Androgen receptors in prostate cancer

Z Culig\(^1\), H Klocker\(^1\), G Bartsch\(^1\) and A Hobisch\(^1,2\)

\(^1\)Department of Urology, University of Innsbruck, Anichstrasse 35, A-6020 Innsbruck, Austria
\(^2\)Department of Urology, General Hospital Feldkirch, Austria

(Requests for offprints should be addressed to Z Culig; Email: zoran.culig@uibk.ac.at)

Abstract

The androgen receptor (AR), a transcription factor that mediates the action of androgens in target tissues, is expressed in nearly all prostate cancers. Carcinoma of the prostate is the most frequently diagnosed neoplasm in men in industrialized countries. Palliative treatment for non-organ-confined prostate cancer aims to down-regulate the concentration of circulating androgen or to block the transcription activation function of the AR. AR function during endocrine therapy was studied in tumor cells LNCaP subjected to long-term steroid depletion; newly generated sublines could be stimulated by lower concentrations of androgen than parental cells and showed up-regulation of AR expression and activity as well as resistance to apoptosis. Androgenic hormones regulate the expression of key cell cycle regulators, cyclin-dependent kinase 2 and 4, and that of the cell cycle inhibitor p27. Inhibition of AR expression could be achieved by potential chemopreventive agents flufenamic acid, resveratrol, quercetin, polyunsaturated fatty acids and interleukin-1\(\beta\), and by the application of AR antisense oligonucleotides. In the clinical situation, AR gene amplification and point mutations were reported in patients with metastatic disease. These mutations generate receptors which could be activated by other steroid hormones and non-steroidal antiandrogens. In the absence of androgen, the AR could be activated by various growth-promoting (growth factors, epidermal growth factor receptor-related oncogene HER-2/neu) and pleiotropic (protein kinase A activators, interleukin-6) compounds as well as by inducers of differentiation (phenylbutyrate). AR function is modulated by a number of coactivators and corepressors. The three coactivators, TIF-2, SRC-1 and RAC3, are up-regulated in relapsed prostate cancer. New experimental therapies for prostate cancer are aimed to down-regulate AR expression and to overcome difficulties which occur because of the acquisition of agonistic properties of commonly used antiandrogens.

Endocrine-Related Cancer (2002) 9 155–170

Androgen receptor structure

The androgen receptor (AR) is a transcription factor that regulates the expression of genes required for normal male sexual development and maintenance of the function of accessory sexual organs. In the absence of ligand, AR activation is prevented by several heat-shock proteins. The AR is composed of three main parts: well-conserved central DNA- and ligand-binding domains which are separated by a hinge region responsible for the translocation of the AR from cytoplasm to the nucleus and a less conserved N-terminal region, which contains a variable number of polyglutamine (their number varies from 17 to 29) and polyglycine repeats involved in the regulation of transcriptional activity of the AR (Fig. 1). Racial differences in the repeat lengths have been investigated in molecular epidemiological studies. Shorter polyglutamine repeats are associated with an increased transcriptional activity of the AR. N-terminal region residues 141–338 are the main location for the transcription activation function-1. Residues in the ligand-binding domain are also implicated in the regulation of transcription and are designated AF-2. That part of the AR recruits a group of coregulatory proteins, p160 coactivators (e.g. steroid receptor coactivator-1 (SRC-1)) in a hormone-dependent manner (Bevan et al. 1999). However, these coactivators which are up-regulated in prostate carcinoma interact with the glutamine-rich region in the N-terminus independently of androgenic hormones. AR functional activity is greatly determined by interactions between the N-terminal and carboxyl-terminal (ligand-binding domain) domains (Ikonen et al. 1997, He et al. 1999). AR agonists and antagonists can be reliably distinguished on the basis of their ability to enhance the N/C interaction (Kemppainen et al. 1999). Loss of AR function might occur because of the presence of mutations that disrupt the N/C interaction although they do not change AR-binding affinity (Langley et al. 1998, Thompson et al. 2001). AR agonists and antagonists differentially regulate receptor phosphorylation. In general, the phosphorylation levels correlate.
Culig et al.: Androgen receptors in prostate cancer

Figure 1 Androgen receptor (AR) structural organization. The three main regions, ligand-binding domain (LBD) and DNA-binding domain (DBD), and N-terminal region are depicted. Proteins which interact with the AR and modulate its activity are shown.

with the induction of reporter gene activity (Wang et al. 1999).

The DNA-binding domain is organized in two zinc fingers; each of them being composed of four cysteine residues bound to a zinc ion. The AR gene consists of nine exons: the N-terminal region is entirely encoded by exon 1, exons 2 and 3 encode the DNA-binding region and the remaining five exons encode the ligand-binding domain. The DNA-binding domain mediates binding to specific sequences on DNA and is involved in receptor dimerization (Wong et al. 1993). Gross deletions in the ligand-binding domain of the AR generate receptors which are constitutively active (Jenster et al. 1991).

Structurally, the human AR is very similar to the human glucocorticoid or progesterone receptors. The three receptors recognize the same DNA response element. However, differences in hormone-specific action in target tissues occur. For example, the probasin gene is induced by androgenic hormones and not by glucocorticoids. This high affinity for the probasin–androgen response element is determined by the three residues in the DNA-binding domain of the AR (Schoenmakers et al. 2000). There are also differences between the AR, glucocorticoid or progesterone receptors in terms of ligand-independent activation and this issue will be discussed in detail in the present review. Interestingly, glucocorticoid hormones are not capable of stimulating growth of prostate cancer LNCaP cells even after transfection of glucocorticoid receptor cDNA (Cleutjens et al. 1997). In contrast, dexamethasone stimulated the expression of the prostate-specific antigen (PSA) gene in LNCaP sublines generated by stable transfection of glucocorticoid receptor cDNA. These findings point to the complexity of AR functional regulation by androgens and glucocorticoids respectively.

Expression and function of the AR are frequently studied in cell lines or tumor specimens obtained from patients with androgen sensitivity syndromes or prostate cancer.

Prostate cancer tumor biology

Prostate cancer is the most commonly diagnosed neoplasm in men in the Western world. Policies on the detection and treatment of prostate cancer vary in different countries, from consequent screening and radical prostatectomy to watchful waiting. Watchful waiting is an approach in which the treatment is deferred because of the fact that not all prostate cancers become clinically manifested (Schmid et al. 2001). Thus some patients die with their prostate cancer but not because of prostate cancer. It is hoped that application of new technologies in prostate cancer research will allow identification of markers of more aggressive tumors which could be subjected to eradication therapy. Radical surgery and radiotherapy are curative in the early stages of prostate cancer. Hormonal dependence of prostate cancer was first recognized by Huggins & Hodges (1941). They showed that the removal of androgens leads to regression of prostate cancer. This recognition was a basis for therapy for non-organ-confined prostate tumors. Androgen ablation could be performed either surgically by orchiectomy or by administration of gonadotropin hormone-releasing hormone analogues. In addition, AR function could be inhibited by either steroidal (cyproterone acetate) or non-steroidal (hydroxyflutamide and bicalutamide) compounds which prevent the acquisition of the transcriptionally active form of the receptor. One might expect that there is a more efficient inhibition of prostate cancer progression by combination of androgen ablation and blockade of AR activity. This combined androgen blockade could, for example, neutralize the effects of adrenal androgens which are not eliminated by castration. The question as to whether combined androgen withdrawal has a clinical benefit is still controversially discussed. In this context, it is worthwhile noting that in recently published studies such a benefit was not observed (Eisenberger et al. 1998).

Prostate growth is regulated by complex interactions between steroids, peptide growth factors and cytokines, and dysregulation of the expression of these molecules and their receptors occurs, especially in late tumor stages. The proliferation rate of prostate cancer cells is rather low and therefore chemotherapy is of little value in the treatment of advanced prostate cancer (Berges et al. 1995). A characteristic feature of metastatic prostate cancer is the inability of cells to undergo programmed cell death, apoptosis. In this context, it is known that several growth factors (e.g. insulin-like growth
factor-I (IGF-I) and cytokines (interleukin-6 (IL-6)) act as survival factors in carcinoma of the prostate (Chung et al. 2000, Sprenger et al. 2002). In addition to conventional anti-androgen therapy, there have been various attempts to introduce novel experimental treatments for prostate cancer, such as antisense oligonucleotides against the suppressor of apoptosis bcl-2 (Miyake et al. 2000a), testosterone-repressed prostate message-2 (clusterin) (Miyake et al. 2000b), or anti-epidermal growth factor (EGF) antibodies (Ye et al. 1999). Prostate cancer is a heterogeneous neoplasm and it is therefore believed that only combined therapeutic approaches will be beneficial in advanced disease. Prostate cancers preferentially produce metastases in bone and their spread is facilitated by prostate and bone fibroblasts (Gleave et al. 1991).

**Historical aspects of AR research**

There are only a limited number of prostate cancer models available. This fact greatly hampers research on AR expression and function in carcinoma of the prostate. There have been many attempts to establish a reliable primary prostate culture system in which androgenic responsiveness could be studied. For several reasons, it has been difficult to achieve this goal. Some primary epithelial cultures yielded only a limited number of passages and others showed a down-regulated AR expression. For a long time, rat Dunning tumors were used in different experiments including studies on the AR. Dunning tumor sublines show different histological patterns, varying from well-differentiated and relatively slow-growing neoplasms to highly aggressive tumors which metastasize predominantly to lymph nodes and lungs (Isaacs et al. 1986). AR status in Dunning tumor sublines was investigated by Quarmby and associates (1990). In general, AR expression decreases in cell lines which show metastatic properties. The same phenomenon was seen in the two human cell lines derived from metastatic lesions, PC-3 and DU-145, in which AR expression is very low or undetectable respectively (Tilley et al. 1990). Early studies on AR in human prostate tissue were carried out using radioligand binding assays (Gorelic et al. 1987). Because of the aforementioned prostate tumor tissue heterogeneity, the use of AR-binding assays as a prognostic factor has not been established. In this respect, prostate cancers differ from breast tumors in which measurements of estrogen and progesterone receptors have been accepted for diagnostic procedures.

Considerable progress in investigations on AR expression was achieved after the AR cDNA sequence was published (Chang et al. 1988, Trapman et al. 1988, Tilley et al. 1989). A number of monoclonal and polyclonal antibodies have been generated and used for studies on tumor material. In the early 1990s, it was demonstrated that the AR is expressed in relapsed prostate cancer and that its expression, as determined on semiquantitative evaluation, does not correlate with time to progression after endocrine therapy (Sadi et al. 1991, Van der Kwast et al. 1991). Those findings were confirmed in prostate cancer metastases obtained from patients before and after endocrine treatment (Hobisch et al. 1995, 1996).

**AR expression and activity during endocrine treatment**

It is obvious that either androgen ablation and/or application of AR antagonists lead to a reduction of tumor volume and an improvement in symptoms in most of the prostate cancer patients. It was assumed that alterations which occur during long-term endocrine treatment would be better understood if prostate cancer cells undergo prolonged androgen ablation in vitro. Long-term steroid depletion was performed in human prostate cancer cells LNCaP which are the most frequently used model in prostate cancer research (Kokontis et al. 1994, Culig et al. 1999, Gao et al. 1999a). An early event after steroid hormone withdrawal of LNCaP cells was a retardation of cellular proliferation which was followed by activation of various adaptation mechanisms. There are many similarities between sublines of LNCaP cells generated in different laboratories after long-term androgen ablation. A characteristic feature of those cells is recovery in the basal proliferation rate and an increased sensitivity to low doses of androgens. The typical growth curve of parental LNCaP cells is biphasic; low doses of androgen stimulate proliferation whereas higher concentrations cause a progressive decline in cell growth (Lee et al. 1995). In contrast, LNCaP secretory function is enhanced by androgenic hormones in a dose-dependent manner. However, this regulation is still only partially understood; it has been proposed that the proliferation is mediated by IL-6 and inhibition by transforming growth factor-β (TGF-β) (Kim et al. 1996, Okamoto et al. 1997a). Both regulatory mechanisms are, however, a matter of debate; for IL-6 both stimulatory and inhibitory effects on LNCaP growth have been reported (Degeorges et al. 1996, Giri et al. 2001) and contrasting results on the expression of TGF-β receptors have been published (Guo & Kyprianou 1998). The growth of a subline of LNCaP cells developed in the authors’ laboratory was stimulated by the non-steroidal antiandrogen bicalutamide which also enhanced transcriptional activity of the AR (Culig et al. 1999) (Table 1). A similar switch of bicalutamide from antagonist to agonist was recently reported after long-term treatment with tumor necrosis factor-α (Harada et al. 2001). AR expression and activity increase after long-term androgen ablation and this adaptation mechanism might be very significant in the clinical situation. Although AR activity is higher in long-term steroid-deprived than in parental cells, the AR-regulated PSA gene is down-regulated (Gao et al. 1999a) (Fig. 2). This suggests that there is a cellular dedifferentiation during prolonged steroid depletion. Clearly, it is difficult to generalize all these findings which were obtained with one prostate...
cancer cell line that expresses a mutated AR. A new AR-positive cell line, MDA PCA 2a, was derived from a prostate cancer bone metastasis (Navone et al. 1997). However, that AR contains two point mutations whose features will be discussed later. It should be kept in mind that long-term steroid-deprived cells become resistant to induction of apoptosis by retinoic acid, taxol and adriamycin (Gao et al. 1999a).

Recent research has improved the understanding of the mechanisms by which androgenic hormones regulate proliferation and survival. Transition through the G1 phase of the cell cycle is governed by cyclin-dependent kinases (cdk) 4/6-cyclin D complexes and by cdk2-cyclin E complexes which peak at the G1 to S transition. Androgenic up-regulation of cdk 2 and 4 has been described at mRNA and protein levels (Lu et al. 1997). The androgen-independent cell cycle subline with AR antisense oligonucleotides led to re-established expression of the AR and its target gene PSA. Such inhibitory effects of IL-1β were observed in experiments with monocyte-conditioned media. Conditioned media caused a reduction of the proliferation of LNCaP cells and down-regulation of the AR protein (Culig et al. 1998). These effects were abolished when the media were pretreated with a neutralizing anti-IL-1β antibody. Similar to monocyte-conditioned media, those obtained from cultured activated T-lymphocytes inhibited LNCaP proliferation and AR expression (Hsieh et al. 1995). However, compounds responsible for these effects were not identified. Polysaturated fatty acids, such as docosahexanoic acid and eicosapentanoic acid, inhibit AR activity and could be considered for chemoprevention trials (Chung et al. 2001). Other potential chemopreventive agents, the anti-inflammatory agent flufenamic acid, the red wine compound resveratrol, and the natural flavonoid quercetin, cause down-regulation of AR expression.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Cell line (fold induction of CAT activity over basal level ± S.D.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LNCaP</td>
</tr>
<tr>
<td>R1881 (0.01 nM)</td>
<td>1.2 ± 0.1</td>
</tr>
<tr>
<td>R1881 (0.1 nM)</td>
<td>3.5 ± 0.5</td>
</tr>
<tr>
<td>R1881 (1 nM)</td>
<td>33.2 ± 2.2</td>
</tr>
<tr>
<td>OHF (100 nM)</td>
<td>2.5 ± 0.1</td>
</tr>
<tr>
<td>OHF (1 µM)</td>
<td>3.8 ± 0.5</td>
</tr>
<tr>
<td>OHF (10 µM)</td>
<td>4.5 ± 0.3</td>
</tr>
<tr>
<td>R1881 (1 nM) + OHF (1 µM)</td>
<td>31.6 ± 2.2</td>
</tr>
<tr>
<td>Bic (100 nM)</td>
<td>0.9 ± 0.1</td>
</tr>
<tr>
<td>Bic (1 µM)</td>
<td>0.9 ± 0.2</td>
</tr>
<tr>
<td>Bic (10 µM)</td>
<td>1.1 ± 0.1</td>
</tr>
<tr>
<td>R1881 (1 nM) + Bic (1 µM)</td>
<td>3.2 ± 0.6</td>
</tr>
</tbody>
</table>

OHF, hydroxyflutamide; Bic, bicalutamide.

The AR is implicated in the up-regulation of the stromal keratinocyte growth factor (KGF) which binds to the epithelial receptor and is therefore considered a mediator of androgen action (Yan et al. 1992). Similar properties were reported for the related fibroblast growth factor-10 (Lu et al. 1999). Other examples of how androgenic hormones interact with growth factors are up-regulations of the EGF receptor (Scharffman et al. 1988) and vascular endothelial growth factor (Joseph et al. 1997, Levine et al. 1998). Targeted expression of an AR transgene led to the development of focal areas of intraepithelial neoplasia which is considered to be a precursor to prostate cancer (Stanbrough et al. 2001).

Long-term androgen ablation was also associated with the down-regulation of the cell cycle inhibitor p21 (Wang et al. 2001a). Treatment of an androgen-independent LNCaP subline with AR antisense oligonucleotides led to re-establishment of the expression of p21 and to partial reversion of the androgen-independent phenotype. It is known that a number of cytokines and food ingredients down-regulate expression and/or activity of the AR and its target gene PSA. Such inhibitory effects of IL-1β were observed in experiments with monocyte-conditioned media. Conditioned media caused a reduction of the proliferation of LNCaP cells and down-regulation of the AR protein (Culig et al. 1998). These effects were abolished when the media were pretreated with a neutralizing anti-IL-1β antibody. Similar to monocyte-conditioned media, those obtained from cultured activated T-lymphocytes inhibited LNCaP proliferation and AR expression (Hsieh et al. 1995). However, compounds responsible for these effects were not identified. Polysaturated fatty acids, such as docosahexanoic acid and eicosapentanoic acid, inhibit AR activity and could be considered for chemoprevention trials (Chung et al. 2001). Other potential chemopreventive agents, the anti-inflammatory agent flufenamic acid, the red wine compound resveratrol, and the natural flavonoid quercetin, cause down-regulation of AR expression.

---

**Table 1** Induction of CAT activity in LNCaP and LNCaP-abl cells transfected with the androgen-inducible plasmid ARE-TATA-CAT. (Reprinted from Culig et al. 1999 with permission from the Cancer Research Campaign.)
Endocrine-Related Cancer (2002) 9 155–170

Figure 2 Northern blot analysis of steady-state levels of prostate-specific antigen (PSA), prostate-specific membrane antigen (PSM), cytokeratin 8 (CK8) and AR mRNAs in AD and AI cells grown in a defined medium (MEGM). AD and AI cells were plated at 5 × 10⁵ per 100 mm dish in RPMI with 10% fetal bovine serum and switched to MEGM. After 30 h of incubation, the cells were harvested, the total RNA was extracted, and 15 µg of each sample was separated on a 1% agarose. After blotting, the filter was sequentially hybridized with 32P-labeled PSA, PSM, CK8, AR, and glyceraldehyde-3-phosphate dehyrogenase (GAPDH) cDNA probes. (Reprinted from Gao et al. (1999). Reprinted by permission of Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc.)


However, the antiproliferative activity on LNCaP cells is also observed in conditions in which AR expression is up-regulated, as evidenced after treatment with 1α,25-dihydroxyvitamin D3 (Zhao et al. 1997). Human prostate cancer cells PC-3 have been stably transfected with AR cDNA in several laboratories. There is a consensus from these studies that stable expression of the AR leads to a retardation of growth of the cells (Yuan et al. 1993, Heisler et al. 1997, Bonaccorsi et al. 2000, Shen et al. 2000). Moreover, in PC-3 cells which stably express the AR there was a down-regulation of molecules responsible for the invasive phenotype, namely α6β4 integrins (Bonaccorsi et al. 2000). Androgen treatment of PC-3/AR cells resulted in an increased expression of neutral endopeptidase, an enzyme that cleaves and inactivates neuropeptides which are implicated in the growth of advanced prostate cancers (Shen et al. 2000).

Results on AR expression and function obtained with long-term androgen-ablated LNCaP cells are interesting because of the clinical evidence of AR gene amplification (Visakorpi et al. 1995, Cher et al. 1996). A subgroup of patients with therapy-resistant prostate cancer present with an increased number of copies of the AR gene and, consequently, these tumors have a higher content of the AR protein (Linja et al. 2001). Thus, it is likely that AR gene amplification is one of the mechanisms to explain the development of hypersensitivity to stimulation by low androgenic concentrations. Mechanisms which are related to structural alterations will be discussed in detail in the next section. In long-term androgen-ablated cells, AR expression increases either because of up-regulation at the mRNA level (Kokontis et al. 1994) or increased stability of the protein, as evidenced in the prostate cancer xenograft CWR 22 (Gregory et al. 2001b). In the LNCaP tumor model, amplification of the AR gene was not observed in hypersensitive sublines (Kokontis et al. 1998, Culig et al. 1999).

While prostate cancers show significant heterogeneity, some tumor cells show a loss of AR expression because of methylation of CpG sites in the AR promoter (Jarrard et al. 1998). AR promoter contains hot spots (131 to −121 and +44 to +54) that are markers for gene silencing (Kinoshita et al. 2000).

AR structural alterations in prostate cancer

As mentioned previously, the AR is mutated in the LNCaP cell line and the discovery of the mutation has greatly influenced research in that field (Harris et al. 1990, Veldscholte et al. 1990a). The mutation is located in exon H of the AR gene and leads to an exchange of the wild-type threonine (position 877) to alanine. It has been demonstrated in several studies that hormones that bind to the mutated LNCaP AR with increased affinity stimulate cellular proliferation and enhance transcriptional activation function of that AR (Veldscholte et al. 1990a,b, 1992, Montgomery et al. 1992, Tan et al. 1997). These hormones are estrogenic and progestagenic steroids, adrenal androgens, and the non-steroidal antiandrogens hydroxyflutamide and nilutamide. The progestagenic compound cyproterone acetate was one of the first agents introduced in prostate cancer treatment. For some
unclear reasons, antiandrogen regulation of LNCaP AR activity does not parallel that of the PSA protein. It was observed that hydroxyflutamide is still capable of down-regulating PSA although it increases AR-mediated reporter gene activity (Young et al. 1991, Montgomery et al. 1992). Interestingly, agonistic effects of hydroxyflutamide were not evident in LNCaP cells which overexpressed the proapoptotic molecule Bax (Andriani et al. 2001). This raises the possibility that Bax acts as an AR corepressor in prostate cancer. After elucidation of the crystal structure of the human AR, the reasons why the LNCaP AR responds to androgen in a manner similar to that of the wild-type AR became clear (Matias et al. 2000). Due to the mutation, a hydrogen-bound partner for the H-bonded leucine 701 with histidine (Zhao et al. 1999). Transcription activation function of the MDA PCa 2a AR is strongly induced by the glucocorticoid hormone, cortisol and cortisone (Zhao et al. 2000), which promote the growth of that cell line. In the prostate cancer xenograft CWR 22, there is an exchange of histidine at position 874 with tyrosine (Tan et al. 1997). Histidine 874 does not contact ligand and the mutation found in the prostate cancer xenograft most probably affects binding of AR coactivators and their regulation of AR function (McDonald et al. 2000).

There is an agreement in the scientific community that AR point mutations are not common in the early stages of prostate cancer (Culig et al. 1993a,b, Elo et al. 1995, Evans et al. 1996, Marcelli et al. 2000). They could be detected in prostatic specimens obtained from patients with metastatic disease and in samples from bone metastases (Culig et al. 1993b, Taplin et al. 1995) (Fig. 3). Analysis of tissue specimens from the stable phase of the disease and ones obtained after the onset of tumor progression in the case of the mutated AR 715 Val→Met has revealed the mutation solely in the latter tissue (Culig et al. 1993b). To exclude the possibility that AR mutations remain undetected because of their presence in a very small percentage of cells, Marcelli and associates (2000) analyzed the mutations in microdissected material. They discovered AR structural changes in 21% of patients with metastatic disease whereas no mutation was detectable in samples obtained from patients who did not present with metastases. Those results are in a good agreement with the findings of Taplin and associates (1995) who first reported a high frequency of AR structural alterations in bone metastases. In contrast to AR mutations in androgen-insensitivity syndromes which generate receptors which either cannot bind androgen or show a reduced transcriptional activity, AR mutations are, in most prostate cancer cases, non-inherited and promiscuous. Several well-characterized AR point mutations in the ligand-binding domain are located in the region responsible for recognition and specificity of ligand (Wurtz et al. 1996). The function of the AR in human prostate cancer has frequently been investigated by cotransfection-transactivation assays in heterologous cells in which a high transfection efficacy could be achieved, such as monkey kidney COS and CV-1 cells, as well as in prostate cancer cells PC-3 or DU-145. Aberrant activation of the AR might be associated with an increased ligand-binding affinity, as described for the LNCaP AR (Veldscholte et al. 1999a), or occurs without appreciable changes in binding characteristics (Culig et al. 1993b). Among substances which stimulate the activity of mutated AR in prostate cancer, testosterone precursors (adrenal androgens) and metabolites, as well as antiandrogens, are particularly important. AR activation by naturally occurring androgenic precursors and metabolites is described for the LNCaP AR, AR 715 Val→Met, 730 Val→Met, and 874 His→Tyr (Culig et al. 1993b, Peterziel et al. 1995, Tan et al. 1997). Hydroxyflutamide and bicalutamide were considered more promising antiandrogenic drugs than cyproterone acetate because of their non-steroidal structure. They both bind to the AR with a low affinity and prevent androgen-induced conformational change of the receptor (Culig et al. 1993b, Kallio et al. 1994). However, hydroxyflutamide acts as a partial agonist even in the presence of the wild-type AR as

![Figure 3](image-url)
evidenced in reporter gene assays and conformational studies (Peterziel et al. 1995, Wong et al. 1995). Micromolar concentrations of hydroxyflutamide which are efficient in reporter gene assays were measured in sera of prostate cancer patients (Belanger et al. 1988). In patient material, mutations which could be aberrantly activated by hydroxyflutamide were detected more frequently than those activated by bicalutamide (Taplin et al. 1999). Moreover, some patients in whom hydroxyflutamide acts as an agonist show a response to the second-line treatment with bicalutamide. Recently, three missense mutations were discovered in patients who received a combined androgen blockade by orchiectomy and bicalutamide (Haapala et al. 2001). Interestingly, there was no evidence of AR gene amplification in patients whose tumors relapsed after endocrine treatment.

AR alterations are occasionally germ-line mutations. The mutation Arg726→Leu was detected in 2% of Finnish prostate cancer patients whereas only 0.3% of healthy blood donors have that mutated AR (Elo et al. 1995, Mononen et al. 2000). There is a possibility that enhanced stimulation of that receptor with androgenic hormones and estradiol leads to overexpression of genes involved in proliferation and/or inhibition of apoptosis.

In clinics, the frequency and implications of the antiandrogen withdrawal syndrome have been frequently debated. According to an initial observation by Scher & Kelly (1993), improvement of clinical symptoms and decline of PSA after discontinuation of hydroxyflutamide was seen in about one-third of patients. This improvement is, however, only temporary. Similar observations were reported for other drugs used in endocrine therapy for prostate cancer (Small & Carroll 1994, Akakura et al. 1994, Dawson & McLeod 1995, Nieh 1995, Gomella et al. 1997, Sella et al. 1998, Laufer et al. 1999). Unfortunately, there is no AR structural analysis available in a large number of patients who experience the withdrawal syndrome and therefore it is difficult to judge which mechanisms are responsible for the occurrence of the antiandrogen withdrawal syndrome.

Cross-talk with other signaling pathways

The AR is structurally similar to the human progesterone and glucocorticoid receptors. The two latter receptors exhibit ligand-dependent synergistic activation by ligand and low doses of non-steroidal compounds, such as growth factors, peptide hormones that increase intracellular cAMP, or neurotransmitters. For this reason, it was not expected that the AR would show a ligand-independent activation. After several years of intensive research in this area, it became clear that the AR could be activated in a ligand-independent manner by different substances. Activation of the AR in the absence of ligand was first demonstrated in DU-145 cells for growth factors IGF-I, KGF, and EGF (Culig et al. 1994). These growth factors are thought to stimulate prostate growth in a paracrine (KGF) (Yan et al. 1992) and/or both autocrine and paracrine manner (EGF, IGF-I) (Connolly & Rose 1994, Nickerson et al. 2001). However, it should be emphasized that ligand-independent activation of the AR is not necessarily associated with tumor cell proliferation. Compounds that activate the AR include peptide hormones, which increase intracellular cAMP and IL-6 which are pleiotropic regulators of cell growth (Nazareth & Weigel 1996, Hobisch et al. 1998), and phenylbutyrate, which is a prodifferentiation agent used in experimental therapies for prostate cancer (Sadar & Gleave 2000). Vitamin D also caused inhibition of cellular proliferation in association with the induction of PSA expression, an event which was blocked by bicalutamide (Zhao et al. 1997). In the same series of experiments in which the activator of the protein kinase A pathway forskolin up-regulated AR activity in a ligand-independent manner, the human progesterone receptor was activated by progesterone and forskolin in a synergistic fashion (Nazareth & Weigel 1996). However, the outcome of experiments on ligand-independent activation of the AR depends on the cell type and promoter used. For example, Reiniikainen and associates (1996) have demonstrated an enhancement of the androgenic effect on reporter gene activity by EGF in non-prostatic cells. In primary cells of the developing mouse reproductive tract, both ligand-independent and ligand-dependent effect of EGF on AR activity were reported (Gupta 1999) (Figs 4 and 5). Consistent with previous results by Culig et al. (1994) and Gupta (1999), EGF was less potent than androgen in the modulation of reporter gene activity (Gupta 1999). In cells which were transfected with AR cDNA and reporter gene, there was no change in AR expression by cAMP derivatives (Nazareth & Weigel 1996). This is in contrast to the regulation of AR by IL-6 in LNCaP cells which involves up-regulation of AR mRNA and protein, and stimulation of reporter gene activity in experiments in which the AR promoter is coupled to a reporter gene (Lin et al. 2001a). AR activation by the EGF receptor-related molecule HER-2/neu was described by Craft and colleagues (1999). The experiments carried out in the LAPC-4 prostate cancer xenograft, which expresses the wild-type AR, clearly demonstrated that the overexpression of HER-2/neu leads to the promotion of tumor growth and PSA expression. In concordance with these findings, it was shown that the mitogen-activated protein kinase (MAPK) pathway is required for AR activation by HER-2/neu and that serine 514 in the N-terminal part is a target for MAPK phosphorylation (Yeh et al. 1999). Involvement of the MAPK pathway in AR activation was also demonstrated for IL-6 (Hobisch et al. 1998). Besides AR, estrogen receptor-β is an important regulator of the growth of prostate epithelium. Androgenic and estrogenic steroids induced a complex between the AR, estrogen receptor-β and the Src protein, which provides a link to the MAPK pathway (Migliaccio et al. 2000). AR activation in the absence of ligand was demonstrated for MAPK kinase
Culig et al.: Androgen receptors in prostate cancer

Figure 4 Effect of testosterone (T) and epidermal growth factor (EGF) on the luciferase reporter activity of the reproductive tract cells transfected androgen response element (ARE) vector in the presence and absence of AR vector. −AR represents the experiments performed in the absence of the AR vector. The results were normalized against β-galactosidase reporter activity in each assay and the data represent means ± s.d. from four different sets of experiments. *P<0.05 compared with vehicle-treated control cells by Student's t-test. RLU, relative luciferase units. (Reprinted from Gupta (1999). Reprinted by permission of Elsevier Science.)

Figure 5 Effect of EGF on the luciferase reporter activity in the presence and absence of testosterone (T). −AR represents the experiments performed in the absence of AR vector. The conditions of the experiments are the same as described in Fig. 4. The results represent means ± s.d., n=4. *P<0.05 compared with the vehicle-treated control; **P<0.005 compared with testosterone response by Student's t-test. (Reprinted from Gupta (1999). Reprinted by permission of Elsevier Science.)

1 whose overexpression caused apoptosis in prostate cancer cells (Abreu-Martin et al. 1999).

The physiological significance of AR activation by a non-steroidal compound is more convincing if expression of an endogenous AR target gene is up-regulated. Examples of such an effect include stimulation of PSA gene expression by IGF-I and IL-6 in LNCaP cells (Culig et al. 1994, Hobisch et al. 1998, Chen et al. 2000) or by forskolin in prostate explants (Nakhla et al. 1997). Thus, non-steroidal activation of the AR is pathophysiologically relevant and it is not a simple reflection of overexpression of AR cDNA.

In previous sections of this review, it was emphasized that AR antagonists could act as agonists because of AR hypersensitivity or the presence of mutated receptors. The ability of these drugs to antagonize non-steroidal AR activation was demonstrated with peptide growth factors, forskolin, IL-6, butyrate, and luteinizing hormone-releasing hormone (Culig et al. 1994, 1997, Nazareth & Weigel 1996, Hobisch et al. 1998, Sadar & Gleave 2000). However, they were less efficient in the presence of HER-2/neu and phorbol ester which suggests that they cannot down-regulate AR activity in conditions in which the MAPK pathway is hyperactive (Darne et al. 1998, Craft et al. 1999, Yeh et al. 1999).

Among the regulators of AR activity, IL-6 has a particularly important role in prostate tumor biology. Its levels are
elevated in the sera of patients with advanced carcinoma of the prostate and the cytokine’s expression is up-regulated even in tissue extracts obtained from patients with non-metastatic prostate cancer (Twilley et al. 1995, Adler et al. 1999, Giri et al. 2001). Divergent results on IL-6 regulation of proliferation of LNCaP cells have been reported and these differences most probably occur because of the use of different passages of LNCaP cells and subtle differences in cell culture conditions (Degeorges et al. 1996, Okamoto et al. 1997b, Chung et al. 1999, Giri et al. 2001). In the authors’ laboratory, parental LNCaP cells were constantly inhibited by IL-6 (Hobisch et al. 1998, 2001). After long-term treatment with IL-6, the cells conferred a growth advantage in vitro and could not be growth inhibited by exogenous IL-6. Interestingly, AR activation was preserved in the newly generated LNCaP-IL-6+ cell line, most probably because of the up-regulation of AR expression. IL-6 and its receptor are expressed in benign and malignant prostate and therefore it was concluded that IL-6 autocrine and paracrine loops occur (Hobisch et al. 2000). The cross-talk between the pathways of IL-6 and AR is bidirectional: it was reported that androgenic hormones are able to enhance the expression of acute phase response genes (Matsuda et al. 2001). Consistent with these findings, expression of the protein inhibitor of STAT3 signaling (PIAS) reduced AR transcriptional activity (Junichio et al. 2000). STAT3 is the major intermediary IL-6 signal transducer. Recent findings indicate that the PIAS proteins differentially affect AR activity and may cause both its enhancement or its inhibition (Gross et al. 2001). In AR-negative prostate cancer cells, IL-6 acts as a survival factor through the phosphatidyl inositol 3-kinase pathway (Chung et al. 2000).

The spectrum of AR-activating compounds is even brighter than that of other steroid receptors. Activation of the AR was reported for β-catenin (Truica et al. 2000), caveolin (Lu et al. 2001), thyroid hormone (Zhang et al. 1999), and cadmium (Ye et al. 2000). Caveolin is a component of caveolae membranes which is clearly implicated in prostate cancer progression; administration of caveolin antisense oligonucleotides led to the re-establishment of the androgen dependency of tumors (Nasu et al. 1998). Caveolin enhances ligand-dependent receptor activity and associates with the AR in a ligand-dependent manner.

AR coactivators and corepressors in prostate cancer

AR accomplishes its action in target tissues by interaction with molecules which have histone acetylase and deacetylase activity, namely coactivators and corepressors. The progress in this field is reflected mainly in discoveries of a number of new molecules which associate with the AR. It is not easy to assess the importance of these new findings for prostate cancer because of the two main reasons: (a) the levels of enhancement of AR activity by different coregulatory proteins are similar and (b) there is a certain redundancy in their action so that inhibition of function of one coregulator could, most probably, be compensated.

The role of the first coactivator which was discovered in prostate cancer cells DU-145, ARA 70, is controversially discussed (Yeh & Chang 1996). The same group has proposed that ARA 70 is implicated in AR stimulation by estradiol and non-steroidal antiandrogens (Miyamoto et al. 1998, Yeh et al. 1998). Initial reports that this protein specifically enhances activity of the AR but not that of other steroid receptors were disputed by Alen et al. (1999) and Gao et al. (1999b). According to those reports, the up-regulation of AR activity by ARA 70 was not substantially higher than the enhancement of activity caused by other coactivators. An important issue for studies on AR coactivators is determination of AR expression in cells in which a coactivator cDNA has been introduced. Hofman and associates (2000) have recently shown that some of the apparent coactivators, in fact, cause the up-regulation of AR protein expression (Fig. 6). A role for ARA 70 has been recently suggested in an autochthonous transgenic carcinoma of the prostate (Han et al. 2001). ARA 70 enhanced activation of the mutated murine AR containing the substitution Glu231→Gly in response to androgen and estradiol, whereas another coactivator ARA 160 was not effective with estradiol. As expected, cotransfection of two AR coactivators frequently yields further additive or synergistic effect on AR functional activity (Yeh et al. 2000, Wang et al. 2001b).

An unexpected effect of D-type cyclins on AR transcriptional activity was reported. Cyclin D1 acts as a transcriptional coactivator for estrogen receptor (Zwijsen et al. 1998) but in the case of the AR it forms a specific complex with the receptor and inhibits its functional activity (Knudsen et al. 1999). This action might be specific for the D1 cyclin because activation of the AR was enhanced by cyclin E
Culig et al.: Androgen receptors in prostate cancer

(Yamamoto et al. 2000). Hydroxyflutamide lacked antagonistic properties in those experiments. AR-mediated gene transcription was enhanced by a cdk-activating kinase which interacts with the N-terminal part of the AR (Lee et al. 2000). On the other hand, tumor suppressor gene 101 protein has an inhibitory effect on AR transactivation (Sun et al. 1999). Again, all these findings point to a complex regulation of proliferative responses by AR coregulatory proteins. Enhancement of p21 expression by the AR coactivator breast cancer susceptibility gene 1 reversed the apoptosis-resistant phenotype of prostate cancer cells (Yeh et al. 2000). Repression of AR activity was reported for the tumor suppressor gene PTEN (Li et al. 2001). PTEN is an inhibitor of the phosphoinositide 3-kinase which exerts proapoptotic effects. Its inhibitory effect on AR transcripational activity is paralleled by an induction of apoptosis and suppression of production of PSA. Inactivation of PTEN occurs in prostate cancer cell lines and xenografts mainly because of mutations and deletions (Vlietstra et al. 1998). Another repressor of the AR is the p21-activated kinase PAK6 (Yang et al. 2001). Little is known, however, about alterations of PAK6 in advanced carcinoma of the prostate. TGF-β is a pleiotropic growth factor which is considered inhibitory for prostate cells in vitro. TGF-β has effects on immunosuppression and angiogenesis and therefore promotes growth of prostate tumors in vivo (Steiner 1995). The nature of its effect on the AR remains unclear since two groups have published conflicting results. Either coactivator or corepressor function for the TGF-β intermediary molecule Smad3 has been proposed (Kang et al. 2001, Hayes et al. 2001). Similar to the findings on TGF-β, divergent effects of Akt on the regulation of AR activity have been reported and repetition of those experiments by other researchers will perhaps clarify these issues (Wen et al. 2000, Lin et al. 2001b).

In two recent reports, coactivator expression was linked to prostate cancer progression. Association between the overexpression of coactivators TIF-2 and SRC-1 and recurrence of prostate cancer was described (Gregory et al. 2001c). The expression of the coactivator RAC3 was highest in LNCaP cells and lowest in DU-145 cells (Gnanapragasam et al. 2001). RAC3 levels correlated significantly with tumor grade and stage and the coactivator expression was associated with poorer disease-specific survival. One of the obstacles in research on coactivator expression is the fact that these proteins are present in a limited amount in prostate tissue and therefore detection problems might occur. For this reason, expression of some of the proposed coactivators has been studied at mRNA level (Tekur et al. 2001). It was reported that the levels of ARA 70 decrease in immortalized prostate cancer cell lines compared with primary cultures and that androgenic up-regulation of ARA 70 is inhibited by hydroxyflutamide.

**Conclusions and directions for future research**

In the last decade there has been a considerable change in the understanding of the role of the AR in prostate cancer. The recognition that the AR is expressed in metastases of therapy-resistant prostate cancers has greatly stimulated

---

**Figure 7** Key mechanisms for AR involvement in prostate cancer progression. The AR which is in some cases overexpressed could be activated in a hypersensitive manner by ligands, non-androgenic steroids, and non-steroidal regulators of protein kinase activity.
research on its function. It is clear that the AR is implicated in the cross-talk with signaling pathways of growth factors, peptide hormones, cytokines, and various inducers of differentiation (Fig. 7). The most important mechanisms by which the AR facilitates prostate cancer progression are an increase in sensitivity and aberrant activation due to structural alterations. New approaches in therapy are aimed to down-regulate AR expression and to overcome the difficulties associated with the use of common antiandrogens (Eder et al. 2000). The AR, however, has a role in the maintenance of differentiation function in the prostate and therefore the timing of such new therapies should be appropriate.

Acknowledgements

This work was supported by the Austrian Research Fund, contract grant number: FWF SFB 002 F203.

References


Cleutjens CB, Steketee K, van Eckelen CC, van der Korn JA, Brinkmann AO & Trapman J 1997 Both androgen receptor and glucocorticoid receptor are able to induce prostate-specific antigen expression, but differ in their growth-stimulating properties of LNCaP cells. Endocrinology 138 5293–5300.

Connolly JM & Rose DP 1994 Regulation of DU145 human prostate cancer cell proliferation by insulin-like growth factors and its interaction with the epidermal growth factor autocrine loop. Prostate 24 167–175.


Culig Z, Hobisch A, Cronauer MV, Radmair C, Trapman J, Hittmair A, Bartsch G & Klocker H 1994 Androgen receptor activation in prostatic tumor cell lines by insulin-like growth
Androgen receptors in prostate cancer


Han G, Foster BA, Mistry S, Buchanan G, Harris JM, Tilley WD & Greenberg NM 2001 Hormone status selects for spontaneous somatic androgen receptor variants that demonstrate specific ligand and cofactor dependent activities in androgen-independent prostate cancer cells. Journal of Biological Chemistry 276 11204–11213.


Gao T, Brantley K, Bolu E & McPhaul MJ 1999b RFG (ARA70, ALE1) interacts with the human androgen receptor in a ligand-dependent fashion, but functions only weakly as a coactivator in cotransfection assays. Molecular Endocrinology 13 1645–1656.

Giri D, Ozem M & Ittmann M 2001 Interleukin-6 is an autocrine growth factor in human prostate cancer. American Journal of Pathology 159 159–165.
steroid-dependent transcriptional activation region. Proceedings of the 72nd Annual Meeting of the Endocrine Society, Atlanta, GA, p93.


Ikonen T, Palvimo JJ & Janne OA 1997 Interaction between the amino- and carboxyl-terminal regions of the rat androgen receptor modulates transcriptional activity and is influenced by nuclear receptor coactivators. Journal of Biological Chemistry 272 29821–29828.


Lauber M, Sinibaldi VJ, Carducci MA & Eisenberger MA 1999 Rapid disease progression after the administration of bicalutamide in patients with metastatic prostate cancer. Urology 54 745.


Nickerson T, Chang F, Lorimer D, Smeekens SP, Sawyers CL & Pollak M 2001 In vivo progression of LAPC-9 and LNCaP prostate cancer models to androgen independence is associated with increased expression of insulin-like growth factor-I (IGF-I) and IGF-I receptor (IGF-IR). *Cancer Research* **61** 6276–6280.


Culig et al.: Androgen receptors in prostate cancer


Wang LG, Ossowski L & Ferrari AC 2001a Overexpressed androgen receptor linked to p21WAF1 silencing may be responsible for androgen independence and resistance to apoptosis of a prostate cancer cell line. *Cancer Research* 61 7544–7551.


Yeh S & Chang C 1996 Cloning and characterization of a specific coactivator, ARA70, for the androgen receptor in human prostate cells. *PNAS* 93 5517–5521.


