Androgen receptors in prostate cancer

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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-1β, 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.

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 interactions (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...
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 orchietomy 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 antiandrogen 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 antiepidermal growth factor (EGF) antibodies (Ye et al. 1999).

Prostate cancer is a heterogenous 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 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 cdk2 and 4 has been described at mRNA and protein levels (Lu et al. 1997). In the prostate cancer xenograft CWR-22, the levels of cdk 1 and 2, cyclin A and B1 mRNA decreased after castration and increased after supplementation with testosterone propionate (Gregory et al. 2001a). The levels of those cell cycle regulators in recurrent tumors were high in the absence of testicular androgen, thus suggesting that compounds other than androgen activate the AR. Low androgenic concentrations induce an increase of phosphorylation of retinoblastoma protein and the expression of the transcription factor E2F-1 and its target gene product cyclin A (Hofman et al. 2001). Consistent with the inhibitory proliferative response, high concentrations of the synthetic androgen R1881 cause retinoblastoma hypophosphorylation, down-regulation of E2F, and induction of the expression of the cell cycle inhibitor p27 (KIP1). Stimulation of p27 by androgen was also reported in long-term androgen-ablated LNCaP cells (Kokontis et al. 1998). In the case of the CWR-22 xenograft, tumor regression was associated with a sustained increase in p27 expression rather than with changes in the expression of regulators of survival (Agus et al. 1999). In this context, it is important to note that many prostate cancers lack p27 expression (Guo et al. 1997, Cordon-Cardo et al. 1998). 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 (Schaurnans 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 reversal 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. Polyunsaturated 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.)

<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.
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 Al cells grown in a defined medium (MEGM). AD and Al cells were plated at 5 × 10^5 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 dehydrogenase (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 androgen receptor (AR), the reasons why the LNCaP AR responds to androgen was investigated. In prostate cancer, 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 was investigated. In prostate cancer, the availability of AR mutations that are conserved in the region responsible for recognition of the hormone-binding domain. Total RNA was purified from a fine-needle biopsy obtained from a patient with a therapy-resistant prostatic carcinoma. RNA was reverse transcribed to cDNA, and the AR fragments were amplified by PCR, isolated, and directly sequenced. This figure shows the section around the mutated base 2671. (Top) Normal AR sequence (N). (Bottom) Mutant AR found in a specimen from a therapy-resistant, metastatic carcinoma (PC). (Reprinted from Culig et al. (1993). Reprinted by permission of The Endocrine Society.)

![Figure 3](image)

**Figure 3** AR mutation in the hormone-binding domain. Total RNA was purified from a fine-needle biopsy obtained from a patient with a therapy-resistant prostatic carcinoma. RNA was reverse transcribed to cDNA, and the AR fragments were amplified by PCR, isolated, and directly sequenced. This figure shows the section around the mutated base 2671. (Top) Normal AR sequence (N). (Bottom) Mutant AR found in a specimen from a therapy-resistant, metastatic carcinoma (PC). (Reprinted from Culig et al. (1993). Reprinted by permission of The Endocrine Society.)
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 orchietomy 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 anti-androgen 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. 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 (Cilig 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
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**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
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
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 transcriptional 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 over-expression 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...
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.

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