What is the pathophysiology of a hormone-resistant prostate tumour?

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Introduction

Prostate cancer (PCa) is the most frequent cancer in males, and one of the leading causes of death by cancer. Despite majors advances in screening, early detection and local treatment, a large number of patients will progress to an advanced stage and require systemic treatment. Since the seminal work of Charles Huggins, androgen deprivation therapy (ADT), by means of orchiectomy or administration of oestrogens, has been the cornerstone treatment of advanced PCa [1]. Very early, however, Huggins recognised that there were many failures with ADT: "*The first series of patients with prostatic cancer treated by orchiectomy comprised 21 patients with far advanced metastases; only 4 of them survived for more than 12 years. Despite regressions of great magnitude, it is obvious that there were many failures of endocrine therapy to control the disease but; on the whole, the life span had been extended by the novel treatments and there had been a decrease of man-pain hours*" [2]. Indeed, despite inducing a regression of large magnitude, ADT is usually followed by a regrowth of the tumour, a mechanisms known as androgen independence (AIPC), hormone resistance (HRPC), or more recently castration resistance (CRPC).

Very early, two opposing theories have emerged to explain the progression in a low testosterone environment [3]. The first theory, known as the *clonal selection* theory, postulates the existence of pre-existing clones of CRPC cells that escape any hormonal manipulation at any time. The second theory, known as the *adaptive* theory, postulates the existence of subsequent phases of up and down regulation or mutations of important genes that help the cancer cells survive and (re)grow after ADT. Today it is clear that these two theories coexist in PCa patients. In most patients, PCa are heterogeneous polyclonal tumours made of different populations that respond heterogeneously to ADT. Here, we will review the main molecular mechanisms involved in these two predominant pathways allowing to adapt to a low testosterone environment.

Physiological bases of androgen dependence of normal and cancerous epithelial cells

The prostate is a sexual accessory gland involved in the production of part of the semen. The growth and maturation of the normal prostate is dependent on the secretion of androgens by the testis.

The androgen receptor (AR)

The regulatory effect of androgens is mediated through the androgen receptor (AR), a member of the nuclear steroid hormone receptor super family. The AR gene is located on the X chromosome (Xq11–12), spanning approximately 180 kb of DNA with eight exons (exons 1–8). As the other nuclear steroid hormone receptors, it comprises 4 domains: a N-terminal domain (NTD), a highly conserved DNA-binding domain (DBD), a short hinge region and a moderately conserved C-terminal domain, the ligand binding domain (LBD) [4]. The NTD is the main transcriptional and co-activator binding domain. The DBD is a classical two zinc-finger motif allowing recognition and binding to specific DNA sequences known as Androgen Response Elements (AREs). The hinge region is important for the nuclear translocation of the AR. The LBD consists of the ligand-binding pocket that is created by a specific spatial arrangement of 12 conserved α-helices [4]. Binding of ligand to the AR induces a conformational change allowing the release of the AR from its chaperone protein (e.g. HSP90), change to a more active form, nuclear translocation, homodimer formation and DNA binding. The binding of the AR to AREs allows the recruitment of AR co-activators and/or co-repressors that regulate the transcriptional machinery by direct physical interaction with general transcription factors and RNA polymerase, by facilitating AR/ARE binding and chromatin remodelling, and by changing AR folding and AR subcellular localisation [5].

Regulation of the growth and function of the normal and cancerous prostate epithelium by androgens

The adult prostate develops in two phases. Embryonically, it develops from the male urogenital sinus under the stimulation of androgen. This process is controlled by an epithelial-to-stromal paracrine interaction. A specific subset of smooth muscle cells expressed both the 5α-reductase (5AR) enzyme and the AR. The 5AR transforms the low level of circulating testosterone (T) into dihydrotestosterone (DHT), which stimulates the AR 10 times more than T. DHT binding to the AR stimulates the secretion of paracrine and autocrine growth factors, known as andromedins, such as IGF-1, FGF-7 and -10, and VEGF [6]. These andromedins activate the formation of blood vessels and the maturation of undifferentiated epithelium into a simple stratified glandular circular epithelium composed of a basal layer of cuboidal cells making the epithelial components of the basement membrane separating the epithelial compartment from the stroma. A second layer of columnar secretory–luminal cells forming a glandular lumen is attached to these basal cells. During puberty, serum T rises again and re-stimulates production of andromedins by stromal cells, leading the prostate to mature into its definitive architecture. Interestingly, the prostate does not continue to grow once it has reached its adult size, even though there is continuous stimulation of the stromal cells by circulating levels of androgens. Because of a reciprocal positive feedback loop between stromal and epithelium cells, the epithelial compartment enters a steady-state maintenance phase in which the rate of epithelial proliferation balances the rate of death, such that neither overgrowth nor regression of the gland normally occurs [7].

The normal prostatic epithelium is made of different compartments (Fig. 1), with different levels of AR expression and therefore different sensitivities to androgens [8]. The basal compartment that is directly separated from the stromal compartment by the basal membrane comprises *prostate adult stem cells* (PAS) and *transit-amplifying* (TA) cells. PAS cells are present in very small proportions (<5%), have a very high self-renewal capacity and expresses high levels of α2β1 integrins but no AR. Infrequently, PAS cells will differentiate into transit-amplifying (TA) cells, a progenitor cell type that undergoes a limited number of proliferative replications before terminal differentiation. As is the case with PAS cells, TA cells do not express AR and are dependent for proliferation on andromedins produced by stromal cells. TA cells express p63 and basal markers such as cytokeratins 5 and 14, Jagged-1 and Notch-131. After a limited number of cell divisions, TA cells mature into *intermediate cells* that express cytokeratins 5, 8, 15 and 18, Prostate Stem Cell Antigen (PSCA), and AR mRNA, but not yet AR protein. AR protein will be found in intermediate cells migrating in the *luminal-secretory layer*. In these cells, AR activation promotes differentiation into secretory cells that will produce prostate-specific antigen (PSA) and other secretion products. Interestingly, in these luminal-secretory cells, AR activation also plays an anti-proliferative role, for instance by upregulating expression of cyclin-dependent kinase inhibitors p21 and p27 [6]. Luminal-secretory cells are the terminal stage of maturation of hierarchical expanding stem cell units and thus, these luminal-secretory cells are quantitatively the major epithelial genotype present in the gland, even though they are non-proliferating [6]. These luminal-secretory cells depend on andromedins secreted by stromal cells for survival.

There has been an intense search to identify the specific epithelial cell subtype in which the initial carcinogenic process could initiate [9]. In contrast to normal epithelial cells, AR expression is found in a wider variety of epithelial cancer cells. High-Grade PIN and Prostatic Inflammatory Atrophy (PIA), two common PCa precursors, express high levels of AR [10]. This suggests that early during prostatic carcinogenesis, there is a gain-of-function that converts the AR from a growth suppressor gene to an oncogene during prostatic carcinogenesis. There is indeed good evidence that PCa may originate in intermediate prostatic epithelial cells, presumably derived from the basal transit-amplifying cell population that normally does not express AR or expresses only low levels of it, through enhancement of AR expression. A typical gain-of-function is the fusion by translocation of the promoter of the transmembrane protease serine 2 (*TMPRSS2*) gene, which contains AREs, to selected members of the erythroblast transformation-specific (*ETS*) transcription factor gene family [11]. Besides these malignancy-dependent transcriptional changes, additional molecular changes result in AR becoming part of the protein complex required to 'license' DNA replication in PCa cells [12]. Such gain-of-function ability allows the AR to engage the molecular signaling pathways stimulating the proliferation and survival of these initiated prostatic cells directly [13].

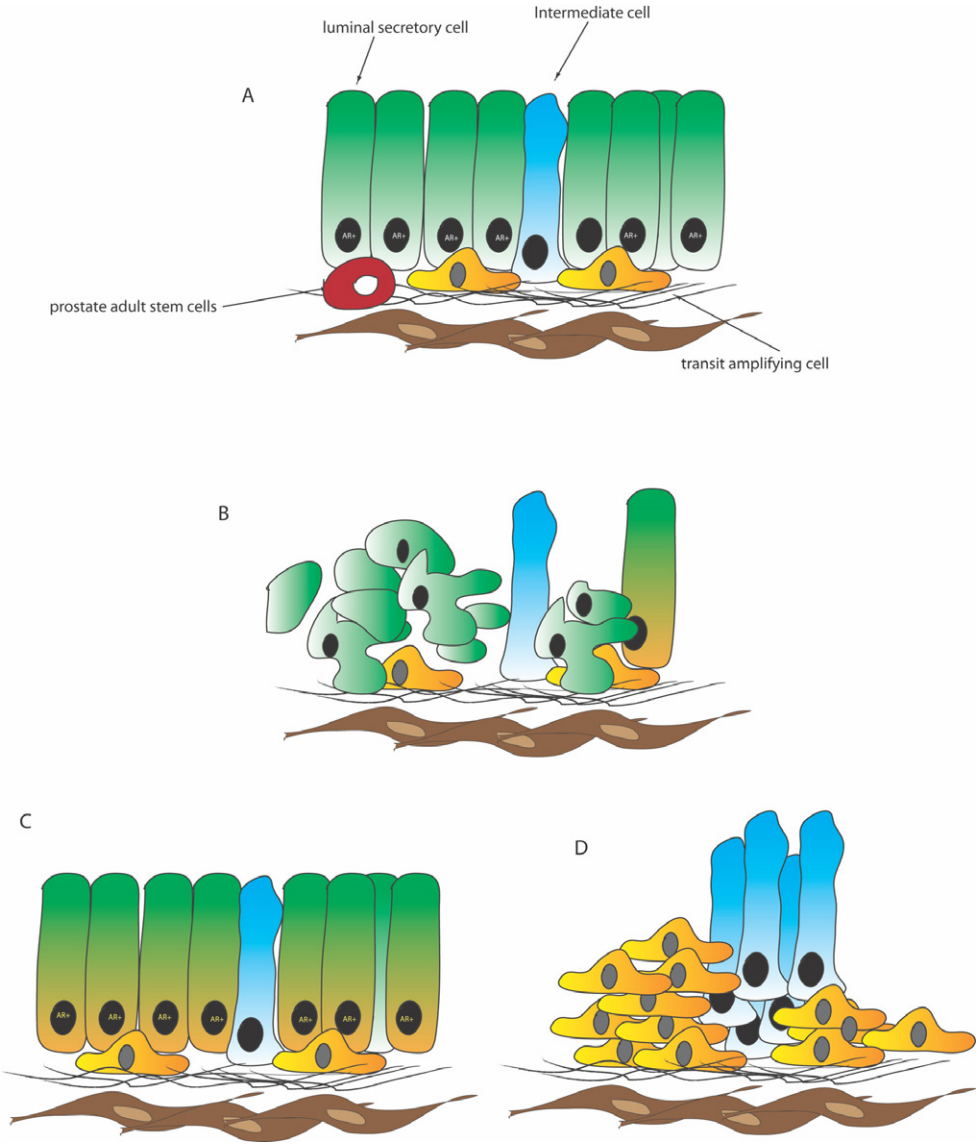

Fig. 1. (A) The prostatic epithelium originates from AR-negative (AR–) prostate adult stem cells (PSA). These generate AR– transit-amplifying (TA) cells and then AR– intermediate cells (I). AR-positive luminal secretory cells are the final adults secreting prostate epithelial cells. (B) Upon suppression of circulating androgen, AR+ luminal cells massively induce apoptosis. (C) The adaptative theory of castration resistance presupposes that some luminal secretory cells adapt their (epi)genetic program to allow growth in absence of androgens. (D) The clonal theory of resistance to castration presupposes that the cancer develops from a pool of AR– cells that is already present in the prostate.

Physiological basis of androgen suppression

In the normal prostate, the rate of prostatic cell proliferation is balanced by an equal rate of prostatic cell death such that neither involution nor overgrowth of the gland normally occurs with time. If an adult male is castrated, serum T rapidly decreases to below a critical value. As a result, the prostate rapidly involutes due to a major loss in the glandular epithelial cells that are androgen dependent and undergo apoptosis following castration. In the ventral prostate of an intact adult rat, glandular cells constitute approximately 80% of the total cells, and approximately 70% of these glandular cells die by 7 days post castration [14]. By 12 to 24 h following castration, the intracellular DHT levels are only 5% of intact control values, leading to changes in nuclear AR function. This mechanism relies on stromal–epithelial interactions since it requires paracrine activity of stromal AR, the apoptosis of glandular cells being initiated when besides DHT the level of andromedins decreases to a critical level [15]. Sev-

eral andromedins have been identified as excellent candidates to modulate the DHT-dependent survival, such as the keratinocyte growth factor (KGF) and transforming growth factor β-1 (TGFβ-1) [16,17]. Once the level of these growth factors decreases to below a critical level within a particular glandular cell, a major epigenetic reprogramming of this cell occurs, resulting in the activation phase of apoptosis. Genes that are regulated include c-myc, c-fos, testosterone repressed prostatic message-2 (TRPM-2) TGFβ-1, H-ras, and calmodulin; genes that are down regulated include secretory protein of the glandular cells, ornithine decarboxylase (ODC), histone-H4, and p53 [18].

Using the human PC-82 prostatic xenograft system, Kyprianou and Isaacs demonstrated that ADT activates similarly the apoptotic pathway in androgen-dependent human PCa cells [19]. Furthermore, they demonstrated that apoptosis of androgen-dependent human PCa cells induced by ADT does not require these cells to be actively proliferating, but that these cells die without leaving G0. Additional studies by the same group demonstrated that ADT does not induce apoptosis in androgen-independent PCa cells because of a defect in the signaling phase [19].

From resistance to castration to resistance to hormone therapy. How do cells progress in a low testosterone environment?

It was clear from the seminal work of Huggins that despite clinical response of great magnitude the effect of ADT was of short duration and that the tumour soon re-grew in absence of circulating androgen. Over the years, this phenomenon has been named hormone resistance or androgen independence, although nowadays the usual term is resistance to castration. In fact these terms overlap two clinical different scenarios. There are patients who primarily failed ADT and progress shortly after initiation of ADT, and others who will progress after a variable duration of ADT but still remain sensitive to further hormonal manipulations.

For many years, researchers have tried to identify the *Golden Path* to castration resistance. What is clear today is that progression to CRPC is a very heterogeneous process between patients and within a patient's own cancer. Progression under low conditions of testosterone invariably involves kaleidoscopic changes in protein and gene regulation. In order to understand these changes, it is important to artificially separate this intricate problem into individual components that, at least from the standpoint of the author, are relevant.

There are two opposing theories explaining the emergence of CRPC (Fig. 1). The most widely accepted model is the "adaptation" model, which supposes that CRPC cells arise through genetic/epigenetic conversion of previously androgen-dependent cells during conditions of ADT. The alternative model is known as the "clonal selection" model, that suggests that emergence of CRPC reflects the proliferation of a previously quiescent population of rare castration-resistant cells within an otherwise androgen-dependent tumour [3].

The clonal theory

The clonal theory suggests that ADT induces selective outgrowth of aggressive hormone-refractory PCa clones expressing distinct cellular and molecular properties not present in parental androgen-dependent cancer cells [20].

This theory is supported by preclinical and clinical data demonstrating that multiple foci of PCa may originate with different sensitivities. Bostwick and colleagues have looked at the distribution and prevalence of allelic imbalance at 6 microsatellite polymorphic markers on chromosomes 7q, 8p, 8q, and 18q in 84 microscopically excised foci of PIN and 95 foci of PCa from 52 radical prostatectomy specimens [21]. They observed significant genetic heterogeneity. Allelic imbalance was observed in at least 1 focus in 11 of 25 cases with multiple foci of PIN (44%) and 20 of 25 cases with multiple PCa foci (80%), therefore suggesting that multiple foci of PIN arise independently within the same prostate. Craft and colleagues have used the human PCa xenograft LAPC-9 to demonstrated that PCa progress to CRPC through two distinct stages, initially escaping dependence on androgen for survival and, subsequently, for growth [22]. They demonstrated that the latter stage of CRPC results from clonal expansion of androgen-independent cells that are present at a frequency of about 1 per 10^5–10^6 androgen-dependent cells. These data support the hypothesis that prostate tumours are comprised of a spectrum of cells with varying androgen sensitivities even before androgen therapy is started. In addition, recent investigations of *TMPRSS2-ETS* rearrangements in end-stage, androgen-independent prostate cancers provide strong evidence that metastatic PCa arises through clonal expansion of a single focus of a primary aggressive clone [23,24].

CPRC cells originate from a pool of androgen-independent cells

CRPC cells may originate from a long-term resident pool of PSA cells and TA cells that are insensitive to

AR pathways [25]. The existence of these prostatic epithelial stem cells is implied by the ability of the adult prostate to undergo repeated cycles of extensive regression in response to ADT, followed by full regeneration following androgen restoration. The cancer stem cell model is consistent with the observed phenotypic heterogeneity found in many tumours, including prostate adenocarcinoma.

This theory could also explain the specific features of neuroendocrine cancers. Neuroendocrine cells are rare cells originating from PAS or intermediate cells that are located in the luminal layer of the epithelium together with the secretory cells [26]. Approximately 10% of prostate cancer tumours show significant neuroendocrine (NE) differentiation, which can be induced by ADT. NE cells produce a number of growth factors, including serotonin and bombesin, which promote the growth of nearby exocrine tumour cells via paracrine mechanisms. NE cells are AR independent and do not induce apoptosis upon ADT [27]. NE differentiation may contribute significantly to the development of CRPC due to its multiple drug-resistance features [28].

Androgen-dependent PCa cells acquire mutations to become androgen-independent

In contrast, a stochastic clonal CRPC model suggests that the phenotypic heterogeneity of tumours is due to variations in the genetic or epigenetic composition of tumour subpopulations, but that these subpopulations are not hierarchically organised and have similar tumour-initiating ability under appropriate circumstances [29]. These CRPC clones may appear from mutations acquired during cancer progression. An exhaustive description of these pathways is out of the scope of this paper, but we will mention three pathways that seem to play a particularly interesting role: *TMPRSS2-ETS* rearrangements, PI3K/AKT/PTEN pathways, and BCL-2.

TMPRSS2-ETS rearrangements

Chromosomal rearrangements are common genetic alterations in solid tumours and haematologic malignancies. In 2005, Tomlins and colleagues discovered in PCa tissues recurrent gene fusions of the 5′ untranslated region of *TMPRSS2* to *ERG* or *ETV1*, two members of the erythroblastosis virus E26 transforming sequence (*ETS*) family of transcription [12]. Initial in vitro studies suggested that the androgen-responsive promoter elements of *TMPRSS2* mediate the overexpression of *ETS* family members in PCa, thereby promoting carcinogenesis. Since then, *TMPRSS2-ETS* rearrangements have been detected in about 20–80% of PCa, usually in relation with stage and grade [11]. Since 2005, more gene fusion variants involving *ETS* family transcriptional factors including *ERG*, *ETV1*, *ETV4*, and *ETV5* have been reported.

Studies of the heterogeneity of TMPRSSE rearrangement provide very good support for the clonal theory of PCa. In their study on 93 multifocal PCa, Mehra and colleagues found *TMPRSS2* rearrangement in 70% of cases, including 63% by deletion (loss of the 3′ *TMPRSS2* signal), 27% by translocation (split of 5′ and 3′ *TMPRSS2* signals), and 10% by both mechanisms in different tumour foci [23]. Of the 30 *TMPRSS2* rearrangements, 30% showed concordance in all tumour foci, whereas 70% were discordant in at least one focus, suggesting that multifocal PCa is a heterogeneous group of diseases arising from multiple, independent clonal expansions. The impact of fusion genes in castration resistance and their impact on clinical outcomes have been studied in several trials but remain controversial [30].

PI3K/AKT/PTEN signaling pathway

The phosphatidylinositol 3-kinase (PI3K) pathway has been implicated in CRPC, so that PI3K and AKT inhibitors are currently tested in humans. PI3Ks are involved in the phosphorylation of membrane inositol lipids, mediating cellular signal transduction [31]. Binding of ligands such as IGF-1 to receptor tyrosine kinases (RTKs) results in the activation of PI3K that phosphorylates and activates AKT/PKB kinases. AKT translocates to the cytoplasm and nucleus and activates a series of important downstream targets that are involved in survival, proliferation, cell cycle progression, growth, migration, and angiogenesis [31]. One of the most important downstream proteins is the mTOR complex 1, a serine/threonine kinase that plays a critical role in protein synthesis, angiogenesis, and cell cycle progression. AKT is negatively regulated by tumour suppressor protein PTEN, which dephosphorylates PIP3. Deregulation of the pathway can occur through several mechanisms, including gain of function of the p110α catalytic subunit of PI3K and loss of function of the tumour suppressor PTEN by gene deletion, mutation, microRNA expression, or epigenetic silencing. Several observations suggest that PI3K/AKT/PTEN signaling pathways are playing an important role in CRPC by helping cells to maintain continued proliferation in low-androgen environments. Functional loss of PTEN is associated with increased AKT-1 phosphorylation and appears to be involved in resistance to castration [32]. Homozygous and heterozygous deletions of *PTEN*

have been detected in respectively up to 15% and 39% of metastases [31]. Immunohistochemical studies have indicated a loss of PTEN expression in 79% of CRPC samples [33]. Recently, Holcomb and colleagues have looked at genomic alterations found in primary PCa and corresponding soft tissue castration-resistant metastases using array comparative genomic hybridisation (array CGH), gene expression arrays, and fluorescence in situ hybridisation (FISH) [34]. They found that 86% of CRPC had lost the 10q23.31 region that encompasses *PTEN*. Attard and colleagues have characterised *PTEN* gene status in circulating tumour cells from 89 CRPC patients being treated in phase I/II clinical trials of abiraterone acetate, including 54 chemotherapy-naive and 35 docetaxel-resistant patients. Overall, 30% of the chemo-naive patients and 24% of the chemo-resistant patients had homozygous or heterozygous loss of *PTEN* [35]. Similarly, recent reports have suggested a critical role for the p110β subunit of PI3K in prostate cancer. In a mice model, ablation of p110β prevents the tumorigenesis caused by *PTEN* loss [36].

BCL-2 overexpression

Overexpression of BCL-2, an anti-apoptotic gene, is another proposed AR-bypassing pathway mediating the development of CRPC. BCL-2 is not normally expressed in the secretary prostate epithelial cells, but is expressed in CRPC [37]. Inhibition of BCL-2 with antisense oligonucleotide prevented the emergence of CRPC in xenograft models [37]. IHC studies have demonstrated increased BCL-2 expression in CRPC specimens compared with hormone-naive prostate cancer tissues [38].

The adaption theory and the role of the AR in resistance to castration

The major argument in favour of the "adaptation" model is the evidence of retention of AR signaling in CRPC. Even when PCa progresses to CRPC, AR activation and signaling remains sustained through a variety of mechanisms [39–41]. Notably, castration-resistant tumours express AR as well as AR target genes such as PSA, indicating that pathway activity is intact [42].

Several mechanisms have been proposed to explain the reactivation of an AR-dependent pathway (Fig. 2):

– Amplification of AR gene copy.
– Gain-of-function mutations of AR.

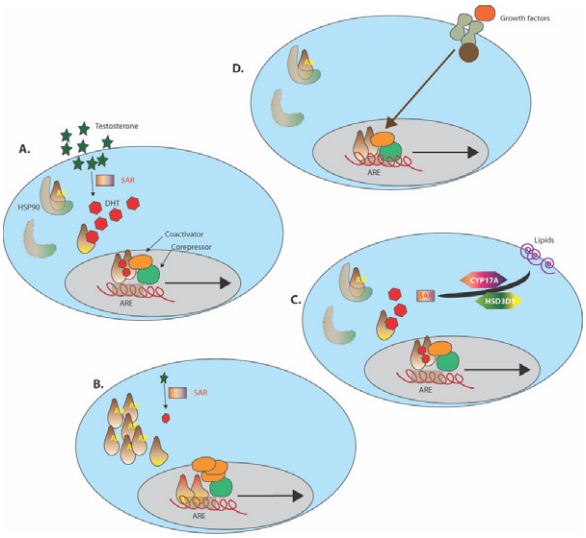

Fig. 2. (A) Serum testosterone (T) freely diffuses in the prostatic cells where it is rapidly converted to more active dihydrotestosterone (DHT). DHT binds to the androgen receptor (AR) and induces release from chaperone protein HSP90, conformational change, homodimerisation and translocation to the nucleus where it binds to the ARE of the DNA to stimulate transcription. This is facilitated by recruitment of co-activators and co-repressors. (B,C,D) Three modes of AR-dependent growth in low testosterone environment. (A) Increased number of AR copies or mutation of the AR leading to auto-activated AR. (B) Intracellular production of androgens through overexpression of enzymes from the steroidogenesis pathways. (D) Direct growth factor activation or the AR independent of androgen binding.

– Deregulation of the AR/co-regulator/co-repressor control.
– Intracellular steroidogenesis.
– Ligand-independent activation of AR.

Increased production of AR

Normal prostate epithelial cells possess one copy of the AR gene. Amplification of AR gene copy number occurs seldom in primary PCa but is found in approximately one-third of CRPC [43–45]. This results in a higher quantity of the wild-type AR protein and therefore in a higher sensitivity and responsiveness to low levels of circulating or intracellular androgens, causing a survival and growth advantage upon castration. Palmberg and colleagues have demonstrated that patients with an AR gene amplification progressing after a first line of LHRH agonist had a 4.5 higher likelihood to respond to a subsequent maximal androgen blockade [46]. In addition, an increased AR level can convert an antiandrogen such as bicalutamide from an AR antagonist to an AR agonist, and this is one of the explanations for the well-known antiandrogen withdrawal response [47]. Increased levels of AR protein

result primarily from AR gene amplification, but also from increased transcription rates, or stabilisation of the mRNA or protein [48,49]. In the aforementioned study by Attard and colleagues on CRPC patients, AR gene copy number was available for 33 patients. Noteworthy, no patient had only one copy of the AR gene in all their CTCs. Eighty-five percent of patients had CTCs harbouring a maximum of 3 copies of AR, 30% had CTCs with a maximum of 4–5 copies, and 45% had CTCs with more than five copies of AR [35]. These observations have motivated the search for second-generation anti-androgens with greater affinity, using cells that overexpress AR, such as RD162 and MDV3100 5 [50].

Gain-of-function mutations of AR

Several mutations have been identified that may confer increased AR protein stability, greater sensitivity to androgens, aberrant responses to antiandrogens or other steroid hormones, ligand-independent activity, or increased recruitment of AR co-activator proteins [51–55]. Because AR mutations occur in a stochastic and heterogeneous manner in different metastases it is extremely difficult to correctly estimate the real frequency of AR mutations in patients. An updated and exhaustive list of AR gene mutations can be found in the McGill Androgen Receptor Gene Mutation Database[1]. AR gene mutations in the LBD may alter ligand binding, leading to AR activation by AR antagonists and other ligands that do not activate wild-type AR, another explanation to the anti-androgen withdrawal syndrome.

In rare cases, mutated AR may lack the entire LBD, leading to an auto-activated mutant that mediates AR signaling independent of any ligands [56]. Such AR splicing variants lacking part of or the entire LBD, *ARΔLBD*, have been generated artificially in vitro to demonstrate that the AR can be constitutively active [57]. These *ARΔLBD* are sufficient to confer ligand-independent and castration-resistant growth. The clinical relevance of these truncated AR variants has been characterised by developing specific technologies [58]. In 2009, Hu and colleagues uncovered seven AR variants lacking the LBD due to splicing of "intronic" cryptic exons to the upstream exons encoding the AR DNA-binding domain [58]. Two of these *ARΔLBD* showed an average 20-fold higher expression in CRPC compared to hormone-naive PCa. More recently, Watson and colleagues demonstrated that *ARΔLBD* increase acutely in response to ADT, are suppressed by testosterone, and, in some models, are coupled to full-length AR mRNA production [59]. Noteworthy, Watson and colleagues also showed that anti-androgens, such as MDV3100, or selective siRNA silencing of full length AR, block the growth of PCa cells, suggesting that the growth-promoting effects of *ARΔLBD* are mediated through full-length AR. These authors hypothesise that the increase in *ARΔLBD* expression in CRPC is an acute response to castration rather than clonal expansion of cells expressing *ARΔLBD*. More recently, Hornberg and colleagues have demonstrated that *ARΔLBD e*xpression was increased in CRPC compared to hormone-naive bone metastases and associated with a particularly poor prognosis [60].

Deregulation of the AR/co-regulator/co-repressor control

Several co-activators, including TIF2, SRC1 and TIP60, have been shown to be overexpressed or accumulated in the nucleus of recurrent PCa specimens [61]. AR overexpression increases the expression of androgen-stimulated co-activators such as MAK, BRCA1, AIB1, or CB. In addition, AR mutants activated by androgens or alternative ligands show differences in co-activator recruitment that will affect receptor activity at the transcriptional level, with subsequent effects on target gene regulation [51].

Intracellular steroidogenesis

One of the most interesting observations from the last five years is the ability of CRPC to synthetise androgen *de novo* or convert adrenal androgens into T and DHT by expressing or up-regulating steroidogenic enzymes including CYP17A1, HSD3B1, HSD17B3, CYP19A1, or UGT2B17 [62–65]. Already in 1985, F. Labrie, the father of maximal androgen blockade, suggested that adrenal androgens could induce AR signaling after intra-prostatic conversion despite low levels of circulating testosterone [66]. In 2005, Titus and colleagues measured tissue level of T and DHT from 18 men with local recurrence during ADT and 18 men with benign prostatic hyperplasia (BPH) receiving no hormonal treatments [65]. T levels were similar in CRPC (3.75 pM/g tissue) and BPH (2.75 pM/g tissue, Wilcoxon two-sided, $P = 0.30$). DHT levels decreased by 91% in CRPC (1.25 pM/g tissue) compared with BPH (13.7 pM/g tissue; Wilcoxon two-sided, $P < 0.0001$) although DHT levels in most specimens of recurrent CRPC were sufficient for AR activation. In 2006, Stanbrough

[1] http://androgendb.mcgill.ca/

and colleagues showed that progression to CRPC was associated by a 5.8-fold increase in AR gene expression but also that multiple genes mediating androgen metabolism (HSD3B2, AKR1C3, SRD5A1, AKR1C2, AKR1C1, and UGT2B15) were overexpressed [64]. Their conclusions supported Labrie's hypothesis of an intracellular conversion of adrenal androgens to T and DHT. It was however Montgomery and coworkers who demonstrated that in addition to converting adrenal androgens, CRPC cells were also capable of synthetising *de novo* androgens from membranes' cholesterol molecules. They showed that median T levels within metastases from CRPC men are approximately three-fold higher than levels within the primary untreated PCa [63]. They also elucidated the mechanism by demonstrating that this increased production of androgens from intracellular precursors was caused by up-regulating the expression of steroidogenic enzymes FASN, CYP17A1, HSD3B1, HSD17B3, CYP19A1, and UGT2B17 and down-regulating SRD5A2 expression ($P < 0.001$ for all) [63]. This pathway was the base of the development of new therapies including abiraterone.

Ligand-independent activation of AR

The activation of different signal transduction pathways in CRPC cells can enhance the activity of the AR or its co-activators in the presence of low levels or even in the absence of androgens. For example, IL-6, KGF, EGF and IGF-1 are overexpressed in CRPC and can stimulate AR transcription in the absence of ligand [67,68]. The MAPK and PI3K/AKT pathways are probably the leading pathways, which regulate the phosphorylation of AR coactivators, such as SRC-1 and TIF2, or the AR protein itself [67–69].

Conclusions

As long as primary ADT remains the main therapeutic option for advanced PCa, CRPC will remain the main challenge to be tackled by physicians. Extensive investment in basic sciences has started to unveil some of the primary mechanisms underlying CRPC acquisition. Already, it appears that it is very unlikely that a unique prevailing pathway will emerge. To treat this disease, we will need to solve the individual heterogeneity and develop tests to allow individualising treatment to specific situations.

Conflict of interest statement

The author has no potential conflict of interest to disclose.

References

1 Huggins C, Hodges C. Studies on prostate cancer. I. The effect of castration, of oestrogen and of androgen injection on serum phosphatases in metastatic carcinoma of the prostate. *Cancer Res* 1941;**1**:293.
2 Huggins C. Endocrine-induced regression of cancers. *Cancer Res* 1967;**27**(11):1925–30.
3 Isaacs JT, Coffey DS. Adaptation versus selection as the mechanism responsible for the relapse of prostatic cancer to androgen ablation therapy as studied in the Dunning R-3327-H adenocarcinoma. *Cancer Res* 1981;**41**(12 Pt 1):5070–5.
4 Gelmann EP. Molecular biology of the androgen receptor. *J Clin Oncol* 2002;**20**(13):3001–15.
5 Heinlein CA, Chang C. Androgen receptor (AR) coregulators: an overview. *Endocrine Rev* 2002;**23**(2):175–200.
6 Litvinov IV, De Marzo AM, Isaacs JT. Is the Achilles' heel for prostate cancer therapy a gain of function in androgen receptor signaling? *J Clin Endocrinol Metab* 2003;**88**(7):2972–82.
7 Berges RR, Vukanovic J, Epstein JI, et al. Implication of cell kinetic changes during the progression of human prostatic cancer. *Clin Cancer Res* 1995;**1**(5):473–80.
8 Vander Griend D, d'Antonio J, Isaacs J. Hormonal regulation of the prostate. In: Berges R, Tombal B, editors, *Androgens and Prostate Cancer*. Lier, Belgium: ISMAR Healthcare; 2009, pp. 11–34.
9 Wicha MS, Liu S, Dontu G. Cancer stem cells: an old idea – a paradigm shift. *Cancer Res* 2006;**66**(4):1883–90; discussion: 1895–6.
10 De Marzo AM, Putzi MJ, Nelson WG. New concepts in the pathology of prostatic epithelial carcinogenesis. *Urology* 2001; **57**(4 Suppl 1):103–14.
11 Tomlins SA, Bjartell A, Chinnaiyan AM, et al. ETS gene fusions in prostate cancer: from discovery to daily clinical practice. *Eur Urol* 2009;**56**(2):275–86.
12 Tomlins SA, Rhodes DR, Perner S, et al. Recurrent fusion of TMPRSS2 and ETS transcription factor genes in prostate cancer. *Science* 2005;**310**(5748):644–8.
13 Shen MM, Abate-Shen C. Molecular genetics of prostate cancer: new prospects for old challenges. *Genes Dev* 2010;**24**(18):1967–2000.
14 Kyprianou N, Isaacs JT. Activation of programmed cell death in the rat ventral prostate after castration. *Endocrinology* 1988; **122**(2):552–62.
15 Abate-Shen C, Shen MM. Molecular genetics of prostate cancer. *Genes Dev* 2000;**14**(19):2410–34.
16 Kyprianou N, Isaacs JT. Expression of transforming growth factor-beta in the rat ventral prostate during castration-induced programmed cell death. *Mol Endocrinol* 1989;**3**(10):1515–22.
17 Yan G, Fukabori Y, Nikolaropoulos S, Wang F, McKeehan WL. Heparin-binding keratinocyte growth factor is a candidate stromal-to-epithelial-cell andromedin. *Mol Endocrinol* 1992; **6**(12):2123–8.
18 Denmeade SR, Tombal B, Isaacs JT. Apoptotic pathways in prostate cancer. In: Mattson MP, Estus S, Rangnekar VM, editors, *Programmed cell death*, Vol. II. Amsterdam: Elsevier; 2001, pp. 23–54.

19 Kyprianou N, Isaacs JT. "Thymineless" death in androgen-independent prostatic cancer cells. *Biochem Biophys Res Commun* 1989;**165**(1):73–81.

20 Tso CL, McBride WH, Sun J, et al. Androgen deprivation induces selective outgrowth of aggressive hormone-refractory prostate cancer clones expressing distinct cellular and molecular properties not present in parental androgen-dependent cancer cells. *Cancer J* 2000;**6**(4):220–33.

21 Bostwick DG, Shan A, Qian J, et al. Independent origin of multiple foci of prostatic intraepithelial neoplasia: comparison with matched foci of prostate carcinoma. *Cancer* 1998;**83**(9): 1995–2002.

22 Craft N, Chhor C, Tran C, et al. Evidence for clonal outgrowth of androgen-independent prostate cancer cells from androgen-dependent tumors through a two-step process. *Cancer Res* 1999; **59**(19):5030–6.

23 Mehra R, Tomlins SA, Yu J, et al. Characterization of TMPRSS2-ETS gene aberrations in androgen-independent metastatic prostate cancer. *Cancer Res* 2008;**68**(10):3584–90.

24 Shah RB, Mehra R, Chinnaiyan AM, et al. Androgen-independent prostate cancer is a heterogeneous group of diseases: lessons from a rapid autopsy program. *Cancer Res* 2004;**64**(24):9209–16.

25 Isaacs J. Control of cell proliferation and cell death in the normal and neoplastic prostate: A stem cell model. In: Rodgers CH, Coffey DS, Cunha G, editors, *Benign prostatic hyperplasia*. Washington, DC: Department of Health and Human Services; 1985, pp. 85–94.

26 Abrahamsson PA. Neuroendocrine cells in tumour growth of the prostate. *Endocr Relat Cancer* 1999;**6**(4):503–19.

27 Fixemer T, Remberger K, Bonkhoff H. Apoptosis resistance of neuroendocrine phenotypes in prostatic adenocarcinoma. *Prostate* 2002;**53**(2):118–23.

28 Cindolo L, Cantile M, Vacherot F, Terry S, de la Taille A. Neuroendocrine differentiation in prostate cancer: from lab to bedside. *Urol Int* 2007;**79**(4):287–96.

29 Shackleton M, Quintana E, Fearon ER, Morrison SJ. Heterogeneity in cancer: cancer stem cells versus clonal evolution. *Cell* 2009;**138**(5):822–9.

30 Huang W, Waknitz M. ETS gene fusions and prostate cancer. *Am J Trans Res* 2009;**1**(4):341–51.

31 Sarker D, Reid AH, Yap TA, de Bono JS. Targeting the PI3K/AKT pathway for the treatment of prostate cancer. *Clin Cancer Res* 2009;**15**(15):4799–805.

32 Mulholland DJ, Dedhar S, Wu H, Nelson CC. PTEN and GSK3beta: key regulators of progression to androgen-independent prostate cancer. *Oncogene* 2006;**25**(3):329–37.

33 Bertram J, Peacock JW, Fazli L, et al. Loss of PTEN is associated with progression to androgen independence. *Prostate* 2006;**66**(9):895–902.

34 Holcomb IN, Young JM, Coleman IM, et al. Comparative analyses of chromosome alterations in soft-tissue metastases within and across patients with castration-resistant prostate cancer. *Cancer Res* 2009;**69**(19):7793–802.

35 Attard G, Swennenhuis JF, Olmos D, et al. Characterization of ERG, AR and PTEN gene status in circulating tumor cells from patients with castration-resistant prostate cancer. *Cancer Res* 2009;**69**(7):2912–8.

36 Jia S, Liu Z, Zhang S, et al. Essential roles of PI(3)K-p110beta in cell growth, metabolism and tumorigenesis. *Nature* 2008; **454**(7205):776–9.

37 Furuya Y, Krajewski S, Epstein JI, Reed JC, Isaacs JT. Expression of bcl-2 and the progression of human and rodent prostatic cancers. *Clin Cancer Res* 1996;**2**(2):389–98.

38 Gleave M, Tolcher A, Miyake H, et al. Progression to androgen independence is delayed by adjuvant treatment with antisense Bcl-2 oligodeoxynucleotides after castration in the LNCaP prostate tumor model. *Clin Cancer Res* 1999;**5**(10):2891–8.

39 Attard G, Cooper CS, de Bono JS. Steroid hormone receptors in prostate cancer: a hard habit to break? *Cancer Cell* 2009;**16**(6): 458–62.

40 Bonkhoff H, Berges R. From pathogenesis to prevention of castration resistant prostate cancer. *Prostate* 2010;**70**(1): 100–12.

41 Taplin ME. Androgen receptor: role and novel therapeutic prospects in prostate cancer. *Expert Rev Anticancer Ther* 2008;**8**(9):1495–508.

42 Gregory CW, Hamil KG, Kim D, et al. Androgen receptor expression in androgen-independent prostate cancer is associated with increased expression of androgen-regulated genes. *Cancer Res* 1998;**58**(24):5718–24.

43 Koivisto P, Kononen J, Palmberg C, et al. Androgen receptor gene amplification: a possible molecular mechanism for androgen deprivation therapy failure in prostate cancer. *Cancer Res* 1997; **57**(2):314–9.

44 Linja MJ, Savinainen KJ, Saramaki OR, Tammela TL, Vessella RL, Visakorpi T. Amplification and overexpression of androgen receptor gene in hormone-refractory prostate cancer. *Cancer Res* 2001;**61**(9):3550–5.

45 Visakorpi T, Hyytinen E, Koivisto P, et al. In vivo amplification of the androgen receptor gene and progression of human prostate cancer. *Nat Genet* 1995;**9**(4):401–6.

46 Palmberg C, Koivisto P, Kakkola L, Tammela TL, Kallioniemi OP, Visakorpi T. Androgen receptor gene amplification at primary progression predicts response to combined androgen blockade as second line therapy for advanced prostate cancer. *J Urol* 2000;**164**(6):1992–5.

47 Chen CD, Welsbie DS, Tran C, et al. Molecular determinants of resistance to antiandrogen therapy. *Nat Med* 2004;**10**(1):33–9.

48 Gregory CW, Johnson RT Jr, Mohler JL, French FS, Wilson EM. Androgen receptor stabilization in recurrent prostate cancer is associated with hypersensitivity to low androgen. *Cancer Res* 2001;**61**(7):2892–8.

49 Shiota M, Yokomizo A, Naito S. Increased androgen receptor transcription: a cause of castration-resistant prostate cancer and a possible therapeutic target. *J Mol Endocrinol* 2011.

50 Tran C, Ouk S, Clegg NJ, et al. Development of a second-generation antiandrogen for treatment of advanced prostate cancer. *Science* 2009;**324**(5928):787–90.

51 Brooke GN, Parker MG, Bevan CL. Mechanisms of androgen receptor activation in advanced prostate cancer: differential co-activator recruitment and gene expression. *Oncogene* 2008;**27**(21):2941–50.

52 Robzyk K, Oen H, Buchanan G, et al. Uncoupling of hormone-dependence from chaperone-dependence in the L701H mutation of the androgen receptor. *Mol Cell Endocrinol* 2007;**268**(1–2): 67–74.

53 Steinkamp MP, O'Mahony OA, Brogley M, et al. Treatment-dependent androgen receptor mutations in prostate cancer exploit multiple mechanisms to evade therapy. *Cancer Res* 2009;**69**(10): 4434–42.

54 Taplin ME, Bubley GJ, Shuster TD, et al. Mutation of the androgen-receptor gene in metastatic androgen-independent prostate cancer. *N Engl J Med* 1995;**332**(21):1393–8.

55 Zhao XY, Malloy PJ, Krishnan AV, et al. Glucocorticoids can promote androgen-independent growth of prostate cancer cells through a mutated androgen receptor. *Nat Med* 2000;**6**(6):703–6.
56 Ceraline J, Cruchant MD, Erdmann E, et al. Constitutive activation of the androgen receptor by a point mutation in the hinge region: a new mechanism for androgen-independent growth in prostate cancer. *Int J Cancer* 2004;**108**(1):152–7.
57 Zhou ZX, Sar M, Simental JA, Lane MV, Wilson EM. A ligand-dependent bipartite nuclear targeting signal in the human androgen receptor. Requirement for the DNA-binding domain and modulation by NH2-terminal and carboxyl-terminal sequences. *J Biol Chem* 1994;**269**(18):13115–23.
58 Hu R, Dunn TA, Wei S, et al. Ligand-independent androgen receptor variants derived from splicing of cryptic exons signify hormone-refractory prostate cancer. *Cancer Res* 2009;**69**(1):16–22.
59 Watson PA, Chen YF, Balbas MD, et al. Constitutively active androgen receptor splice variants expressed in castration-resistant prostate cancer require full-length androgen receptor. *Proc Natl Acad Sci USA* 2010;**107**(39):16759–65.
60 Hornberg E, Ylitalo EB, Crnalic S, et al. Expression of androgen receptor splice variants in prostate cancer bone metastases is associated with castration-resistance and short survival. *PloS ONE* 2011;**6**(4):e19059.
61 Urbanucci A, Waltering KK, Suikki HE, Helenius MA, Visakorpi T. Androgen regulation of the androgen receptor coregulators. *BMC Cancer* 2008;**8**:219.
62 Locke JA, Guns ES, Lubik AA, et al. Androgen levels increase by intratumoral de novo steroidogenesis during progression of castration-resistant prostate cancer. *Cancer Res* 2008;**68**(15):6407–15.
63 Montgomery RB, Mostaghel EA, Vessella R, et al. Maintenance of intratumoral androgens in metastatic prostate cancer: a mechanism for castration-resistant tumor growth. *Cancer Res* 2008;**68**(11):4447–54.
64 Stanbrough M, Bubley GJ, Ross K, et al. Increased expression of genes converting adrenal androgens to testosterone in androgen-independent prostate cancer. *Cancer Res* 2006;**66**(5):2815–25.
65 Titus MA, Schell MJ, Lih FB, Tomer KB, Mohler JL. Testosterone and dihydrotestosterone tissue levels in recurrent prostate cancer. *Clin Cancer Res* 2005;**11**(13):4653–7.
66 Labrie F, Dupont A, Belanger A. Complete androgen blockade for the treatment of prostate cancer. *Important Adv Oncol* 1985: 193–217.
67 Gregory CW, Fei X, Ponguta LA, et al. Epidermal growth factor increases coactivation of the androgen receptor in recurrent prostate cancer. *J Biol Chem* 2004;**279**(8):7119–30.
68 Ueda T, Mawji NR, Bruchovsky N, Sadar MD. Ligand-independent activation of the androgen receptor by interleukin-6 and the role of steroid receptor coactivator-1 in prostate cancer cells. *J Biol Chem* 2002;**277**(41):38087–94.
69 Krueckl SL, Sikes RA, Edlund NM, et al. Increased insulin-like growth factor I receptor expression and signaling are components of androgen-independent progression in a lineage-derived prostate cancer progression model. *Cancer Res* 2004;**64**(23):8620–9.