Prostate cancer: molecular biology of early progression to androgen independence

M D Sadar¹, M Hussain² and N Bruchovsky¹

¹Department of Cancer Endocrinology, British Columbia Cancer Agency, 600 West 10th Avenue, Vancouver, British Columbia, V5Z 4E6 Canada
²Division of Hematology/Oncology, Barbara Ann Karmanos Cancer Institute and Wayne State University, Detroit, Michigan, USA

(Requests for offprints should be addressed to M D Sadar; Email: msadar@bccancer.bc.ca)

Abstract

To improve the therapy for prostate cancer, it will be necessary to address the problems of progression to androgen independence and the process of metastatic spread of tumour. The complexity of the latter condition is likely to mitigate against the immediate development of relevant therapeutic approaches. However, the basis of androgen independence appears to be a problem of simpler dimensions and more amenable to treatment with current therapeutic technology. Since early tumour progression can be detected by an incomplete prostate-specific antigen (PSA) response to androgen withdrawal therapy, a study of the molecular biology of PSA gene regulation may well provide insight into new methods for preventing or delaying this problem. Mounting evidence suggests that ligand-independent activation of the androgen receptor may be one underlying mechanism of androgen independence. In the absence of androgen, a compensatory increase in the activity of cAMP-dependent protein kinase (PKA) enhances the ability of the androgen receptor to bind to the response elements regulating PSA gene expression. The activation of the androgen receptor through up-regulation of the PKA signal transduction pathway involves the amino-terminus of the androgen receptor, the function of which may be altered either by modifications such as phosphorylation, or through interactions with co-regulators or other proteins. Of therapeutic interest is the fact that this effect can be counteracted experimentally by the anti-androgen, bicalutamide, and clinically by several other similar agents. We speculate that the inhibition of PKA-activated androgen receptor might also be accomplished by decoy molecules that can bind to the relevant activated site on the amino-terminus or competitively interact with proteins recruited by the PKA pathway that are responsible for activating the receptor in the absence of androgen. Such molecules might include small mimetic substances or agents that can gain access to the nucleus of the cell.

Introduction

Prostate cancer is the most commonly diagnosed, and the second leading cause of death from cancer in North American and European men. Androgen deprivation is the only effective systemic therapy available for metastatic prostate cancer. The inability of androgen deprivation to completely and permanently eliminate all prostate cancer cell populations is manifested by the predictable pattern of initial response and relapse, with the ultimate progression to androgen independence (Denis & Murphy 1993). Androgen deprivation is associated with a gradual transition of prostate cancer cells through a spectrum of androgen-dependence, androgen sensitivity and ultimately androgen independence. There is mounting evidence supporting the concept that prostate cancer progression is accompanied by a shift in reliance on endocrine controls to paracrine and eventually autocrine controls and that this complex process is the result of changes which occur at molecular levels of cellular control (Isaacs et al. 1996). However, the molecular mechanisms involved in the development of androgen-independent prostate cancer are unknown. Historically, clonal selection and adaptation have been advanced to explain progression, but these general theories have been supplanted largely by molecular concepts related to the androgen receptor. Recognition of androgen receptor mutations, gene amplification, co-regulators and signal transduction cross-talk has given rise to the possibility of studying the primary events that trigger progression (Table 1).
Prostate-specific antigen is a marker of early tumour progression

Prostate-specific antigen (PSA) is a tissue-specific tumour marker routinely used by oncologists and urologists to monitor treatment responses, prognosis and progression in patients with prostate cancer. The production of PSA in most tumours is initially androgen-regulated and undergoes a sharp decline following medical or surgical castration. However, in the absence of androgens, the tumour invariably progresses to an androgen-independent condition, an early sign of which is a rising titre of serum PSA that may eventually surpass the pre-treatment value (for a review see Gleave et al. 1996). Not only is the increase in serum PSA a sign of emergent androgen-independent disease (Miller et al. 1992), but it is also associated with a poor prognosis.

Seventy percent of patients with advanced prostate cancer respond to androgen deprivation therapy as measured by a decrease in serum PSA to normal levels during the first 8 months of therapy following the kinetics illustrated in Fig. 1. In contrast, in the remaining 30% of the patients, serum PSA will fail to decline to a plateau in the normal range: after an initial response to therapy, higher readings are again observed which denote early progression to the androgen-independent condition (Fig. 2).

Through monitoring of serum PSA in patients with prostate cancer, it is possible to distinguish trends indicative of early onset of androgen-independent disease. For example the data in Fig. 3 shows a PSA velocity curve associated with a good prognosis (Fig. 3a), as compared with one associated with a poor prognosis (Fig. 3b). The former is hallmarked by an initial and continuing decrease in serum PSA during each of two cycles of androgen deprivation. The latter is characterized by a plateau in serum PSA after an initial decrease in response to the withdrawal of androgens. Not only is the PSA plateau achieved in cycle 2 elevated above that in cycle 1, but also there is a spontaneous increase in serum PSA level despite continuing androgen withdrawal; both changes are indicative of incipient progression to androgen-independence. Thus, patients who follow the poor prognosis trend can be identified at an early stage and might benefit from therapies specifically designed to compensate for anomalous transcriptional events that give rise to the first signs of tumour progression.

The androgen-independent regulation of PSA gene expression is a transcription-related event as has been demonstrated in the LNCaP tumour model of human prostate cancer. The data, as presented in Fig. 4, demonstrate the effects of continuous versus intermittent androgen suppression both on serum PSA and tumour mRNA levels. Both are down-regulated when testosterone is withdrawn, and up-regulated when it is replaced. However, when the tumour becomes androgen-independent, PSA mRNA is constitutively up-regulated.
despite continuing absence of testosterone. The fact that PSA production ultimately increases in an androgen-deprived environment suggests that other factors not directly related to androgens, but possibly acting through the androgen receptor (or other signal transduction pathways), become paramount leading to androgen-independent induction of PSA mRNA.

Transcriptional regulation of the PSA promoter

The expression of PSA escapes regulation by androgen prior to the clinical symptoms of androgen-independent prostate cancer. This suggests that factors responsible for increased expression of the PSA gene, in the absence of androgens, may play a role in the progression of the disease to androgen independence. Therefore, elucidation of how the PSA gene is regulated should provide possible targets that may be involved in the molecular progression of prostate cancer to androgen independence. Recent reports have drawn attention to the fact that PSA gene expression can become elevated in the absence of androgens by numerous compounds that include butyrate (Walls et al. 1996, Gleave et al. 1998), phenylacetate (Walls et al. 1996), vasoactive intestinal peptide (VIP) (Gkonos et al. 1996), retinoic acid (Fong et al. 1993, Walls et al. 1996), vitamin D (Walls et al. 1996, Zhao et al. 1997), interleukin-6 (Hobisch et al. 1998), growth factors (Culig et al. 1994, Rajagopal et al. 1998), and activators of cAMP-dependent protein kinase (PKA) (Sadar 1999). The mechanism of androgen-independent induction of PSA by insulin-like growth factor-I (IGF-I) and activators of the PKA pathway has been suggested to be through ligand-independent activation of the androgen receptor (Culig et al. 1994, Sadar 1999). The mechanisms of androgen-independent induction of PSA by the other compounds have not been elucidated. Therefore, if PSA gene expression is induced by interleukin-6, growth factors, phenylacetate, retinoic acid, VIP, vitamin D or butyrate by a pathway independent of the androgen receptor, it would mean that another transcription factor acting on the PSA promoter may become stimulated. It also follows that alternative transcription factors that are involved in dysregulation of the PSA promoter may play a role in the progression of prostate cancer to androgen independence.

The PSA promoter has been sequenced (Lundwall 1989, Schuur et al. 1996) and the following DNA response elements have been characterized: (1) TA TA box, -28/-23 (Riegman et al. 1991); (2) androgen response elements (AREs), -170/-156 (Riegman et al. 1991), -4148/-4134 (Schuur et al. 1996); (3) androgen response region (ARR), -395/-376 (Cleutjens et al. 1996); and (4) SP-1, -43/-54, -84/-93, -113/-123 (M Sadar, unpublished data). A number of putative consensus sites for transcription factors exist on the PSA promoter, as shown in Fig. 5, that could potentially activate this gene by other signal transduction pathways. Examples of these pathways include AP-1 and cAMP since there are putative consensus sites for both of these transcriptional factors. An interesting putative AP-1 site overlaps the ARE at -395/-376, thereby giving rise to a possible mechanism for AP-1 regulation of the PSA gene as illustrated in Fig. 6. In a situation where the AP-1 (Jun and Fos family proteins) level is elevated, AP-1 may bind to its DNA-binding site within the androgen response region and inhibit the binding of androgen receptor. In addition, c-Jun prevents the binding of androgen receptor to AREs which results in the attenuation of androgen-induced PSA gene expression (Sato et al. 1997).

Another DNA regulatory site of major interest is the putative cAMP response element (CRE; Fig. 5) located at -3196/-3189 of the PSA enhancer because transcriptional factors responsive to cAMP, i.e. CREB and activating transcription factor (ATF), can bind to this site. Two other putative CRE sites (imperfect) not shown are located at -80 and -520. Although experimental elevation of cAMP strongly induces the expression of the PSA gene by an androgen receptor-dependent pathway (Sadar 1999), it is not known whether CREB or ATF can modulate this effect. Proteins binding to CREs and AP-1 DNA-binding

Figure 2 Response and progression of serum PSA in eight cases of stage D2 prostate cancer. The upper limit of normal for serum PSA is 2.5 µg/l in the fifth decade of life, 3.5 µg/l in the sixth decade, 4.5 µg/l in the seventh decade and 6.5 µg/l during the eighth decade (Oesterling et al. 1993). Patients were treated as described in the legend to Fig. 1. Reprinted with permission from Bruchovsky et al. (1993).
sites may take the form of homo- or heterodimers of CREB and ATF, or alternatively of homo- or heterodimers of Jun, Fos and other members of the basic zipper or bZip family (Fig. 6). Interaction between these transcription factors contributes to the complexity of gene regulation via cross-talk between signal transduction pathways. Induction of PSA gene expression by butyrate (Walls et al. 1996, Gleave et al. 1998) may involve either SP-1, AP-1 or CREs since it has been shown to increase SP-1 DNA binding (Nakano et al. 1997), elevate levels of AP-1 (Toscani et al. 1988, Tichonicky et al. 1990, Gupta et al. 1994), and modulate PKA activity (Datti & Dennis 1993, Aukema et al. 1997) in other cell lines. Hence, it is not inconceivable that androgen-independent regulation of PSA gene expression may involve transactivation through an alternative DNA response element in the absence of activated androgen receptor. Changes in the activation or recruitment of such transcription factors may also be involved in the molecular progression of prostate cancer to androgen independence. Therefore, regulation of the

Figure 3 PSA velocity and testosterone levels of patients receiving intermittent androgen suppression therapy with leuprolide acetate and cyproterone acetate. (a) Serum PSA associated with a good prognosis; (b) poor prognosis. Androgen withdrawal (treatment), replacement (no treatment).
PSA gene provides an important model that can be used to identify possible mechanisms that may be involved in the progression of prostate cancer to androgen independence.

Androgen receptor related and signal transduction theories of progression

The androgen receptor is implicated in progression of prostate cancer to androgen independence since it is the ligand-activated transcription factor that mediates the action of androgens. As such, it forms the basis of a signal transduction pathway that regulates the expression of a specific subset of androgen-responsive genes. Control of androgen-regulated gene activity may be agonistic (stimulated) or antagonistic (repressed), and results from binding of hormone-activated androgen receptor to upstream flanking nucleotide sequences, termed androgen response elements (AREs). Among the earliest detected effects of androgen withdrawal are decreases in the intranuclear concentration of dihydrotestosterone and androgen receptor, thereby setting conditions for androgen-stimulated genes to become dormant, while androgen-repressed genes are activated (Van Doorn et al. 1976). The fact that androgen replacement can reverse alterations in gene activity, which favour the emergence of androgen independence, suggests that the hormone-activated form of the wild-type androgen receptor plays a significant role in impeding tumour progression (Akakura et al. 1993, 1996). If this is the case, there is a strong rationale for examining the implications of various abnormal or non-functional states of the androgen receptor (Quigley et al. 1995).

Studies of androgen receptor expression in clinical specimens of human prostate cancer have confirmed the presence of a high percentage of receptor-positive cells both in primary tumours and in hormone-refractory recurrent tumours (Hobisch et al. 1995, Wilding 1995, McPhtaul 1996). However, variations in the levels of

Figure 4 Effects of androgen withdrawal and replacement (intermittent) versus continuous withdrawal on serum PSA in the LNCaP tumour model. Mice were castrated when implanted tumour attained a diameter of 1 cm. Two weeks after castration, those in the intermittent withdrawal group were implanted subcutaneously with a testosterone pellet (▲). The pellet was removed 1 week after implantation (Δ); thereafter the same regimen was repeated five more times as shown. No androgen replacement was done after castration in the continuous androgen withdrawal group. Serum PSA was measured weekly in both groups. Each value shown represents the mean value of results from five different animals. Total mRNA was extracted from tumours at peak (P1 to P5) and nadir (N1 to N5) PSA levels and hybridized with a cDNA probe for PSA mRNA. Northern analysis results are shown at the top of the figure. In mice receiving cyclic therapy, PSA mRNA levels remained below pre-castration (intact) levels at least up to the fifth nadir (N5). In mice subjected to continuous androgen withdrawal, PSA mRNA levels were already elevated 4 weeks after castration. Progression indicates the point at which serum PSA levels first exceeded the pre-castration values. Modified from Sato et al. (1996) and reprinted with permission.
expression of the androgen receptor mitigate against a
direct relationship between the level of receptor protein
and the biological behaviour of any given tumour, thereby
providing reason to search for other causes of receptor
dysfunction. This has led to the discovery that mutations
in the androgen receptor ligand-binding domain can alter
the specificity of hormone binding such that activation of
transcription can take place in the presence of not only
androgens, but also of other steroid hormones and anti-
androgens used in therapy (Taplin et al. 1995, Barrack

However, these mutations do not seem to account for the
majority of cases of hormone-independent prostate cancer.
Another genetic variation in the structure of the androgen
receptor has been shown to involve variable lengths of
cytosine-adenine-guanosine (CAG) trinucleotide repeat
sequences which encode the glutamine stretch within the
amino-terminal transactivation domain of the androgen
receptor (Choong et al. 1996, Hardy et al. 1996). The
length of the glutamine-rich region of the androgen
receptor affects gene transcription. Progressive expansion
of this repeat length is associated with a linear decrease in

---

**Figure 5** Regulatory sites of the PSA gene. Putative AP-1 and CRE sites are illustrated. Note the putative AP-1 site overlapping the ARR at -395 to -376. (Copyright Dr M Sadar).

**Figure 6** Regulation of PSA: the role of Jun in progression. The bottom of the figure shows an increasing ratio of Jun to androgen receptor (AR) as progression proceeds. The top of the figure shows possible protein dimers that may bind to the PSA promoter and illustrates mechanisms that may come into play as progression proceeds. Note that at an early stage of progression, when the androgen receptor is more abundant than Jun, the receptor may inhibit Jun from binding to its respective AP-1 DNA-binding site (top left of diagram), but the receptor can still form dimers to bind to its respective ARE. When progression proceeds to a point where Jun is in abundance, the androgen receptor is inhibited from binding to its respective ARE by two possible mechanisms: the first involving protein–protein interactions; and the second involving Jun binding to the AP-1 site in the ARR, thereby preventing the androgen receptor from binding to this region (protein–DNA interaction). (COpyright Dr M Sadar).
androgen receptor transactivation. However, there has been no indication of expansion of CAG repeat length in androgen-independent prostate cancer; in fact, CAG repeat deletions are likely to be more typical of prostate cancer (Li et al. 1997) if indeed their occurrence can be confirmed (Mohler et al. 1997).

Another indication of how the functional state of the androgen receptor is altered during tumour progression is amplification of the androgen receptor gene (Visakorpi et al. 1995, Koivisto et al. 1997). A common DNA amplification site in recurrent hormone-refractory tumours has been mapped to chromosome Xq11-q13, the site of the androgen receptor gene. Amplification of the androgen receptor is exclusively associated with relapsing malignancy under conditions of extreme androgen deprivation. Thus, it is possible that an elevated receptor copy number allows the cell to utilize low levels of residual non-testicular androgens for cell growth. Although this infers that recurrent prostate cancer is highly dependent on trace amounts of androgen, previous experience with surgical adrenalectomy and hypophysectomy and the failure of second-line anti-androgen therapy to alter the survival of patients with androgen-independent disease are not in conformity with this concept of tumour progression (Hussain et al. 1994).

Focus on anomalies of the expression and structure of the androgen receptor has tended to divert attention from the possible significance of the combined effect of multiple protein–protein interactions between the androgen receptor and other transcription factors and/or co-regulatory proteins via cross-talk between the androgen receptor and alternative signal transduction pathways.

### Cross-talk between the androgen receptor and other signal transduction pathways

The androgen receptor belongs to the superfamily of nuclear receptors that mediate responses of lipophilic ligands, including steroids, retinoids, vitamin D₃, and thyroid hormones (Zilliacus et al. 1995). These receptors have distinct functional domains involved in transcriptional activation and repression, ligand binding, and a highly conserved DNA-binding domain. Binding of ligand results in an activation or transformation step such that the receptor can effectively bind to its respective DNA element. The mechanism of ligand-induced transformation of steroid receptors may include the following: (1) a change in protein conformation required for DNA binding; (2) the removal of associated heat-shock proteins which may act as a cytoplasmic anchor or inhibit receptor–DNA interactions; and (3) the covalent modification of receptor (e.g. phosphorylation) presumed to be required for DNA-binding activity. The process of ligand-induced transformation of the androgen receptor is not completely understood, although it is known that the conformation of the androgen receptor becomes more compact upon ligand binding, heat-shock proteins are dissociated, and dimerization and phosphorylation occur prior to DNA binding (Kuil et al. 1995). Thus, in the presence of ligand, the androgen receptor is activated to stimulate or repress androgen-regulated genes. However, the androgen receptor may also be transformed in the absence of androgen in prostate cells (Culig et al. 1994, 1995, 1997b, Ikonen et al. 1994, Nazareth & Weigel 1996, Zhao et al. 1997, Hobisch et al. 1998, Sadar 1999). The mechanism of ligand-independent activation of androgen receptor has not been clarified but may involve the bypassing of one of the above-mentioned mechanisms of ligand-dependent transformation. Of these, phosphorylation has been the more generally accepted theory of ligand-independent activation of the progesterone, oestrogen and retinoic acid receptors. On the other hand, although there are three identified phosphorylation sites on the androgen receptor, phosphorylation does not appear to be a necessary for the induction of androgen-stimulated genes (Zhou et al. 1995), thereby questioning the role that phosphorylation plays in androgen receptor transactivation. The fact that the androgen receptor is still present in the nucleus of cells in androgen-independent tumours (Pertschuk et al. 1995) is compatible with ligand-independent activation of the androgen receptor in prostate cancer and by inference in progression to androgen independence.

**Ligand-independent activation of the androgen receptor by the PKA pathway**

Cross-talk between the androgen receptor and PKA signal transduction pathways occurs in androgen-depleted human prostate cancer cells maintained in culture (Nazareth & Weigel 1996, Culig et al. 1997b, Sadar 1999). These studies have shown that anti-androgens can block PKA induction of PSA mRNA (Sadar 1999) and androgen-responsive reporters (Nazareth & Weigel 1996, Culig et al. 1997b, Sadar 1999). This suggests activation of the androgen receptor by the PKA pathway. Further evidence supporting ligand-independent activation of the androgen receptor through the PKA pathway is shown in Table 2 and includes increased androgen receptor–ARE complex formation with nuclear extracts from cells exposed to activators of PKA. Interestingly, more androgen receptor–ARE complex formation occurred in the presence of nuclear extracts from forskolin-treated cells than from androgen-treated cells, despite the fact that nuclear levels of androgen receptor were approximately tenfold higher in the androgen-treated cells (Sadar 1999). This suggests that the forskolin-transformed androgen receptor may have a greater affinity for the PSA–ARE

Endocrine-Related Cancer (1999) 6 487-502
than the receptor activated by androgen. Such a theory is supported by the report that the amino-terminus of the androgen receptor is activated by PKA in LNCaP cells (Sadar 1999) and this region of the receptor has been suggested to contribute to the stability of the receptor–DNA complex (Kallio et al. 1994). PKA activation of the amino-terminus of the androgen receptor in the absence of androgen, yielding a receptor with enhanced affinity for AREs, may be a mechanism involved in the progression of prostate cancer to androgen independence. If this proves to be the case, then the amino-terminus of the androgen receptor would provide a new target for therapeutic intervention.

**Ligand-independent activation of the androgen receptor by growth factors**

Oestrogen and progesterone receptors, which belong to the same superfamily of ligand-induced transcription factors as the androgen receptor, mediate gene transcription in response to IGF-I, epidermal growth factor (EGF) and dopamine (Power et al. 1991, 1992, Aronica & Katzenellenbogen 1993, Cho & Katzenellenbogen 1993, Ignar-Trowbridge et al. 1993) in the absence of ligand. The mechanism by which the oestrogen receptor is activated by EGF differs from that of PKA, EGF (El-Tanani & Green 1997) and IGF (Ignar-Trowbridge et al. 1996) act primarily by means of the transactivation domain AF-1, while PKA acts through the transactivation domain AF-2 of the oestrogen receptor (El-Tanani & Green 1997). Hence, the oestrogen receptor can be activated through three different signal molecules, oestradiol, cAMP and growth factor, each acting through a different mechanism. These studies bring to light the fact that steroid hormone receptors can be transformed in the absence of ligand through various pathways. Whether this applies to the androgen receptor is unknown, although EGF, IGF-I and keratinocyte growth factor (KGF) have been shown to stimulate the transcription of ARE-driven reporter gene constructs in DU-145 prostatic cells, an effect mediated by the androgen receptor (Culig et al. 1994). Interleukin-6 (IL-6) has also been shown to activate the human androgen receptor in prostate cancer cells to increase androgen receptor-mediated transcription through a mechanism that may involve cross-talk between the androgen receptor and PKA, PKC and/or MAPK (Hobisch et al. 1998) through ErbB2 (Qui et al. 1998). However, in the case of PSA gene expression, ligand-independent activation of the androgen receptor by growth factors remains virtually unexplored.

**Activation of the PKC pathway and inhibition of the androgen receptor**

Cross-talk between the androgen receptor and protein kinase C (PKC) signal transduction pathways has been reported in prostate cells (Shemshedini et al. 1991, Ikonen et al. 1994, Kallio et al. 1995, Bubulya et al. 1996, Sato et al. 1997). However, since phosphorylation does not alter androgen receptor activity, the observed effects of stimulating PKC may in part be due to downstream elevation of AP-1 and subsequent protein–protein interactions between the androgen receptor and AP-1 (Bubulya et al. 1996, Sato et al. 1997). AP-1 is a complex of transcriptional factors encoded by c-fos and c-jun proto-oncogenes and has been implicated in cell growth, differentiation and development with its activity modulated by growth factors, cytokines, oncogenes and activation of PKC by tumour promoters (Pfahl 1993). AP-1 induces transcriptional activation through interaction with the 12-O-tetradecanoylphorbol 13-acetate- (TPA) response element (TRE) or AP-1 DNA-binding site (Angel et al. 1987, Lee et al. 1987). The levels of c-Jun are increased in the androgen-independent PC3 cell line and androgen-induced PSA gene expression is inhibited by AP-1 (Murtha et al. 1997, Sato et al. 1997). Furthermore, AP-1 inhibition of PSA is due, at least in part, to protein–protein interaction between the DNA- and ligand-binding

---

**Table 2** In the absence of androgen, activation of the PKA pathway causes the following effects in LNCaP prostate cancer cells

<table>
<thead>
<tr>
<th>Effect</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Increases the nuclear levels of androgen receptor</td>
<td></td>
</tr>
<tr>
<td>Increases PSA mRNA levels</td>
<td></td>
</tr>
<tr>
<td>Increases the activity of the PSA (-630/+12) promoter–luciferase reporter</td>
<td></td>
</tr>
<tr>
<td>Increases the activity of the probasin (286/+28) promoter–luciferase reporter</td>
<td></td>
</tr>
<tr>
<td>Has no effect on the activity of the ARR3-tk-luciferase reporter</td>
<td></td>
</tr>
<tr>
<td>Increases protein–DNA complex formation between androgen receptor and PSA–ARE</td>
<td></td>
</tr>
<tr>
<td>Bicalutamide blocks induction of PSA mRNA by PKA</td>
<td></td>
</tr>
<tr>
<td>Bicalutamide blocks induction of PSA reporter activity by PKA</td>
<td></td>
</tr>
<tr>
<td>Bicalutamide blocks induction of probasin reporter activity by PKA</td>
<td></td>
</tr>
<tr>
<td>Activates the amino-terminus of the androgen receptor</td>
<td></td>
</tr>
</tbody>
</table>
domains of the androgen receptor and the leucine zipper motif of c-Jun (Sato et al. 1997). Paradoxically, Shemshedini’s group consistently found that the transcriptional activation of androgen receptor, measured by HRE-driven reporters, is increased by overexpression of c-Jun in some cell types (Shemshedini et al. 1991, Bubulya et al. 1996, Wise et al. 1998, Tillman et al. 1998). The fact that c-Jun inhibits androgen receptor action in some prostate cells, and activates it in other cells, emphasizes possible cell-, and/or promoter-specific responses that may be explained by differences in the requirements of a particular promoter for coactivators, or differences between cells in the availability of required coactivators.

Co-regulators and other factors that interact with the androgen receptor

Coactivators can act as bridging molecules between the steroid hormone receptors and general transcription factors to enhance transactivation of target genes (for reviews see Horwitz et al. 1996, Glass et al. 1997, Shibata et al. 1997). The mechanism by which this occurs appears to involve multiple protein–protein interactions between steroid hormone receptors and co-regulators to stabilize the assembly of basal transcription factors resulting in elevated activity of RNA polymerase II (Smith et al. 1996). Most of the identified co-regulators are promiscuous since they generally associate with numerous steroid hormone receptors. Attention to the study of steroid hormone receptor coactivators was highlighted by a recent report identifying AIB1 (amplified in breast cancer-1) as a co-activator whose altered expression may contribute to the development of steroid-dependent cancers (Anzick et al. 1997). To date there has not been an equivalent report of an androgen receptor coactivator that is elevated in prostate cancer. There are reports though that some coactivators may have preference for the androgen receptor such as androgen receptor associated protein (ARA70) and bcl-2-associated anathogene 1 long (BAG-1L).

ARA70 (ELE1, RFG) was originally described as a specific, ligand-dependent, coactivator of the androgen receptor (Yeh & Chang 1996) and can modulate the response of the receptor to androstenediol, anti-androgens, and 17β-oestradiol (Miymoto et al. 1998a,b, Yeh et al. 1998). The original paper describing the cloning and characterization of ARA70, reported that ARA70 enhanced androgen receptor transcriptional activity by tenfold, with only marginal effects with the oestrogen, glucocorticoid and progesterone receptors in Du-145 cells (Yeh & Chang 1996). However, this has been challenged with the report that in Du145 cells, ARA70 has only minor effects on the transcriptional activation of the androgen receptor, and it appears to generally interact not only with the androgen receptor, but also with the estrogen receptor (ER) and glucocorticoid receptor (GR) (Alen et al. 1999). Several interesting characteristics of ARA70 include: (1) it does not have an intrinsic transcription activation domain nor does it have histone acetyltransferase activity; (2) it interacts with the histone acetyltransferase, pCAF, the basal transcription factor TFIIB, and helix 3 of the ligand-binding domain of the androgen receptor; and (3) the expression of ARA70 is not altered by androgens in prostate cancer cell lines (Alen et al. 1999). Thus, although it is debatable whether ARA70 is indeed a specific coactivator of the androgen receptor, it may in fact be a common bridging factor for steroid receptors and the basal transcription machinery.

BAG-1 (Bcl-2-associated anathogene 1) binds to the anti-death protein, bcl-2, to further suppress apoptosis (Takayama et al. 1995). A member of the BAG-1 family, the BAG-1M (RAP46) protein interacts with steroid hormone receptors, including the androgen receptor (Zeiner & Gehring 1995), but does not affect transactivation of the androgen receptor (Froesch et al. 1998). Another isoform of BAG-1, the BAG-1L protein has been detected in prostate cancer specimens, and interacts with the androgen receptor to enhance its transactivation of reporter genes containing ARDs in the presence of androgen (Froesch et al. 1998). The mechanism of how BAG-1L enhances transcriptional activity of the androgen receptor in the presence of ligand has not been clarified, but may involve modulation of the function and/or the interaction of the androgen receptor with Hsp70 (Froesch et al. 1998). Given that BAG-1L is expressed in prostate tumours, together with it enhancing androgen receptor action and inhibiting apoptosis, makes BAG-1L a potential candidate that may be involved in the progression of prostate cancer.

CREB binding protein (CBP)/p300 has been shown to enhance the transactivation of many transcription factors, including steroid hormone receptors (Chakravarti et al. 1996, Hanstein et al. 1996, Kamei et al. 1996, Smith et al. 1996, Yao et al. 1996)., NFκB (Gerritsen et al. 1997, Perkins et al. 1997) and AP-1 (Arias et al. 1994, Bannister et al. 1995). CBP/p300 is therefore considered a transcriptional adapter that has histone acetyltransferase activity (Bannister & Kouzarides 1996, Ogryzko et al. 1996), associates with P/CAB (Yang et al. 1996), interacts directly with TFIIB of the basal transcription machinery (Kwok et al. 1994), and associates with RNA polymerase II (Kee et al. 1996). CBP/p300 forms a complex with SCR-1 and then interacts with the ligand-binding domain of steroid hormone receptors (Kamei et al. 1996). In non-prostatic cells, overexpression of CBP was found to cause a slight enhancement of the transcriptional activity of the rat androgen receptor in the absence of androgen, and a
3.5-fold increase in the presence of testosterone, in experiments measuring the activity of a reporter gene driven by four AREs in front of a minimal thymidine kinase promoter (Ikonen et al. 1997, Aarnisalo et al. 1998). In addition, protein–protein interaction between the rat androgen receptor and CBP was not altered by the presence of androgen, nor was the presence of the ligand-binding domain required for twofold increase of androgen receptor-dependent activation of reporter gene activity by overexpression of CBP (Aarnisalo et al. 1998). Together these observations raise questions as to what role ligand plays in the interaction of androgen receptor and CBP. It is possible that ligand only facilitates nuclear localization of the androgen receptor and/or its dissociation with heat-shock proteins such that it can bind DNA.

Establishment that CBP is a coactivator of the androgen receptor (Ikonen et al. 1997, Aarnisalo et al. 1998, Frønsdal et al. 1998), AP-1 and NFKB has raised the possibility that competition for limited cellular amounts of CBP by these transcription factors could result in a decrease in their respective transcriptional activities (Kamei et al. 1996). The androgen receptor is inhibited by increased expression of either AP-1 (Sato et al. 1997) or NFKB (Palvimo et al. 1996). Therefore, competition for CBP by these factors may at least in part be involved in the repression of androgen receptor-mediated transcription. This does in fact appear to be the case when using systems reliant upon overexpression of the rat androgen receptor in non-prostatic cells and examining the activity of an AP-1 driven reporter gene in the presence of ligand-bound androgen receptor (Aarnisalo et al. 1998). Overexpressed rat androgen receptor, in the presence of ligand, repressed an AP-1 driven reporter activity and this repression was rescued by overexpression of CBP (Aarnisalo et al. 1998). Similarly, overexpression of CBP in non-prostatic cells ectopically expressing androgen receptor rescued inhibition of the activity of an ARE-driven reporter by NFKB(relA), as well as inhibition of NFKB(relA) activity by rat androgen receptor (Aarnisalo et al. 1998). Therefore, it appears that competition for CBP by androgen receptor, AP-1 and NFKB could lead to a decrease in the transcription of genes regulated by these transcription factors. In particular, squelching of CBP by c-Jun may contribute to repression of androgen-regulated PSA gene expression in addition to the inhibitory protein–protein interactions between AP-1 and the androgen receptor.

Table 3 lists other factors that interact with the androgen receptor. However, most of these demonstrate a general effect on hormone receptors and include the following. (1) The small nuclear RING finger protein (SNURF) that was identified to interact with the TATA-binding protein and DNA-binding domain of androgen receptor in the presence of ligand (Moilanen et al. 1998). (2) The transcriptional intermediary factor 2 (TIF2) enhances the transcription of androgen receptor-dependent promoters, in an androgen-dependent and promoter-specific manner, through a mechanism that involves interaction between TIF2 and the amino-terminus domain of the androgen receptor, either directly or indirectly, with the AF-2 AD core region of the ligand-binding domain of the receptor (Berrevoets et al. 1998). (3) F-steroid receptor activator-1 (SRC-1) enhanced rat androgen receptor-mediated transactivation and

<table>
<thead>
<tr>
<th>Protein</th>
<th>Effect on receptor</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>BAG-1L</td>
<td>Enhances activity</td>
<td>Froesch et al. 1998</td>
</tr>
<tr>
<td>ERM</td>
<td>Inhibits activity</td>
<td>Schneikert et al. 1996</td>
</tr>
<tr>
<td>NFKB</td>
<td>Inhibits activity</td>
<td>Palvimo et al. 1996</td>
</tr>
<tr>
<td>RIP140</td>
<td>Enhances activity</td>
<td>Ikonen et al. 1997</td>
</tr>
<tr>
<td>SNURF</td>
<td>Enhances activity</td>
<td>Moilanen et al. 1998</td>
</tr>
<tr>
<td>F-SRC-1</td>
<td>Enhances activity</td>
<td>Ikonen et al. 1997</td>
</tr>
<tr>
<td>SRC-1</td>
<td>Inhibits activity</td>
<td>Ikonen et al. 1997</td>
</tr>
<tr>
<td>TFIIF</td>
<td>Interacts with transcriptional machinery</td>
<td>McEwan &amp; Gustafsson 1997</td>
</tr>
<tr>
<td>TIF2</td>
<td>Enhances activity</td>
<td>Berrevoets et al. 1998</td>
</tr>
<tr>
<td>RB</td>
<td>Enhances activity</td>
<td>Lu &amp; Danielsen 1998</td>
</tr>
</tbody>
</table>
facilitated interactions between the amino- and ligand-binding domains in a ligand-dependent manner (Ikonen et al. 1997). SRC-1, the truncated form of F-SRC-1 (Takeshita et al. 1996), inhibited rat androgen receptor-mediated transcription and interaction between the amino- and ligand-binding domains (Ikonen et al. 1997). (4) RIP140 enhanced rat androgen receptor-mediated transcription of the probasin promoter, in the presence of androgen, in non-prostatic cells (Ikonen et al. 1997). (5) The amino-terminus of the human androgen receptor, amino acids 142-485, interacts with basal transcription factor TFIIF and is involved in the recruitment of the transcriptional machinery (McEwan & Gustafsson 1997). (6) Another transcription factor that has been reported to interact with the androgen receptor include the Ets family member, ERM, which is thought to play a role in regulation of transcription of matrix metalloproteinases (MMPs). The MMPs consist of a family of enzymes that degrade components of the extracellular matrix and are implicated in process of tumour invasion and metastasis (Woessner 1991). Androgens have been shown to negatively regulate the expression of MMP-1, -3 and -7 in human prostate cancer cells by protein–protein interactions between the amino-terminus of the androgen receptor and Ets related molecule (ERM) (Schneikert et al. 1996). Thus, androgen ablation therapy for patients with prostate cancer may result in the up-regulation of these MMPs and enhance tumour invasion and metastasis. (7) The transcriptional activity of the human androgen receptor may be controlled by the cellular level of hypophosphorylated retinoblastoma protein (pRB) through protein–protein interactions involving the amino-terminus of the androgen receptor (Lu & Danielsen 1998). Interaction between the hypophosphorylated pRB and the androgen receptor suggests that the androgen receptor can only be active in the G0 and G1 phase of the cell cycle when the hypophosphorylated pRB is predominate. Furthermore, association of the pRB with the nuclear matrix occurs when it is hypophosphorylated, during G1 phase. Ligand-bound androgen receptor is also associated with the nuclear matrix, placing it in the same vicinity as the hypophosphorylated pRB for possible protein–protein interactions to occur. Therefore, a loss of pRB activity during progression of prostate cancer may result in a decreased response to androgens.

Translational aspects

There are two major impediments to the improved therapy of prostate cancer, namely progression to androgen independence and metastases. In the attempt to develop new therapeutic initiatives for prostate cancer, it would seem logical to focus attention on the malignancy-associated condition of least complexity and most amenable to prevention or reversal within a reasonable time-frame. Given the predictability of the emergence of androgen independence with androgen ablative therapies, coupled with the ever-increasing potential on the experimental side for discovering the basis of androgen independence, a compelling argument can be made for giving priority to new ways of reversing or preventing such resistance. A significant breakthrough in this area may well be within grasp and is more immediately conceivable than therapeutic containment of the metastatic process which remains poorly understood owing to the number of steps involved.

In the present review, we have assumed that the recognition of early tumour progression would make it possible to intervene with a treatment regimen that specifically targets the initial targets of androgen independence. Attention is drawn to the fact that the earliest event which signifies tumour progression is the androgen-independent induction of PSA gene transcription (Figs 1–4) and may be the result of (1) ligand-independent activation of the androgen receptor; (2) activation of an alternative transcription factor that binds to a regulatory region of the PSA promoter; or (3)

![Figure 7](Early tumour progression and effects of treatment on serum PSA. Numerical values above the bars represent the serum PSA levels (ng/ml). CPA, cyproterone acetate; FLU, flutamide; NIL, nilutamide.)
alteration in the expression or activities of co-regulators that may affect androgen receptor activity.

An observation of practical importance and of immediate clinical relevance, is the effect of the non-steroidal anti-androgen, bicalutamide, on the inhibition of ligand-independent activation of the androgen receptor through the PKA signal transduction pathway (Table 2). Although more work is required to clarify whether other anti-androgens are characterized by this inhibitory action, it is likely that this effect is not restricted to a single compound as shown in Fig. 7. We observed that if the serum PSA plateaus during therapy of prostate cancer as shown in Fig. 3b, a switch in anti-androgen may result in an almost immediate further significant drop in serum PSA. In the examples given in Fig. 7, each patient was treated with a combination of the luteinizing hormone-releasing hormone (LHRH) agonist, leuprolide acetate, and cyproterone acetate or flutamide for a minimum period of 9 months. In each case, after the first 3 months, there was an apparent plateauing of serum PSA in months 4, 5 and 6 of treatment. At the end of the sixth month, when another anti-androgen was substituted for the original one, a further decrease of up to tenfold in the level of serum PSA was observed. The effect cannot be attributed to an anti-androgen withdrawal response since, under these circumstances of low serum PSA, no effect of stopping the initial anti-androgen is observed (N Bruchovsky, unpublished data). Thus, during early tumour progression, as signified by androgen resistance of PSA gene regulation, it may be possible to temporarily block any adverse change by introducing an anti-androgen which can check the PKA-dependent activation of the androgen receptor. Whether this effect is brought about by the binding of anti-androgen to the ligand-binding domain or to some other site on the androgen receptor is unknown.

The inhibitory effect of anti-androgens on ligand-independent activation of the androgen receptor suggests that it might be profitable to search for other compounds that can function in the capacity of selective antagonists of the androgen receptor. In theory, it is possible to synthesize peptides or other small molecules which bind to a specific sequence on the androgen receptor, thereby preventing its activation or, serving as inhibitory decoys, bind to co-regulators or alternative transcription factors essential for androgen-independent receptor activity (Fig. 8). With increasing recognition of the fact that ligand-independent activation of the androgen receptor might account in part for androgen-independent growth of prostate cancer cells, this aspect of early tumour progression seems to be a promising starting point for exploring new therapies directed against androgen independence, a potentially manageable problem in prostate oncology (Akakura et al. 1993, Bruchovsky et al. 1997).

Acknowledgements
This work is supported by the United States National Institute of Health, George M O’Brien Research Center Grant (P50 DK47656) and US Army Medical Research and Materiel Command DOD Prostate Research Program Grant (DAM17-98-1-8577). According to the general
References


Aronica SM & Katzellenbogen BS 1993 Stimulation of estrogen receptor-mediated transcription and alteration in the phosphorylation state of the rat uterine estrogen receptor by estrogen, cyclic adenosine monophosphate, and insulin-like growth factor-I. Molecular Endocrinology 7 334-352.


Berrevoets CA, Doesburg P, Steketee K, Trampan J & Brinkmann AO 1998 Functional interactions of the AF-2 activation domain core region of the human androgen receptor with the amino-terminal domain and with the transcriptional coactivator TIF2 (transcriptional intermediary factor 2). Molecular Endocrinology 12 1172-1183.


Cho H & Katzenellenbogen BS 1993 Synergistic activation of estrogen receptor-mediated transcription by estradiol and protein kinase activators. Molecular Endocrinology 7 441-452.


Culig Z, Hobisch A, Hittmair A, Cronauer MV, Radmayr C, Zhang J, Bartsch G & Klocker H 1997 Synergetic activation of androgen receptor by androgen and luteinizing hormone-


El-Tanani MKK & Green CD 1997 Two separate mechanisms for ligand-independent activation of the estrogen receptor. *Molecular Endocrinology* **11** 928-937.


Endocrine-Related Cancer (1999) 6 487-502


