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Molecular biology of androgen-independent prostate cancer: the role of the androgen receptor pathway

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Abstract Prostate cancer (PC) cells express the androgen receptor (AR) and need the presence of androgens to survive. Androgen suppression is the gold standard first-line therapy for metastatic disease. Almost all PC patients initially respond to hormonal therapy, but most of them gradually develop resistance to castration. There is evidence that these tumours that are considered castration-resistant continue to depend on AR signalling. Several mechanisms that enhance AR signalling in an androgen-depleted environment have been elucidated: (1) AR mutations that allow activation by low androgen levels or by other endogenous steroids, (2) AR amplification and/or overexpression, (3) increased local intracrine synthesis of androgens, (4) changes in AR cofactors and (5) cross-talk with cytokines and growth factors. Today, there are a number of novel agents targeting the AR signalling pathway under development, including more effective antiandrogens; inhibitors of CYP17, inhibitors of HSP90, inhibitors of histone deacetylases and inhibitors of tyrosine kinase inhibitors.

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Introduction

Prostate cancer (PC) is the most frequently diagnosed cancer in Western countries and North America. It is estimated that 1 in every 6 men may develop a PC and 1 in 30 will die of metastatic disease. PC is considered a hormone-dependent tumour, where malignant cells express the androgen receptor (AR) and need the presence of androgens to survive. For that reason, androgen suppression (including LHRH agonists associated or not to antiandrogens) is the gold standard first-line therapy for metastatic disease.

Almost all PC patients initially respond to hormonal therapy, but most of them gradually develop resistance and the disease progresses despite androgen depletion. There is evidence that these tumours that are considered castration-resistant continue to depend on AR signalling, which may remain activated despite castrate levels of androgens and be responsible for tumour progression. It is not well known whether alterations in AR and/or AR signalling are present on the primary tumour and clones harbouring them are selected during PC progression or they appear *de novo* in later stages of the disease. However, the co-existence of both mechanisms may be possible. Among the processes of development of progression under castration, genetic, epigenetic or microenvironment-dependent factors may affect AR signalling. Moreover, the biology of the progressing tumour may be influenced also by specific therapeutic exposure ("therapeutic mediated pressure"), as the AR mutations induced by anti-androgen therapy.

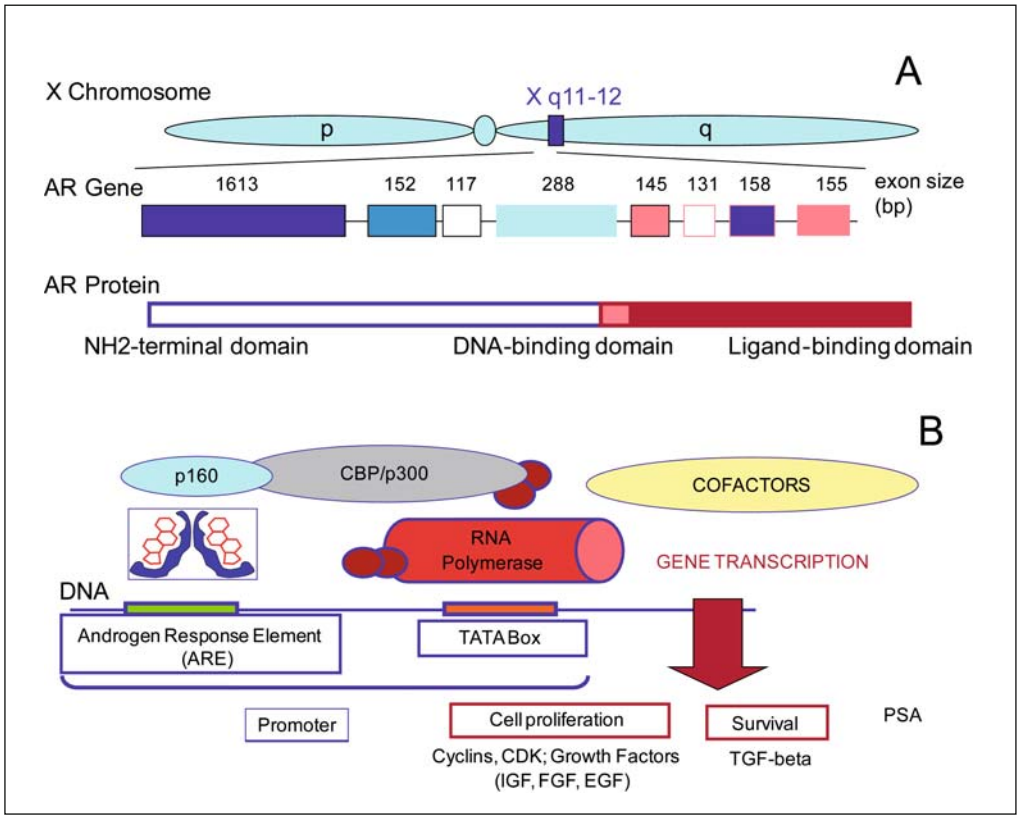

Fig. 1A Human AR gene: structural organisation and protein. **B** The interaction between AR homodimers and ARE induces the attraction of other co-activators, which interact with the genetic transcription machinery. This process causes an increase of transcription of genes involved in cell proliferation and survival

Based on this knowledge, agents that target AR expression represent an attractive treatment option for PC patients with disease progression following castration. In the hormone-independent progression of PC, the activation of survival pathways independent of AR has also been described. In the present review, we focus on the implication of the AR pathway in progressive castrate PC and its therapeutic implications [1–6].

AR structure and function

The AR gene is located in the X chromosome (Xq11-1) and is essential for growth and differentiation of prostate cells, and development and maintenance of male reproductive organs. Loss of function of AR induces lack of development of prostate or PC.

Actions of androgens are mediated by the AR, which is a ligand-dependent transcription factor belonging to the superfamily of nuclear receptors. This family includes receptors for steroid hormones, thyroid hormones, all-trans and 9-cis retinoic acid, 1,25 dihydroxy-vitamin D, ecdysone and peroxisome proliferator-activated receptors.

It contains a DNA-binding domain (DBD) comprised of 2 zinc finger motifs that determine the DNA sequences recognised by the receptors, and a carboxyl terminal hormone (ligand) binding domain. This domain also contains a region, termed activation function 2 (AF-2), which is important for the transcriptional activity of the receptor. The DNA and the ligand domains are linked by a region that contains a nuclear localisation signal. The amino-terminal domain contains a region important for transcriptional activity, termed AF-1, which appears to be the major transactivation domain (Fig. 1).

In the absence of hormone (testosterone or its active metabolite, dihydrotestosterone (DHT)), the receptor is located in the cytoplasm and associated with heat shock protein (HSP) complexes, which maintain the AR in a conformation capable of binding hormone and protect AR from proteolysis. Binding of hormone favours dissociation of the complex, receptor dimerisation and nuclear translocation. In the nucleus, AR binds to specific DNA response elements and recruits a series of co-activator complexes that modify chromatin structure, recruit DNA polymerase II and induce transcription. The genomic actions of AR are modulated by coregulators, coactivators and corepressors, which activate or reduce, respectively, receptor function. A change of the coactivator/corepressor ratio may modify AR sensitivity to hormone binding. Among them, the coactivator p300 bridges the transcriptional machinery of AR and the coactivator family p160 modifies chromatin structure (Fig. 1).

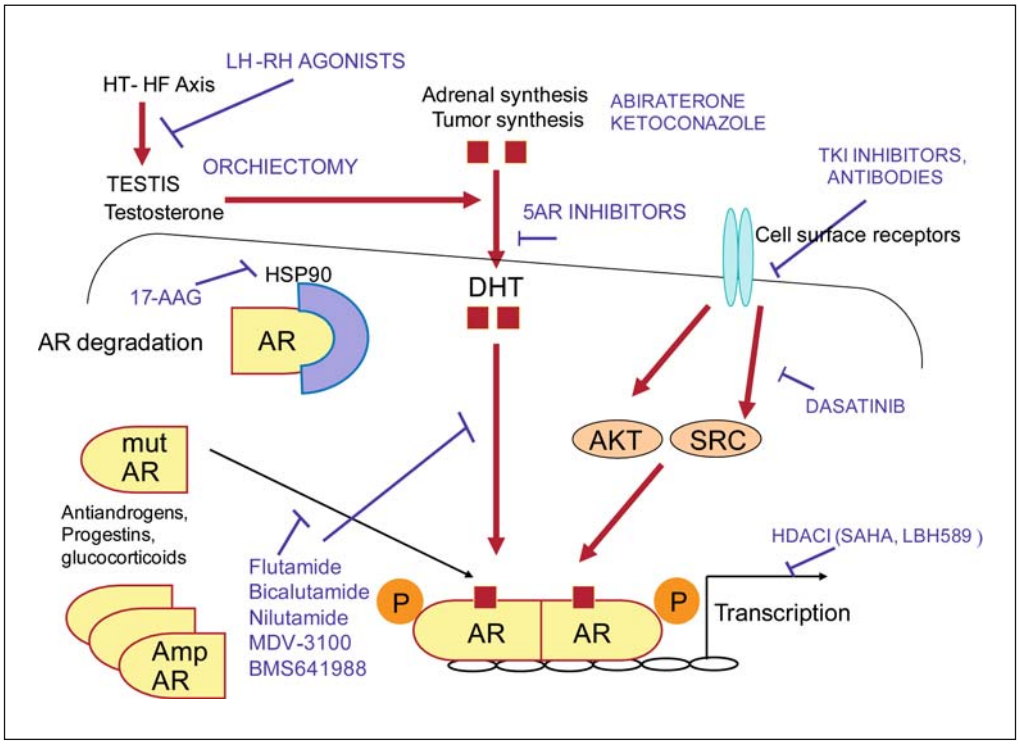

Fig. 2 Mechanisms associated with continued signalling through the AR axis despite castration and options for therapeutical intervention. 5AR, 5-alpha reductase; TKI, tyrosine kinases; mut AR, mutated AR; Amp AR, amplified AR; HDACI, histone deacetylases inhibitors

AR can also mediate a non-genomic signalling, which does not require nuclear translocation and DNA binding: AR is able to activate mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase (ERK) pathways [4–11].

Androgen receptor signalling in castration-resistant tumours

Hormone-independent progression is typically characterised by increases in serum prostatic specific antigen (PSA) levels, an androgen-regulated protein. This suggests that an active AR, in the absence of normal circulating levels of androgens, is responsible for PSA expression. Moreover, up to 90% of PC overexpress the ets oncogene product. The most common mechanism of ets overexpression is the fusion of the ets gene ERG with the 5'-untranslated region highly AR-regulated TMPRSS2 gene, confirming the androgenic induction of this oncogenic pathway [12]. Several mechanisms that enhance AR signalling in an androgen-depleted environment have been elucidated: (1) AR mutations that allow activation by low androgen levels or by other endogenous steroids; (2) AR amplification and/or overexpression; (3) increased local intracrine synthesis of androgens; (4) changes in AR cofactors implicated in ligand-independent activation of AR signalling; and (5) cross-talk with cytokines and growth factors [1–6] (Fig. 2).

AR gene mutations

Mutations in the AR gene were first described in the hormone-dependent human prostate cancer cell line LNCaP, derived from a lymph node metastasis. This cell line harbours a mutation at codon 877 (Thr to Ala). As a consequence of this mutation, AR can be stimulated by hormones other than androgens, such as progesterone, oestrogens and several anti-androgens (promiscuous ligand interaction).

Today, more than 70 single-base substitutions have been described, while only a small proportion have been functionally studied. Most studied mutations affect critical ligand-receptor and protein-receptor interactions, and are supposed to lead to a gain of function, showing an enhancement of transactivating capacities compared to wild-type AR.

AR mutations are present in about 10–20% of patients with PC. The percentage of mutations is more important in hormone-independent disease compared with untreated early-stage PC; and in metastasis than in primary tumours. These findings suggests that wild-type AR signal may be implicated in the early PC progression while AR mutations are a later event that confer a growth advantage to cells during progression. Different mutations have been described in patients receiving anti-androgens (flutamide, bicalutamide) or LH-RH agonists. The mutations can appear as a response to therapy and can have as a consequence a

change of the effect of the anti-androgen, which acquires an agonist function and stimulates AR. This agonist function of the anti-androgen may explain the response to anti-androgen withdrawal that can be observed in up to 30% of patients [13–20].

Currently, new anti-androgens are in clinical development: MDV-3100 binds AR in cells with 10-fold higher affinity than bicalutamide, completely inhibits growth of castration-resistant, impairs AR nuclear translocation and blocks DNA binding.

BMS-641988 is a novel antiandrogen with 20-fold greater affinity for AR than bicalutamide and is effective in xenograft models that have progressed on bicalutamide [4, 21].

AR amplification/overexpression

This mechanism may allow tumour proliferation despite low levels of androgens (hypersensitivity of AR). It has been shown in preclinical experimental models that AR gene is consistently up-regulated during androgen-independent progression and that AR overexpression is necessary and sufficient to induce castrate-independent progression. In *in vitro* studies, AR inhibition in androgen-independent cell lines inhibited tumour growth [22, 23].

In PC patients, AR expression in PC is heterogeneous. In about one-third of patients progressing after androgen suppression, there is an amplification of the AR-gene (detected as an increased copy number by fluorescence in *in situ* hybridisation methods), having as a consequence an over-expression of AR. Moreover, AR over-expression at the mRNA and at the protein level has been observed in the absence of AR amplification.

In tumours with AR over-expression, the receptor may be activated by low levels of androgens. Moreover, amplification of the AR gene in patients that progressed under androgen deprivation is associated with a favourable outcome. Patients with AR overexpression may have more differentiated tumours and/or are more likely to respond to second-line hormone therapies. In addition to AR gene amplification, increased stability and enhanced nuclear localisation have been described in animal models in progression to castration [22, 23].

Synthesis of intra-tumoral androgens

Androgen suppression therapy reduces the levels of circulating androgens by 95%, but the concentration in the prostatic tumour is maintained at levels that can activate AR. Intratumoral androgens may come from an adrenal source, however recent studies support the high relevance of the *de novo* synthesis of androgens within the tumour. Moreover, over-expression of enzymes key to androgen synthesis has been observed in castrate-resistant tumours [24, 25].

In the prostate, conversion of testosterone to its active metabolite, DHT, is catalysed by the enzymes 5alpha-reductase types 1 and 2 (5alphaR1, 5alphaR2). PC cells may increase the synthesis of DHT from testosterone, by increasing the activity of the 5alpha-Rs [26].

A recently published study [27] showed that soft tissue metastases from castration-resistant cancers exhibit elevated testosterone concentrations compared with untreated primary tumours. To determine whether PC metastases were capable of synthesising androgens *de novo*, the authors quantified transcripts encoding enzymes involved in testosterone biosynthesis from cholesterol precursors. Compared with untreated tumours, castration-resistant metastases expressed higher levels of enzymes responsible for the synthesis of adrenal androgens from progesterone (FASN, HSD3B1, HSD3B2, CYP17A1, AKR1C3, HSD17B3) and CYP19A1, which mediates aromatisation of testosterone to oestradiol. They also observed a decrease of the DHT/testosterone ratio, as a consequence of reduction of the expression of 5AR. While DHT is 10-fold more potent than testosterone, high concentrations of this hormone may activate AR at the same level as DHT. They also found high levels of intratumoral androgens in castration-resistant xenografts lacking adrenal CYP17 expression, a finding that supports the *de novo* synthesis of androgens in tumour tissue.

These mechanisms may explain in part the response with inhibitors of the adrenal androgen synthesis, such as ketoconazole and aminogluthetimide. More recently, abiraterone acetate, a potent, selective, small-molecule inhibitor of CYP17, has been clinically tested in metastatic PC cancers progressing after androgen suppression. It has shown a promising clinical activity, with greater than 50% declines in PSA lasting more than 3 months in 57% of patients [28, 29].

Cross-talk with cytokines and growth factors

AR may be activated by ligand-independent mechanisms. The cross-talk with growth factor receptors can transactivate AR, inducing the expression of androgen-response elements in the absence of androgens but not AR.

The HER2 receptor tyrosine kinase is progressively overexpressed in advanced, castrate resistance PC [30]. In experimental systems, forced overexpression of HER2 results in increased AR activity and stability, while pharmacologic inhibition or knockdown of the protein results in growth suppression. In hormone-dependent LNCaP cells, HER-2 inhibition led to impairment of AR-mediated functions, such as androgen-stimulated growth [31]. Heregulin activates HER2 and HER3 and increases androgen-dependent AR transactivation of reporter genes in hormone-independent CWR-R1 cells, and activates downstream signalling, including MAPK and phosphatidylinositol-3 kinase and Akt pathways. Tyrosine phosphorylation of HER2 and HER3, AR transactivation, and cell proliferation induced by heregulin can be inhibited by the EGFR/HER2 dual tyrosine kinase inhibitor lapatinib [32]. Recent reports of lapatinib in castrate-resistant PC have shown clinical activity in these patients [33].

The nuclear factor kappa B (NF-kB) system activation has also been implicated in androgen-independent growth of PC by regulating AR action. NF-kB pathway activation results in increased levels of AR in LNCaP cells. By blocking NF-kB signalling *in vitro*, AR activation is inhibited. In addition, the continuous activation of NF-kB signalling *in vivo* can sustain high levels of nuclear AR, maintaining continued proliferation of the prostatic epithelium [34]. The ubiquitin/26S proteasome pathway induces AR expression by activating NF-kB and promotes AR activity by participating in the assembly of an AR transcription complex [35]. Interleukin 6 (IL-6) and (IL-8), cytokines regulated by NF-kB, have also been shown to activate AR. IL-6 is known to stimulate AR activity and expression of its downstream target genes. IL-6 may cause growth of AR-positive tumours *in vitro* and *in vivo* through activation of the AR, a process that may be inhibited by bicalutamide [36]. IL-8 treatment also increases AR transcriptional activity and a subsequent upregulation of PSA and cyclin-dependent kinase 2 mRNA transcript levels in LNCaP cells. Interleukin-8 signalling promotes androgen-independent proliferation of PC cells via induction of AR expression and activation [37].

Canonical Wnt signalling has also emerged as an important pathway that underlies the initiation of PC and in the establishment of skeletal metastasis. Beta-catenin, the downstream effector of this pathway, can directly interact with AR and modulate its function [38].

In addition to the mechanisms of AR transactivation explained above, the interaction with a number of other growth factor receptors (e.g., EGFR, IGF-1R, IL-6R, BRAF, SRC) can enhance AR signalling and confer castration resistance in preclinical models. These receptors induce downstream activation of critical growth and survival pathways, including the AKT, MAPK and STAT pathways. AKT activation in PC has been associated with loss of PTEN, which occurs in a high percentage of tumours. Inhibition of different pathways is now being clinically tested as a strategy to treat castration-resistant PC [39] (Fig. 2).

Other ways to regulate AR activity

Prior to ligand binding, AR exists in a complex with heat shock protein 90 (Hsp90) and other co-chaperones. The AR–Hsp90 interaction maintains AR in a high-affinity ligand-binding conformation, which is necessary for efficient response to hormone. 17-Allyamino-17-demethoxy geldanamycin (17-AAG) is an inhibitor of the Hsp90 chaperone protein. 17-AAG inhibits AR-positive PC growth, and reduces AR and HER2 expression. Clinical trials with HSP inhibitors are currently ongoing in PC [4, 40].

Histone deacetylases (HDAC) are proteins involved in the regulation of interplay between transcription factors, such as AR and a chromatin state that supports active gene transcription. Several HDAC inhibitors have demonstrated promising anti-tumour activity and direct suppression of AR transcription and are under clinical development in phase I/II trials in castrate-resistant PC (depsipeptide, SAHA and LBH589) [4].

AR expression in PC stem cells

Initial reports characterising PC stem cells suggest that AR is not expressed in this population. However, a recent reported study in the CD44+/24– LNCaP putative stem cell population showed that AR is expressed at the protein level [41]. The stem cell hypothesis of cancer would predict that AR should be expressed in the PC stem cell, since genetic selection for gain-of-function changes in AR should occur at the level of the stem cell population. However, this theory remains to be proved [42].

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