the importance of PTEN in PCa establishment and progression. However, while loss of PTEN expression appears to be a prominent means by which CaP cells promote AI growth, which and how selection for hyperactivated PI3K signaling is invoked remains to be elucidated.

PI3K-induced recruitment and activation of AKT is a central antiapoptotic pathway triggered by growth factors [reviewed in235]. AKT directly phosphorylates and inactivates several proapoptotic factors, including Bad [236], procaspase-9 [237], GSK3β, and Forkhead transcription factors [238, 239] and activates c-FLIP, MDM2, mTOR, and the antiapoptotic transcription factor, NFκB [240]. In turn, mTOR complexed with rictor can regulate activation of AKT [241]. Association of constitutive AKT activation with resistance to chemo- and radiotherapeutics in diverse cancers, particularly CaP, has promoted research into the role(s) of subsequent downstream signaling in regulation of these phenomena [242, 243].

The mammalian target of rapamycin (mTOR) is an S/T kinase that regulates cell growth and division by integrating information regarding nutrient sufficiency, energy levels, and mitogenic signaling [244, 245]. mTOR relays proliferative signals from the PI3K pathway and information on amino acid sufficiency to critical mediators of protein translation. Inhibition of mTOR can reverse AKT-dependent malignant transformation of murine prostate [246] and doxorubicin resistance in CaP cell lines [227]. These downstream mediators, the 40S ribosomal subunit protein kinase (S6K1) and the eukaryotic initiation factor 4E binding protein-1 (4EBP1), are required for ribosomal biosynthesis and the production of proteins required for $G_1$/S transition [247, 248]. Monitoring the activation state of terminal kinase targets such as S6 and 4EBP1 can therefore be used as pharmacodynamic endpoints for activation of upstream signaling cascades due to loss of PTEN function, and in response to therapeutics that target proximal PI3K activation.

## Angiogenesis

Angiogenesis is critically important for the growth and metastatic development of tumors. It involves migration and proliferation of endothelial cells from the microvasculature, controlled expression of proteolytic enzymes, breakdown and reassembly of extracellular matrix, and endothelial tube formation. Stimuli such as hypoxia can drive tumor, inflammatory, and connective tissue cells to generate a variety of angiogenic factors, including growth factors, cytokines, proteases, and cell adhesion molecules. Regulation of angiogenesis is thought to be largely dependent on a balance between pro- and antiangiogenic factors during the vascular network formation [249]. Angiogenesis plays an essential role in CaP development and metastasis. Therapy targeting tumor neovasculature therefore represents a promising area of research aimed at developing anticancer and antimetastasis therapeutics with many antiangiogenic agents being evaluated in various phases of clinical trials [250].

Among the various proangiogenic factors, vascular endothelial growth factor (VEGF) is a major angiogenesis promoting factor, primarily acting on endothelial

cells to induce their migration and proliferation via activation of tyrosine kinase receptors, VEGFR1 and VEGFR2. Increased expression of VEGF by tumors, resulting from e.g., hypoxia, can lead to tumor angiogenesis. As such, VEGF and its receptors represent key targets for new antiangiogenic drugs for treatment of cancer and have evoked a lot of interest [251, 252]. The VEGF level in plasma can serve as an independent prognostic factor in men with metastatic CRPC [253]. Antiangiogenic agents utilizing specific anti-VEGF monoclonal antibodies, such as bevacizumab (Avastin®), have been evaluated in CRPC. Interestingly, most antiangiogenic drugs failed to demonstrate significant activity as single agents in CRPC, but when bevacizumab was combined with docetaxel a 65% PSA response was achieved [254]. Unfortunately, a phase III study with accrual of 1,050 patients (CALGB 90401) recently reported that the addition of bevacizumab to docetaxel did not prolong OS.

In addition to VEGF, platelet-derived growth factor (PDGF) has been implicated in the progression of CaP and bone metastasis and is expressed in 80% of CRPC lesions [255]. Preclinical studies indicated that imatinib mesylate (Gleevec®), a PDGF inhibitor, is active in CaP cell lines, and a phase I trial of 21 patients with metastatic CRPC reported a 38% PSA response rate [256]. However, a randomized Phase II trial of imatinib and docetaxel in patients with CRPC showed increased toxicity without delaying progression. Sunitinib (Sutent®) and sorafenib (Nexavar®) are oral multitargeted tyrosine kinase inhibitors that inhibit RAF kinase, VEGF receptor tyrosine kinase, and the PDGF receptor; both are currently approved for the treatment of metastatic renal cell carcinoma [257]. Several phase II studies evaluated the activity of sorafenib in CRPC [258–260], demonstrating single agent decreases in PSA. Phase III trials of sunitinib and sorafenib are either planned or underway as second line therapy in docetaxel recurrent CRPC. Despite negative results with bevacizumab, the use of angiogenesis inhibitors continues to be evaluated as a promising treatment strategy for a variety of solid tumors, including CRPC.

## Inflammation

Increasing evidence suggests that cancer-associated inflammation should be viewed as a seventh hallmark of cancer [261]. Most recently, such inflammation has been functionally linked to metastasis [262]. In fact, a number of inflammation-associated proteins, including tumor necrosis factor (TNF)-α, interleukin-1 (IL-1), interleukin-6 (IL-6), interleukin-11 (IL-11), TGFβ, cyclooxygenase 2 (COX-2), NFκB, Stat3, stromal-derived factor-1 (SDF1) and hedgehog, have been shown to facilitate CaP growth, tissue invasion and importantly, metastasis. Furthermore, inhibition of, for example, the COX-2 enzyme, which catalyzes the conversion of arachidonic acid to prostaglandins, i.e., important inflammatory mediators, has led to inhibition of tumor growth and suppression of metastasis in multiple cancers, including CaP [263]. Accordingly, inhibition of cancer-associated inflammation has emerged as a most promising new approach for treatment of metastatic CaP.

The nuclear transcription factor, NFκB, is a key regulator of immune, inflammatory and acute phase responses and has also been implicated in the control of cell proliferation and apoptosis [264]. It is overexpressed in many human cancers, including metastatic CaP [265, 266]. Stat3, which is both a cytoplasmic signaling molecule and a nuclear transcription factor, belongs to the seven-member Stat gene family of transcription factors. Recently, it has been reported that Stat3 is activated in clinical CaP metastasis and in recurrent CaP and may have a major effect on metastatic dissemination of the disease [267]. In view of this, NFkB and Stat3 could act as potential targets for inhibition of metastatic progression of CaP. RTA 402, an NFκB and Stat3 inhibitor, has demonstrated anticancer activity in preclinical studies and a recent clinical Phase I pancreatic cancer trial [268]. This inhibitor is now moving into Phase II trials. Moreover, several small molecule inhibitors for such targets are under preclinical development [269].

The chemokine stroma-derived factor, SDF-1/CXCL12, plays multiple roles in tumor pathogenesis. It has been demonstrated that CXCL12 promotes CaP growth, enhances tumor angiogenesis, contributes to immunosuppressive networks within the tumor microenvironment, and participates in tumor metastasis [270, 271]. The interaction of CXCL12 and its receptor CXCR4 leads to mitogen-activated protein kinase and phosphoinositide 3-kinase/Akt–mediated MMP-9 expression, migration, and tissue invasion of CaP cells [272]. Therefore, it stands to reason that the CXCL12/CXCR4 pathway is an important target for development of novel antimetastasis therapies. A wide variety of strategies, based on peptides (e.g., T22) [273], small molecules (e.g., AMD3100) [274], antibodies [275], and small interfering RNAs [276], have been

used to target this pathway. Treatments in combination with current therapies seem to be especially promising in preclinical studies, and compounds are advancing into early stages of clinical development [277].

The hedgehog pathway has also been implicated in CaP development and metastasis [278]. The multi transmembrane protein, Patched (PTCH), is the receptor for various hedgehog ligands (Sonic, Indian, and Desert). In the absence of hedgehog, PTCH inhibits Smoothened (SMO), a G protein-coupled receptor protein encoded by the SMO gene of the hedgehog pathway [279]. When hedgehog binds to PTCH, SMO is disinhibited and initiates a signaling cascade that results in activation of GLI transcription factors and increased expression of target genes (including PTCH and GLI1). Inhibition of the hedgehog pathway induces apoptosis and decreases tumor invasiveness of CaP cells. For example, IPI-926 (Infinity Pharmaceuticals, Inc.), a small molecule inhibitor of the hedgehog signaling pathway, has shown potent efficacy and specific inhibition of the hedgehog pathway in multiple preclinical animal cancer models. Currently, IPI-926 is in a clinical Phase 1 trial for patients with advanced and/or metastatic solid tumors. GLI2 knockdown in preclinical models induces apoptosis, inhibits cancer growth, and chemosensitizes cells to chemotherapy in vitro and in vivo, providing preclinical proof-of-principle for CRPC [280]. The approach of regulating cancer-associated inflammation will be one of the most promising treatment strategies for a variety of tumors, including CaP.

## Bone Metastases

Bone is the most frequent site for metastases of CaP. While the precise mechanism by which cancer cells home to bone is still unclear, it is generally accepted that bone can express certain chemo-attractants (e.g., SDF-1) or growth factors [e.g., TGFβ, IGF] that selectively retain/promote circulating CaP cells. As well, the cancer cells secrete many factors (e.g., uPA, TGFβ, FGFs, BMPs, PDGF, IGF, PTHrP, ET1) that activate bone stromal components, thus establishing a complex interplay between tumor and bone tissue.

Advances in the understanding of the biology of CaP, bone and interactions between tumor and bone stroma have led to the development of drugs directed against specific molecular sites in the CaP and host cells in the bone environment. Bone remodeling is a tightly regulated process of osteoclast-mediated bone resorption, counterbalanced by osteoblast-mediated bone formation. Disruption of this balance can lead to excessive bone loss or extra bone formation. Recently, a triad of key regulators of bone remodeling in bone oncology was discovered. It consists of the receptor activator of NF-κB (RANK), an essential receptor for osteoclast formation, its ligand RANKL, and the decoy receptor osteoprotegerin (OPG). OPG, a member of the tumor necrosis factor (TNF) receptor superfamily, can bind to RANKL and thus prevents activation of osteoclastic bone resorption. RANK, RANKL, and OPG are critical determinants of osteoclastogenesis, and increased RANK signaling is involved in metastasis of various cancers, including CaP [281–283]. These findings highlight the potential of RANKL inhibition as a novel treatment for patients with bone diseases and metastatic CaP [283–287]. Denosumab, a human monoclonal antibody, inhibits osteoclastic bone destruction by binding and neutralizing RANKL and has been evaluated in a randomized Phase 2 trial of CaP patients with bone metastases [288]. Denosumab suppressed bone turnover markers (BTMs) in CaP patients with bone metastases and elevated BTMs. Phase 3 trials of denosumab in patients with bone metastases of CaP are in progress (e.g., ClinicalTrials.gov Identifier: NCT00286091).

Endothelins (ETs) and their receptors (i.e., ET-B and ET-A) have emerged as potential targets for therapeutic intervention of CaP bone metastasis [289, 290]. Several clinical trial studies have shown that use of ET-A receptor antagonists (e.g., atrasentan, ZD4054) led to a significant increase in the time to disease progression [291]. While atrasentan failed to achieve its primary endpoints in two Phase III trials, indicators of anticancer activity were seen. Currently, the SWOG-S0421 trial is testing this further in patients with metastatic CRPC in a randomized phase III trial to compare the efficacy of docetaxel and prednisone with or without atrasentan. Several phase III trials of ZD4054 monotherapy or in combination with docetaxel are underway in CRPC.

c-Met is a receptor tyrosine kinase involved in multiple pathways linked to cancer, such as cell migration, tissue invasion, and metastasis and is upregulated in a large number of human cancers, including metastatic

CaP [292, 293]. Multiple agents to target c-Met or its ligand hepatocyte growth factor (HGF, scatter factor) are under development [294]. Like c-Met, the nonreceptor tyrosine kinase, Src, is considered part of the metastatic process [295]. Consequently, a number of Src inhibitors are under development. PSCA [296, 297], MEK5 [298], CDK5 [299], ASAP1 [300], and ID1 [301] have also been proposed as potential therapeutic targets for metastatic CRPC.

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