Chapter 18
Castration-Resistant Prostate Cancer

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The Molecular Pathology of Castration-Resistant Prostate Cancer

Androgens are the primary regulators of normal prostate as well as prostate cancer cell growth and proliferation. When testosterone enters the cell, it is converted to its active metabolite, dihydrotestosterone (DHT), by the enzyme 5α[alpha]-reductase. In turn, DHT binds the AR in the cytoplasm leading to phosphorylation, dimerization, and subsequent nuclear translocation. In the nucleus the AR associates with DNA sequence motifs known as androgen-response elements (AREs), resulting in upregulation or downregulation of target gene transcription [1]. Although androgen deprivation therapy (ADT) functions in depriving cells of androgens (usually 90–95% reduction in serum testosterone) [2], AR and AR-dependent transcriptional programs are thought to remain functional. This is the basis of castration-resistant prostate cancer (CRPC), which is the recurrence of aggressive, lethal prostate cancer in an androgen-depleted setting. Genome-wide expression analysis revealed that CRPC is more similar to hormone-naïve

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primary cancers than to tumors undergoing ADT [3]. Many genes regulated by the AR that initially respond to ADT, such as FKBP5, are re-expressed in CRPC, suggesting a reactivation of the AR signalling axis under androgen-depleted conditions.

Clinicopathologic Characteristics

Men with advanced prostate cancer are typically treated with ADT, which results in tumor shrinkage. However, despite its initial response rate of 80–90%, ADT is palliative but not curative [4]. Many men experience only short-term regression, with nearly 20% of patients eventually progressing to a clinical castration-resistant state within 5 years of follow-up [5]. Compared with patients who are diagnosed with early, localized disease, the prognosis for patients with CRPC is poor, and survival is reduced. Mean survival is approximately 14 months from CRPC diagnosis [5].

The poor survival associated with CRPC is due to metastatic progression of the disease, most frequently to the bone. There is no clear temporal relationship between the emergence of metastases and the development of castration resistance—either can occur first—and this may be dependent on treatment practice. Bone metastases are present in over 84% of CRPC patients [6], and of those patients with no metastases present at diagnosis, 33% develop them within 2 years [7]. Accordingly, bone pain occurs in many patients, and fractures, spinal cord compression, and vertebral collapse are common [5]. Circulating tumor cells (CTCs), which are "seeds" for metastasis, have been accepted by the Food and Drug Administration as a prognostic tool in advanced prostate cancer. Patients with ≥5 CTC/7.5 mL of blood have a shorter overall survival (11.5 months versus 21.7 months) and higher frequency of metastatic disease [8, 9].

In addition to CTC enumeration, a number of biomarkers have been used to prognosticate CRPC patient survival, including prostate-specific antigen (PSA), lactate dehydrogenase (LDH), hemoglobin, albumin, and alkaline phosphatase [10]. A phase III clinical trial of patients receiving treatment for CRPC revealed that CTC number and LDH level are the most effective markers for discrimination between high-risk and low-risk patients. Patients with <5 CTC/7.5 mL of blood are classified as low risk, and those with ≥5 CTC with LDH >250 U/L are classified as high risk with a 2-year overall survival of 46% and 2%, respectively [9].

Identifying patients with CRPC may seem straightforward; however, it has been hindered by a lack of consensus regarding the clinical parameters for diagnosis. To address this issue, the European Association of Urology recently published a set of guidelines aimed to standardize the definition of CRPC [11], the key defining factors of which are listed in Table 18.1.

Table 18.1 Definition of castration-resistant prostate cancer

• Castrate serum levels of testosterone (<50 ng/dL or <1.7 nmol/L)
• Three consecutive rises of PSA, 1 week apart, resulting in two 50% increases over nadir with PSA >2 ng/ml
• Antiandrogen withdrawal for at least 4 weeks (flutamide) or 6 weeks (bicalutamide)
• PSA progression, despite consecutive hormone manipulation
• Progression or appearance of two or more osseous lesions on bone scan or soft tissue lesions using response evaluation criteria in solid tumors (RECIST) with nodes >2 cm in diameter

PSA prostate-specific antigen

Gene Expression Signatures of Castration-Resistant Prostate Cancer

Gene expression profiling has provided much insight into identifying molecular signatures that can define and stratify patients with CRPC. A paradigm-shifting study using microarray-based profiling of isogenic prostate cancer xenograft models reported that increased androgen receptor (AR) mRNA is consistently associated with the development of CRPC [12]. This was the first indication that castration-resistant progression remains dependent on persistent AR signalling. Since then, many studies have described in detail the transcriptional programs and pathways downstream of the AR that are active in CRPC. A transcription-based AR activity signature of 250 genes was developed that could accurately predict patients with CRPC from those with local, hormone naïve prostate cancer [13]. In addition, microarray analysis of genes co-dependent on AR and serum response factor (SRF) identified a 158-gene signature that correlated with aggressive disease, poor outcome, and biochemical recurrence [14]. As expected, a disproportionate number of genes in the signature were involved in cellular processes associated with metastasis, such as cell adhesion, actin cytoskeleton rearrangement, and cell-cell interaction.

Although AR plays a functional role in most cases of CRPC, AR signalling is quite different in CRPC from that observed in androgen-dependent prostate cancer. In contrast to androgen-dependent prostate cancer where AR drives the G1/S cell-cycle transition via regulation of cyclin D1, p21, and p27 [15], in CRPC AR selectively upregulates M-phase cell-cycle genes. These include CDC20, UBE2C, and CDK1, which together function to inactivate the M-phase checkpoint and promote cell proliferation [16]. Capitalizing on the genomic repositioning of the AR in CRPC, David Neal and colleagues curated a signature of 16 AR-regulated genes that increased in CRPC patient tissue, was downregulated by castration, and reemerged after the transition to CRPC [17]. This gene signature could be used to make prognosis or monitor the progression of CRPC and, notably, was better able to identify CRPC than the larger AR expression signatures. Intriguingly, CRPC-specific AR-binding sites do not overlap with motifs for common AR cofactors such as FOXA1 but instead are enriched for STAT, MYC, and E2F motifs [17], suggesting that altered signalling in CRPC tissue reprograms the AR.

It has recently emerged that transcript levels of a few selected genes isolated in blood samples from prostate cancer patients can accurately identify and predict the severity of CRPC. The LPD1 expression signature—whose nine signature genes include HMBS, TMCC2, SNCA, SLC4A1, STOM, GABRARAPL2, TERF2IP, RIOK3, and TFDP1—was derived by analyzing mRNA expression data in whole-blood samples from men with metastatic CRPC compared to those with clinically indolent cancer [18]. The signature was associated with known prognostic markers of CRPC, such as elevated PSA and CTCs, and overall survival was significantly lower for men who tested positive for LPD1 than those who tested negative (9.2 months versus 21.6 months). Using a similar blood-based RNA expression profiling strategy, a six-gene signature consisting of ABL2, SEMA4D, ITGAL, C1QA, TIMP1, and CDKN1A could stratify men with low-risk and high-risk CRPC (7.8 months versus >34.9 months survival) [19]. Interestingly, many of the genes in the above signatures have a role in B cell and T cell function suggesting that poor prognosis could be related to diminished immune response.

The Genetic Landscape of Castration-Resistant Prostate Cancer

Characterization of the prostate cancer transcriptome and genome has identified chromosomal rearrangements and copy number changes that initiate progression to CRPC, most notably AR mutation and/or amplification, PTEN loss, and ETS gene family fusions [20]. Although the overall mutation rates are low in CRPC (~2.00 per megabase), genes that are recurrently mutated include TP53, BRCA2, AR, ZFHX3, RB1, PTEN, and APC (see Fig. 18.1) [21, 22]. Of these, AR mutations are very rare in

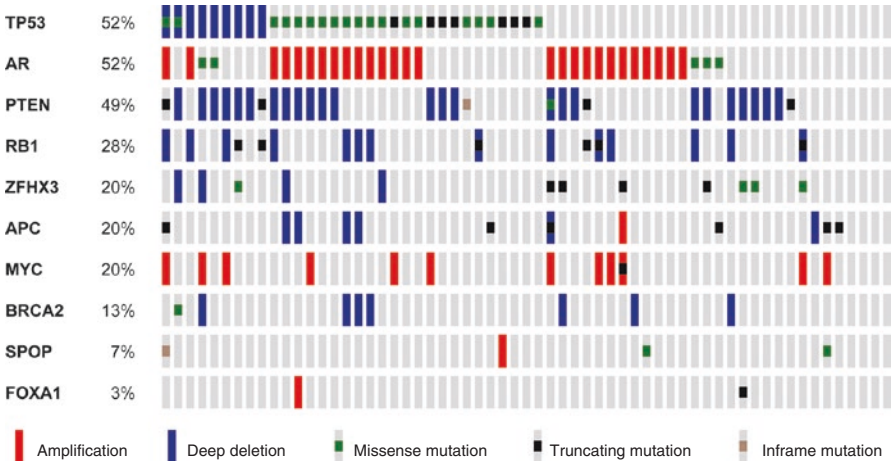

Fig. 18.1 Mutation and copy number changes in CRPC. Genome-wide genetic aberrations from 61 high-grade prostate cancer samples (represented by *gray squares*), including 50 metastatic CRPCs, were visualized using cBioPortal for Cancer Genomics [129, 130]. Genes are ranked by frequency of genomic alteration

early-stage untreated prostate cancer but are readily detected in CRPC; 10–30% of CRPCs harbor AR mutations, and 22–73% exhibit high-level amplification of the gene [23, 24].

Apart from the AR, CRPC driver mutations are clustered in key genes that confer enhanced proliferation and survival properties. The long arm of chromosome 10 (10q23), which contains the PTEN tumor-suppressor gene, is one of the most frequently deleted chromosomal regions in advanced prostate cancer; upward of 40% of CRPCs exhibit complete loss of PTEN via deletion or frameshift mutation [25]. This yields uninhibited activation of the AKT pathway, which is associated with cell survival, proliferation, and invasiveness. In addition to PTEN inactivation, loss of the retinoblastoma tumor-suppressor protein (RB) has been identified as a predominant compensatory mechanism for tumor maintenance under low-androgen condition. Relative to localized prostate cancer, RB expression is dramatically attenuated in CRPC, with both allelic deletion and methylation contributing to RB inactivation [26, 27]. These data are consistent with CRPCs clustering with a gene signature that is characterized by RB loss [28]. Mechanistically, loss of RB upregulates AR expression via the transcription factor E2F1 and increases recruitment of AR to the promoters of AR target genes associated with cell-cycle control. RB and/or PTEN loss in androgen-sensitive prostate cancer cells is sufficient to attenuate ADT and confer castration-resistant tumor growth, cementing them as key drivers of CRPC [28, 29].

Mutations in the DNA repair pathway occur with high frequency in CRPC and are largely associated with increased susceptibility to disease formation. The highest rate of mutation is located in the BRCA2 gene (12%), with mutations also identified in BRCA1 and ATM (8%) [25]. BRCA mutation carriers have an increased risk of developing prostate cancer, which presents with an aggressive, metastatic phenotype [30]. However, BRCA dysfunction itself is insufficient to promote carcinogenesis [31] but rather is believed to impair DNA repair thus facilitating genomic instability. This paves the way for secondary oncogenic events that lead to malignant conversion, such as TP53 deficiency or TMPRSS2-ERG fusion.

Although likely an early event in the genesis of prostate cancer, the expression of ETS gene fusions is maintained in many cases of advanced disease. In about one-third of CRPC patients, the androgen-regulated gene TMPRSS2 is fused with the ETS transcription factor family members, ERG, ETV1, or ETV4 [25]. These fusions, most commonly TMPRSS2-ERG, correlate with migratory cell phenotype, aggressive disease, and poorer prognosis [32]. In one particular study, all metastatic CRPC patient samples harbored ERG rearrangement by interstitial deletion, suggesting that it may be a requirement for progression to androgen independence [33]. However, this hypothesis was not supported by analysis of circulating tumor cells from patients with CRPC, which did not universally contain TMPRSS2-ERG [34]. This highlights the genetic basis of CRPC: there is not one defining “CRPC mutation” but a few distinct genomic alterations that can initiate disease progression.

Epigenetic Reprogramming in Castration-Resistant Prostate Cancer

Epigenetic alterations are also believed to represent important contributing factors in the genesis of CRPC. Genomic DNA from most CRPC is hypermethylated compared with benign prostate tissue [27], which functions to silence genes involved in hormone signalling, DNA repair, cell adhesion, cell-cycle control, and apoptosis. For example, glutathione S-transferase (GST), which protects cells from oxidative damage, is repressed in CRPC via DNA methylation of its CpG island-associated promoter. Methylated GST is detected in about 30% of men with CRPC and correlates with biochemical relapse and metastasis [35]. Interestingly, genes involved in the androgen biosynthesis pathway, such as CYP17A1 and HSD17B3, and the p53 signalling cascade, such as RB1 and TNFRSF10C, are particularly enriched for CpG methylation in CRPC [27]. These genes are also the target of copy number alterations and/or mutations [25, 26], suggesting that genetic aberrations and methylation work in concert to silence key tumor-suppressor pathways.

Histones are dynamic regulators of gene activity that undergo posttranslational modifications, including methylation and acetylation, to control chromatin accessibility. In particular, methylation of lysine 4 of histone H3 (H3K4) is an epigenetic mark correlated with an active transcriptional state. In CRPC the majority of genes that have H3K4 methylation near their promoter and/or enhancer also show AR binding [17, 36], highlighting the importance of the epigenome in the genomic positioning of the AR. For example, H3K4 is significantly methylated at the AR enhancer of the proto-oncogene UBE2C, which potentiates AR binding and UBE2C gene expression [16]. In addition to regulation of AR-binding dynamics, H3K4 methylation contributes to transcription of the AR gene itself. The second intron of the AR gene is associated with substantial levels of H3K4 methylation in cells adapted to androgen deprivation [37], consistent with this element functioning as an enhancer of AR gene expression and restored activity in CRPC. The establishment of unique H3K4 methylation patterns in CRPC is mediated by mutation of the H3K4 methyltransferase complex [22, 38] and/or altered activity of the AR cofactor lysine-specific demethylase 1 (LSD1) [37]. Ligand-bound AR recruits LSD1 to ARE-driven enhancers where it catalyzes the demethylation H3K4 to silence genes mediating androgen synthesis, DNA synthesis, and proliferation; however, castrate levels of androgen relive this LSD1-mediated repression [37].

Trimethylation of histone H3 on lysine 27 (H3K27) is also strongly associated with CRPC [39]. This epigenetic mark is mediated by the polycomb group protein EZH2, which acts in a large complex to silence genes involved in controlling cell identity. Typically, EZH2 expression is confined to stem/progenitor cells [40]; however, it is also found to be overexpressed in hormone-refractory, metastatic prostate cancer [41]. This could explain why CRPC cells exhibit a similar pattern of polycomb/EZH2 genomic occupancy and H3K27me3 marks as embryonic stem cells [42], indicating that developmental regulators are repressed by EZH2 in CRPC. Although the mechanism responsible for reactivation of EZH2 in CRPC is

poorly understood, transcriptional activation by ERG [43] and/or genomic loss of microRNA-101 [44], which targets EZH2, is likely responsible. In support of its role as an epigenetic driver of CRPC, a "polycomb repression signature" composed of 14 direct targets of polycomb/EZH2 correlates with prostate cancer progression, metastasis, and poor prognosis [42]. In particular, EZH2 has been shown to repress CDH1 (encoding E-cadherin) and DAB2IP, which trigger metastasis, as well as SLIT2, which promotes cell proliferation [45, 46].

Molecular Subtypes

The impressive, recent crescendo of whole-exome sequencing studies has made it possible to decipher molecular subtypes of CRPC [22, 26, 38]. The main division is based on the expression of ETS gene fusions: ETS fusion positive (35%) and ETS fusion negative (65%). These subtypes differ markedly in their gene expression and response to therapy; for example, ETS fusion-positive CRPC is associated with higher response rates to abiraterone acetate [47].

Of the ETS fusion-negative CRPCs, about 15% contain SPOP mutations, which anchor a distinct molecular subtype. In a study of 112 prostate tumors, more than 5000 somatic DNA mutations were identified with SPOP being the most frequently mutated gene in advanced-stage disease [38]. A subsequent in-depth analysis across multiple independent cohorts uncovered that all patients with SPOP mutations lacked the ETS family gene rearrangements and TP53 mutation. CHD1 deletion, which is overwhelmingly associated with ETS deletions [22], was harbored within the SPOP mutated population. Notably, tumors with SPOP mutations are enriched in PIK3CA mutations [38], suggesting they may be exquisitely sensitive to PI3K/AKT/mTOR inhibitors. In the future, expanded molecular subtyping of CRPC may illuminate molecular vulnerabilities and improve the stratification of patients in the neoadjuvant setting.

Mechanisms of Castration-Resistant Prostate Cancer

A number of adaptive mechanisms have been proposed which would allow CRPC cells to circumvent the restraint conferred by low levels of androgens (see Fig. 18.2): [1] amplification or overexpression of AR and its coactivators, which sensitizes cells to low levels of androgens; [2] AR mutation that decreases ligand specificity, thereby allowing AR signalling to be activated by nonandrogenic steroids; [3] activation of AR by nonsteroids such as growth factors and cytokines via deregulated kinase signalling pathways; and [4] complete bypass of dependence on the AR pathway. These mechanisms are not mutually exclusive but indeed work in concert during the development of CRPC, along with contribution from immune cells in the tumor microenvironment as well as cancer stem cells (CSCs).

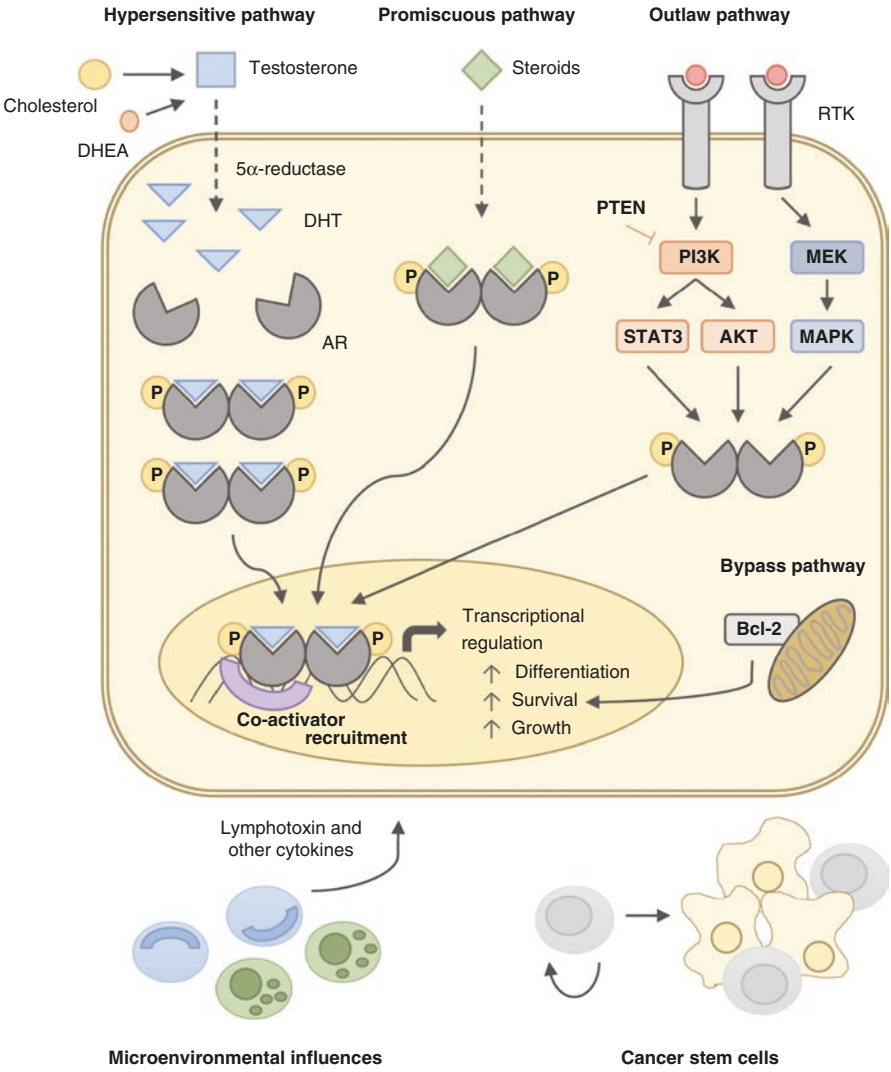

Fig. 18.2 Mechanisms of CRPC development. AR signalling can be activated via low levels of DHT (hypersensitive pathway) or nonandrogenic steroids (promiscuous pathway), while multiple signalling cascades, including PI3K and MAPK, stimulate and allow tumor cells to survive without androgens (outlaw pathway). In the absence of AR, survival can be enhanced through cell-intrinsic pathways, such as loss of PTEN or upregulation of anti-apoptotic Bcl-2 proteins (bypass pathway), as well as pro-growth signals from the microenvironment. Prostate cancer stem cells, which are not dependent on canonical androgen receptor signalling for survival, continually resupply the tumor cell population despite therapy. *AR* androgen receptor, *DHEA* dehydroepiandrosterone, *DHT* dihydrotestosterone, *RTK* receptor tyrosine kinase

Hypersensitive Pathway

One way in which CRPC cells circumvent the effects of androgen blockade is by increasing their sensitivity to very low levels of androgens. Prostate cancer cells that employ this mechanism are not, strictly speaking, androgen independent as they still depend on the activity of the AR signalling axis, but they have a lower threshold for androgens.

One potential mechanism to accomplish castration-resistant growth is through increased expression of the AR itself, leading to enhanced ligand-occupied receptor content. A gene-profiling study of isogenic pairs of androgen-sensitive and CRPC xenografts revealed that increased expression of AR is causally associated with castration resistance [12]. As previously discussed, overexpression of the AR can result from alterations in transcription factors, such E2F1 [28]; however, AR gene amplification is the most common mechanism for its overexpression in CRPC. Notably, AR amplification is significantly more prevalent in patients progressing on antiandrogen therapy than those receiving conventional chemotherapies, such a prednisone or docetaxel [48]. This suggests that AR aberrations are selected for during therapy and function to drive a resistance phenotype and CPRC progression.

Increased local production of androgens by prostate cancer cells themselves has been proposed as another mechanism for castration independence. Despite low-level serum androgens resulting from ADT, the intraprostatic concentration of DHT is usually reduced to a lesser extent than circulating testosterone (60–75% reduction) and is sufficient to maintain AR signalling [49]. These sustained levels of intratumoral DHT could result from elevated expression of enzymes converting adrenal androgens (e.g., dehydroepiandrosterone) [50] and cholesterol [51] to DHT, increased back conversion of the inactivated DHT metabolite androstanediol to DHT [52], or intratumoral de novo androgen synthesis by increased expression of enzymes involved in steroidogenesis, such as CYP17A1 [51, 53, 54]. A "back-door pathway" can serve as an alternative synthesis pathway utilizing progesterone as the primary steroidal precursor of DHT, bypassing testosterone as an intermediate altogether [55]. Therefore, the development of CRPC can be attributed to an incomplete blockade of androgen production with conventional ADT.

Promiscuous Pathway

Normally, the AR is activated only by testosterone and DHT; however, missense mutations in the ligand-binding domain can broaden this stringent specificity. As a result, CRPC cells can continue to activate the AR signalling axis and proliferate by using other circulating steroid hormones as substitute androgens.

The most common mutation of the AR in CRPC is a missense mutation in amino acid 877, which is detected in approximately 25% of CRPC patients [56]. This mutation results in the substitution of alanine for threonine at position 877 (T877A) located

in the ligand-binding domain. Molecular studies have demonstrated that hormones such as progestins, estrogens, and antiandrogens illicitly bind to this mutant AR and act as agonists [57]. In addition, a leucine-to-histidine substitution at amino acid 701 (L701H) enhances the ability of AR to bind adrenal corticosteroids, in particular cortisol and cortisone [58]. Recently, an F876L mutation in the AR has been linked to resistance to the clinically utilized second-generation antiandrogen drug enzalutamide [59]. This mutation promotes a switch from antagonist to agonist receptor function upon exposure to enzalutamide allowing for sustained proliferation during treatment.

Modulation of AR co-regulatory complexes has been shown to influence AR promiscuity by reprogramming the AR to new regions in the genome. Notably, many of the AR interacting proteins mutated in CRPC control chromatin and histone modification, including several members of the MLL complex (MLL2, MLL, ASH2L) as well as UTX and ASXL1 [22]. MLL2, which encodes a H3K4 methyltransferase to rearrange chromatin structure from a closed to open state, is most significantly mutated in nearly 10% of CRPC patients [22]. The resultant alteration in chromatin structure redistributes AR binding and promotes a new gene profile. Similarly, recurrent indel mutations in another AR collaborating factor, FOXA1, have a similar influence on AR chromatin accessibility. FOXA1 is mutated in about 3% of prostate cancers [22], which represses androgen signalling and promotes tumor growth. Finally, EZH2, which is overexpressed in metastatic CRPC [41], was found to bind and recruit AR to distinct genomic sites in CRPC [60]. Although the mechanism is not fully understood, the CRPC phenotype is likely mediated at least in part by cooperation between the AR and epigenetic modifiers.

Outlaw Pathway

Activation of AR signalling can occur independent of ligand binding. This can be accomplished through crosstalk with other signalling cascades, such as interleukin (IL)-6, growth factors (including insulin-like growth factor 1, keratinocyte growth factor, and epidermal growth factor (EGF)), human epidermal growth factor receptor 2 (HER2), and the proto-oncogene tyrosine-protein kinase Src. Indeed, IL-6 is elevated in the sera of patients with metastatic CRPC [61], and IL-6 signalling can activate the STAT3 and MAPK pathways to induce AR phosphorylation and activation [62]. Growth factors are also postulated to play a role in the regulation of AR transcriptional activity, particularly under androgen-depleted conditions. Engagement of the HER2 receptor by EGF activates the PI3K-AKT signalling pathway. Activated AKT directly phosphorylates AR at serine 213 and serine 791 to stimulate AR activity in the absence of androgens [63]. Similarly, EGF-dependent signalling activates Src kinase, and the subsequent phosphorylation of AR on tyrosine 534 is sufficient to facilitate androgen-independent growth [64].

Ligand-independent activation of AR signalling can also be achieved through alternative splicing of the AR. AR splice variants (ARVs) with a truncated, variable length ligand-binding domain are isoforms of AR that have been reported in prostate cancer cell lines, CRPC specimens, and metastatic lesions [65, 66]. To date, seven different

ARVs have been described with diverse activities ranging from constitutively active to dominate negative [67]. In particular, expression of the AR-V7 variant is increased upon antiandrogen therapy with abiraterone or enzalutamide [68]. This variant is constitutively active and its transcriptional activity is not regulated by androgens or antiandrogens. Notably, compared to the full-length AR, ARVs activate a distinct pro-proliferative transcriptional program that could confer castration resistance [68].

Bypass Pathway

The abovementioned mechanisms require the presence of the AR and its signalling cascade for the development of CRPC. However, it is also possible that complementary or alternative pathways can be invoked that are capable of bypassing the AR completely. AR activation stimulates androgen-dependent prostate cancer cells to proliferate, and depletion of androgens yields apoptosis. As such, an effective bypass of the AR signalling axis would upregulate parallel pathways that can provide a substitute survival signal, even in the absence of androgens and AR.

Blocking the apoptosis signal would be one such pathway for CRPC cell survival, with BCL2 being an obvious bypass candidate gene. BCL2 has anti-apoptotic function driven by its ability to inhibit caspase activity either by preventing the release of cytochrome c from the mitochondria and/or by sequestering apoptosis-activating factor (APAF1). It is not normally detected in the secretory epithelial cells of the prostate but is frequently overexpressed in CRPC [69]. In support of this mechanism, the emergence of BCL2 expression correlates with progression to CRPC in mouse models of prostate cancer [70].

In addition to BCL2, tumor-suppressor genes could have a similar bypass role in the development of CRPC. As previously discussed, PTEN is frequently inactivated in CRPC [25]. PTEN functions by antagonizing the PI3K-AKT-mTOR signalling axis, which functions as an alternative pathway to enhance cell proliferation and survival. As such, PTEN-null tumors are less dependent on AR signalling and, as such, are capable of proliferating under castrate conditions. The underlying mechanism for the increased cellular proliferation in the context of PTEN deficiency can be explained by unchecked AKT activation, resulting from downregulation of PHLPP, which encodes an enzyme that directly dephosphorylates AKT and protein kinase C [29]. Therefore, PTEN loss and the resultant AKT upregulation might provide alternative stimulatory signals to drive AR-independent cellular survival and growth to contribute to CRPC development.

Microenvironmental Influences

Despite the numerous cell-intrinsic pathways that endow CRPC cells with their remarkable propensity for growth and survival in androgen-depleted conditions, the interaction between the tumor and microenvironment plays an equally important

role in progression of the disease. Overall, the prostate tumor microenvironment is strongly immunosuppressive, creating a "fertile soil" for tumor growth and metastasis. There is a high degree of tolerance to prostate-specific antigens, which impedes antitumor immunity. For example, functionally suppressive CD4+ and CD8+ T regulatory cells and metabolically unresponsive T cells are found in primary prostate tumors [71] and prostate cancer islets [72]. This immunological tolerance may be linked to their expression of the T cell inhibitory checkpoint receptor programmed death-1 (PD-1), as there is a significantly increased frequency of PD-1+ prostate-infiltrating CD8+ T cells in patients with primary, hormone- and radiotherapy-naïve prostate cancer [73]. Interestingly, androgen ablation can mitigate this immunological tolerance and augment immune responses to CRPC tumors by allowing prostate-specific T cells to expand and develop effector function [74]. This is due, in part, to enhanced thymopoiesis following androgen deprivation; in turn, antigen-specific T cell effector and cytotoxic T cell functions are increased in response to prostate cancer-specific antigens [75–77]. In addition, apoptosis of prostate cancer cells following ADT has been shown to trigger an inflammatory response, leading to infiltration of regressing tumors with a myriad of immune cells, including T lymphocytes, B lymphocytes, natural killer cells, and myeloid cells [78]. Activation of IKK-β[beta] (inhibitor of nuclear factor KB kinase subunit β[beta]) in tumor-infiltrating B cells results in the production of lymphotoxin and other cytokines such as IL-6, IL-12, and TNF-α[alpha], which activate IKK-α[alpha] and STAT3 in prostate cancer cells to enable them to survive in the castrated state [78]. Notably, STAT3 is an anti-apoptotic, pro-tumorigenic transcription factor that when activated drives expression of genes central for proliferation, angiogenesis, and epithelial-to-mesenchymal transition [79].

Cancer Stem Cells

The acquisition of genetic or epigenetic alterations in prostate cancer cells or the surrounding microenvironment that promote survival in low-androgen conditions does not capture the entire complexity of CRPC progression. Both prostate cancer cell lines and patient tumors are heterogeneous with subclones of cells exhibiting varying degrees of androgen dependence even before ADT [80, 81]. Therefore, the outgrowth of pre-existing castration-resistant clones under the selective pressure of androgen deprivation likely occurs in parallel with adaptive mechanisms of resistance to drive CRPC progression.

Cancer stem cell theory proposes that cancer cell populations have a hierarchical developmental structure and a small fraction of cells, termed cancer stem cells (CSCs), can drive tumor growth and disease progression, perhaps through therapy resistance and metastasis [82]. Although not necessarily derived from normal tissue stem cells, CSCs share many similar characteristics with normal stem cells, including quiescence, expression of ATP-binding cassette transporters, common cell-surface markers and signal transduction cascades, and self-renewal capacity [83, 84].

These features could confer resistance to cancer therapy; hence, CSCs represent a plausible candidate to survive castrate conditions and reignite tumor growth.

CSCs have been identified in prostate cancer cell lines, xenografts, and patient tissue based on aldehyde dehydrogenase (ALDH) activity [85] and the combination of cell-surface markers such as CD44+, CD133+, and α[alpha]2β[beta]1hi [86]. The self-renewal capacity of CSCs in human prostate cancer has been successfully assessed by the formation of three-dimensional tumor spheroids in culture [80] as well as long-term tumor propagating capacity in mice [87]. Interestingly, all the identified subsets of putative prostate CSCs lack AR expression or have low AR activity [81, 86–88], which suggests that these cells might not be dependent on AR signalling for survival and growth. Indeed, the CSC population is expanded dramatically post-ADT both in mouse models and patient tumors [89, 90]. These cells have been shown to be capable of asymmetric cell division to regenerate a phenotypically mixed tumor, including AR- and PSA-positive cells [87]. Clearly, further studies are required to evaluate the biological characteristics and androgen dependence of prostate CSCs and their role in the genesis of CRPC.

Treatment of Castration-Resistant Prostate Cancer

An increased understanding of the molecular mechanisms that underlie CRPC has expanded the repertoire of therapeutic options (see Table 18.2). While docetaxel-based chemotherapy remains the cornerstone of CRPC treatment, a myriad of new drugs have entered the clinic that are well tolerated and significantly prolong survival in patients with CRPC. These include the taxane cabazitaxel, the CYP17 inhibitor abiraterone, the androgen receptor antagonist enzalutamide, and the vaccine sipuleucel-T. Clinical trials of targeted therapies directed against key biological mechanistic drivers of CRPC, such as metastases and cancer stem cells, are ongoing.

Table 18.2 Therapeutic agents for CRPC

Type of agent	Therapeutic agent	Mechanism of action	Clinical trial status	Therapeutic efficacy
Chemotherapy	Docetaxel	Stabilization of tubulin, induction of cell-cycle arrest, and inhibition of proliferation	FDA approved	Increase in OS (1.9–2.4 months)
	Cabazitaxel	Stabilization of tubulin, induction of cell-cycle arrest, and inhibition of proliferation	FDA approved for patients after failure of docetaxel	Increase in OS (2.4 months)

(continued)

Table 18.2 (continued)

Type of agent	Therapeutic agent	Mechanism of action	Clinical trial status	Therapeutic efficacy
AR-pathway targeting	Abiraterone acetate	Irreversible inhibition of CYP17 and subsequent androgen synthesis	FDA approved in pre- and post-docetaxel settings	Increase in OS (~4 months), radiographic progression-free survival, and time to PSA progression
	Enzalutamide (MDV3100)	AR antagonist preventing nuclear translocation and DNA binding	FDA approved in the pre- and post-docetaxel setting	Increase in OS (4.8 months), radiographic progression-free survival, and time to PSA progression
Immunotherapy	Sipuleucel-T (Provenge)	Enhancement of antigen-presenting cells to induce cytotoxic response against prostate cancer cells	FDA approved	Increase in OS but not progression-free survival
	PSA-TRICOM (PROSTVAC)	Poxviral-based PSA-targeting vaccine	Phase III in combination with GM-CSF or docetaxel	Results pending
	Ipilimumab	Monoclonal antibody that blocks CTLA4, a negative regulator of T cell activation	Phase III in combination with GVAX or PSA-TRICOM	Results pending
Bone targeting	Radium-223	Delivery of radiation to areas of high bone turnover	Phase III in comparison with placebo	Increase in OS (3.6 months) and decrease in time to first skeletal related event
CSC targeting	GDC-0449	Binds to and inhibits smoothened receptor to antagonize hedgehog signalling	Phase I/II in combination with hormone therapy	Results pending
	GSK2816126	Inhibition of EZH2	Phase I	Results pending
	JQ1	Inhibition of Myc	Phase I	Results pending

AR androgen receptor, *CSC* cancer stem cell, *CTLA-4* cytotoxic T lymphocyte associated protein 4, *FDA* US Food and Drug Administration, *GM-CSF* granulocyte macrophage colony-stimulating factor, *OS* overall survival, *PSA* prostate-specific antigen

Chemotherapy

Docetaxel-based chemotherapy is the current first-line standard-of-care treatment for patients with detectable metastatic CRPC, based largely on two pivotal trials TAX327 and SWOG 9916. In the TAX327 trial, patients treated with docetaxel plus prednisone (a corticosteroid that suppresses adrenal androgen production) demonstrated a statistically significant improvement in overall survival of 2.4 months compared with the de facto chemotherapy mitoxantrone plus prednisone [91]. A similar endpoint was achieved in the SWOG 9916 trial, which combined docetaxel with estramustine [92].

Most patients with metastatic CRPC experience disease progression during or following docetaxel therapy, and, until recently, no life-prolonging second-line treatment options were available. All this changed in 2010, when the FDA approved cabazitaxel for patients with metastatic CRPC previously treated with docetaxel. Cabazitaxel has the ability to overcome taxane resistance largely due to its low affinity for P-glycoprotein, a drug efflux pump that is overexpressed in taxane-resistant tumor cells [93]. The approval of cabazitaxel was based on data from the TROPIC study, which showed statistically significant and clinically relevant improvement in overall survival (15.1 months versus 12.7 months) in men treated with cabazitaxel plus prednisone compared with mitoxantrone plus prednisone [94].

AR-Pathway Targeting Therapy

Given that AR signalling remains active in patients with CRPC, targeting the androgen receptor axis continues to have an important role in the treatment of CRPC, with abiraterone acetate and enzalutamide being the most exciting developments. Abiraterone acetate is a highly selective irreversible inhibitor of CYP17, a critical enzyme for androgen biosynthesis in the adrenal gland and possibly also within prostate tumors [95]. In the phase III trial COU-AA-301, abiraterone acetate indicated superiority over placebo, demonstrating a 4-month gain in median overall survival from 12 to 16 months in the post-docetaxel setting [96]. Both groups also received prednisone because CYP17 inhibition has the potential to cause life-threatening adrenal insufficiency. In light of the positive results, in 2011, abiraterone acetate was approved as a second-line treatment for patients with CRPC.

A second study COU-AA-302 was designed to evaluate the effects of abiraterone acetate versus placebo in patients with asymptomatic CRPC without previous chemotherapy [97]. More deaths were observed in the prednisone arm alone than in the abiraterone acetate (34% versus 27%) prompting the recommendation that patients in the placebo arm switch to abiraterone acetate treatment. Radiographic progression-free survival was significantly better for patients who received abiraterone acetate, at a median of 16.5 months compared with 8.3 months for the placebo group. Based on the results from this trial, the use of abiraterone acetate with prednisone for treating chemotherapy-naïve CRPC was approved in 2012.

Enzalutamide (formally MDV3100) is a second-generation potent competitive inhibitor of the AR that impairs nuclear translocation and prevents DNA binding. In contrast to previous generation AR antagonists, such a bicalutamide, enzalutamide binds to the AR with greater relative affinity and has no agonistic activity at the wild-type receptor. It also induces shrinkage of CRPC tumor xenografts, whereas other conventional AR antagonists can only retard growth [98]. Enzalutamide was approved by the FDA in 2012 based on results from the AFFIRM study, which compared enzalutamide and placebo-treated patients that had progressed on docetaxel chemotherapy. Enzalutamide demonstrated a significant advantage over placebo in median overall survival of 4.8 months and all secondary endpoints, including radiographic progression-free survival and time to PSA progression [99]. The PREVAIL trial, which was set up to evaluate the benefit of enzalutamide in the pre-chemotherapy setting, revealed that enzalutamide not only delays the initiation of chemotherapy but also decreases the risk of radiographic progression and death [100]. Following this, in 2014, the FDA approved enzalutamide as a first-line therapy for use in chemotherapy naïve CRPC patients.

Immunotherapy

Although potent antiandrogen drugs improve CPRC patient survival, resistance is inevitable, leaving few other treatment options for men with this metastatic and lethal form of prostate cancer. The promise of improved survival with immunotherapy in prostate cancer is alluring; in 2014 there were over 2500 patients enrolled in global immunotherapy trials [101]. This is not surprising as prostate cancer is the only solid tumor type for which a vaccine is approved for the treatment of late-stage disease. In 2010 the FDA approved the autologous dendritic cell vaccine, sipuleucel-T (Provenge), for patients with metastatic CRPC [102, 103], and the poxviral vector-based vaccine, PSA-TRICOM (Prostvac-VF), is in late-stage clinical development [104]. Vaccines are a cornerstone of prostate cancer immunotherapies due to the well-characterized tumor associated antigens expressed uniquely by prostate tumor cells, including prostatic acid phosphatase (PAP—the target of Provenge) and PSA (the target of Prostvac-VF) [105]. These vaccines, therefore, are designed to facilitate the presentation of PAP or PSA antigenic peptides by dendritic cells to T cells in order to initiate antigen-specific killing of prostate cancer. Interestingly, although both vaccines have not shown improvements in improved time to radiographic or PSA progression in metastatic CRPC, they have resulted in significantly increased overall survival [102], leading to the approval of Provenge and Phase III clinical trials for Prostvac-VF. Importantly, retrospective analysis of the Provenge IMPACT trial showed that patients that most benefited from vaccination had baseline PSA values in the lowest quartile of those on study [106]. This lower burden of disease may allow for time for antigenic spread to occur as tumor cells are killed by vaccine-induced antigen-specific T cells, which has been documented in Provenge as well as Prostvac-VF-treated patients [107, 108], suggesting that Prostvac-VF may also prove most beneficial in patients with lower PSA.

The other major immunotherapeutic intervention for prostate cancer is the use of antibody-based therapies directed against T cell-inhibiting or "checkpoint" molecules like CTLA-4 and PD-1/PD-L1. These drugs enhance T cell antitumor responses by blocking inhibitory molecules like CTLA-4 or PD-1 on T cells from interacting with their ligands (CD80/86 or PD-L1 and 2, respectively), which are upregulated by tumors to evade T cell killing and/or by innate immune cells. While there is sound reason for the excitement over the durable responses after CTLA-4 blockade with ipilimumab and PD-1 pathway targeting agents in many cancers [109], neither have significantly improved survival in trials of CRPC patients [110–112]. However, ipilimumab treatment did show a trend to improved survival in metastatic CRPC, especially in patients with indolent disease features [111] and in an $n = 1$ report, a patient with metastatic CRPC showed a complete response after one dose of ipilimumab [113]. In addition, patients progressing after enzalutamide treatment show upregulation of PD-L1 on circulating immune cells, suggesting the presence of this immunotherapeutic target in this patient subset [114]. These more promising results suggest that choice of appropriate sequencing and combination therapies with checkpoint inhibitors may be the key to their success in improving CRPC outcomes.

At each stage of prostate cancer, from localized disease to advanced metastatic CRPC, there is strong rationale to integrate immunotherapies into the treatment landscape. Importantly, although immunotherapies were first tested in late-stage CRPC patients, results showing the most benefit from vaccines or ipilimumab in patients with low PSA or less clinically aggressive disease highlight the potential immunotherapies to alter prostate cancer progression much earlier. The many ongoing immunotherapy clinical trials now available to men with localized, castration-sensitive and nonmetastatic CRPC with checkpoint blockade or vaccination underscore this concept. In addition, the potential for synergy between standard-of-care radiation, androgen deprivation, and chemotherapy treatments with immunotherapies should be exploited and is not limited to one particular stage of prostate cancer. As such, vaccines and checkpoint inhibitors will undoubtedly play a major role in not only altering survival outcomes in prostate cancer patients but also how we study the mechanisms of prostate cancer progression.

Bone-Targeting Therapy

Patients with CRPC are particularly vulnerable to developing bone metastasis. This is associated with a significant risk of skeletal complications, such as pathologic fractures, debilitating bone pain, and spinal cord compression. Accordingly, a concerted effort has been made to identify therapeutic strategies that can prevent and/or treat prostate cancer spread to the bone. Denosumab is a human monoclonal antibody that targets the osteoblast-secreted receptor activator of nuclear factor KB ligand (RANKL) and prevents it binding to its receptor (RANK), leading to inhibition of bone loss [115]. It was the first bone-targeted agent able to delay bone

metastasis in patients with nonmetastatic CRPC by 4.2 months compared with placebo [116]. However, no difference in overall survival was found between denosumab and placebo groups.

Radiopharmaceuticals are bone-seeking agents that emit radiation or are conjugated to a radioactive emitter, enabling the preferential delivery of radiation to areas of high bone turnover. Strontium-29 and samarium-153 are FDA approved for palliation of pain caused by bone metastasis and are indicated in patients with multifocal bone metastasis [117]. Notably, radium-223 was the first radiopharmaceutical shown to improve overall survival in patients with symptomatic CRPC (14.9 months versus 11.3 months for placebo treated) [118]. Interestingly, radiopharmaceuticals are well known to increase antitumor immunity. Because of this, multiple trials have shown that combination of samarium-153 with PSA vaccines improves antigen-specific T cell responses [119] and progression-free survival [120], respectively.

Cancer Stem Cell-Targeting Therapy

The notion that CRPCs contain a rare and distinct subpopulation of CSCs that drive tumor regrowth following ADT and/or chemotherapy has gained increased acceptance in recent years [86, 90, 121]. This has led to the proliferation of novel targeted therapies aimed at key molecules and signalling pathways required to sustain CSCs. For example, GDC-0449 (Genentech) is a small-molecule inhibitor that binds to the smoothened receptor to antagonize the hedgehog signalling pathway. In preclinical studies GDC-0449 depleted the CSC population and reduced CRPC xenograft growth [122]. Similarly, inhibition of Myc, a transcription factor with a central function in CSC maintenance, was found to reduce the CSC population and suppress CRPC tumor growth and metastasis in mouse models [123]. While Myc-inhibitor design has been difficult due to the absence of a clear ligand-binding domain, BET inhibitors, such as JQ1, reduce Myc expression in prostate cancer models and have demonstrated astounding therapeutic efficacy in blocking CRPC tumor growth [124]. Finally, EZH2 represents a particularly alluring therapeutic target as it is overexpressed in prostate CSCs, which are addicted to it for growth and survival [125]. Pharmacological inhibition of EZH2 is associated with antitumor activity in mouse models of CRPC, mediated in part by eradication of the CSC population [126]. The EZH2 inhibitors GSK2816126 (GlaxoSmithKline) and E7438 (Epizyme) are currently being assessed in phase I clinical trials.

Ideal use of cancer stem cell-directed therapies will undoubtedly be in combination with other standard-of-care treatments such as antiandrogens, radiation, and chemotherapy. The rationale for combination therapy goes beyond the efficacy of each individual treatment and underscores the heterogeneity and plasticity of CRPC, which is comprised of a mixed population of AR-positive and AR-negative cells. As aforementioned, ADT has marked effects on enhancing the CSC population [89, 90]. Accordingly, targeting putative CSCs using an N-cadherin monoclonal antibody in combination with ADT markedly increased time to treatment failure [127].

In another study, eradicating the CSC population using a PI3K/mTOR inhibitor in combination with docetaxel had an enhanced antitumor effect relative to single-drug treatment [128]. These studies pave the way for designing rational combination therapies to optimize the clinical management and outcomes of patients with CRPC.

For the discussion of the highly aggressive variant of CRPC that presents with clinical features of small cell carcinoma (referred clinically to as neuroendocrine or anaplastic prostate cancer), see Chap. 19.

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