International Congress on Hormonal Steroids and Hormones and Cancer

Androgen receptor involvement in the progression of prostate cancer

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Abstract

Since the growth of prostate cancer is androgen-sensitive, metastatic disease has been treated by hormonal therapy. Almost all prostate cancer patients initially respond to hormonal therapy, but the majority gradually develop resistance. The mechanism of the change in tumors from being androgen-responsive to androgen-unresponsive is generally explained by clonal selection, adaptation, an alternative pathway of signal transduction and androgen receptor (AR) involvement. Since androgen action is mediated by ARs, abnormalities in ARs are believed to play an important role in the progression of prostate cancer. Hyperactivated AR gene mutations have been detected in 20–30% of hormone-refractory tumors and functional analyses have demonstrated a wide responsiveness to estrogens, progesterone and anti-androgens as well as to androgens. The AR is highly amplified in 30% of patients with hormone-refractory prostate cancer that has been treated by castration without anti-androgens. Immunohistochemical studies of ARs in hormone-refractory prostate cancer specimens have shown that AR protein is down-regulated. DNA hypermethylation of the AR promoter region leading to AR down-regulation has been identified in 30% of hormone-refractory prostate cancers. The AR N-terminal domain in the LNCaP cell line model is activated by interleukin-6 via mitogen-activated protein kinase and single transducers and activators of transcription 3. Epidemiological observations have shown that short CAG repeats are more frequently associated with higher transactivational function in the African-American population, which may explain racial differences in the incidence of prostate cancer. Among Japanese, a short CAG repeat appears to predict a response to hormonal therapy, indicating a positive prognostic value and good prognosis at the metastatic stage of prostate cancer. Several co-factors between ARs and the transcriptional complex have been cloned and reports indicate that steroid receptor co-activator 1 is correlated with the hormone-refractory progression of prostate cancer.

Thus, ARs plays an important role in the progression of prostate cancer. Based on the findings described above, genetic diagnosis and/or molecular-targeted therapy via AR pathways can be developed for hormone-refractory states.

Endocrine-Related Cancer (2003) **10** 209–216

Introduction

Loss of hormone sensitivity in prostate cancer

The enzyme 5α-reductase type 2 is responsible for converting testosterone to the more potent 5α-dihydrotestosterone in the prostate. The actions of both testosterone and 5α-dihydrotestosterone are mediated by the intracellular androgen receptor (AR) (Kokontis & Liao 1999). The AR belongs to the superfamily of nuclear receptors that mediates the actions of steroids, retinoids, vitamin D3 and thyroid hormones (Zilliacus *et al.* 1995). The AR is a ligand-activated transcription factor that mediates the biological responses of androgens. These receptors have similar structures that are composed of an N-terminal domain (NTD) that is involved in transcriptional activation, a DNA-binding domain, a hinge region and a ligand-binding domain. After the ligand binds to the AR, the ligand–receptor complex translocates to the nucleus and binds specific androgen-response elements on the chromosome. The AR might regulate the expression of various genes (Fig. 1). For example, prostate-specific antigen is up-regulated by androgens in the

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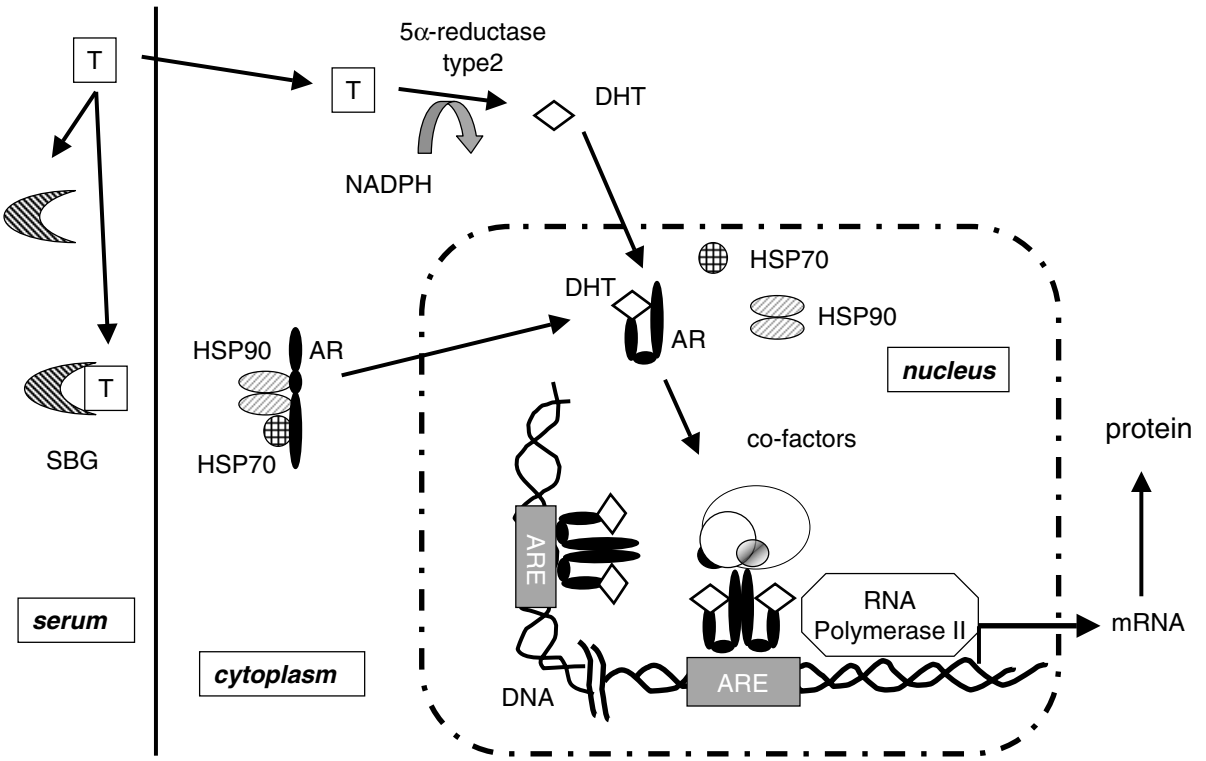

Figure 1 Mechanism of ligand-dependence of AR. ARE, androgen-response element; DHT, dihydrotestosterone; HSP, heat shock protein; SBG, sex hormone-binding globulin; T, testosterone.

prostate. The AR is expressed in both androgen-dependent and -independent prostate cancers. Therefore, ARs might play an important role in the progression of androgen-independence in prostate cancer. Most patients with metastatic prostate cancer initially respond to androgen-ablation therapy. However, the cancer often recurs as an androgen-independent tumor that is difficult to treat. Thus, the progression from androgen-dependence to androgen-independence is a critical step in the development of prostate cancer, yet the molecular mechanism is poorly understood.

Loss of androgen sensitivity is generally considered to have four causes: selection of cancer clones; adaptation of cells to an environment without androgen; an alternative pathway of signal transduction; and involvement of ARs (Grossmann *et al*. 2001, Gelmann 2002, Navarro *et al*. 2002). The present article focuses on AR involvement in the progression of androgen-responsive prostate cancer to being androgen-unresponsive.

Over-expression (amplification) of ARs in prostate cancer

Visakorpi *et al*. (1995) found a high level of AR amplification at the DNA and RNA levels in seven of 23 (30%) hormone-refractory prostate cancer patients and in none of the specimens obtained from the same patients before therapy. However, almost all of the patients whose tumors overexpressed ARs underwent androgen-deprivation monotherapy without the administration of anti-androgens. Another report by Palmberg *et al*. (2000) evaluated whether AR gene amplification at the primary progression of prostate cancer is associated with a response to a second-line maximum androgen blockade (MAB). Patients with AR gene amplification also had a decrease in serum prostate-specific antigen (PSA) more often after MAB than those with no amplification ($P = 0.079$). Amplification of the AR gene detected in tumors that progress during androgen-deprivation monotherapy is associated with a favorable treatment response to second-line MAB. This finding suggests that at least some tumors with amplified ARs retain a high degree of dependence on residual androgens remaining in the serum after monotherapy. These findings indicate that the loss of androgen sensitivity in these patients was caused by the growth of cancer clones stimulated by remaining androgen produced by the adrenal glands, thus suggesting the importance of MAB therapy.

AR gene mutations in prostate cancer tissue

The initial drive to deal with AR gene mutations in prostate cancer originated from a study of the LNCaP cell line that is

derived from a metastatic lesion of the lymph nodes of a patient with prostate cancer (Veldscholte *et al*. 1990). The AR gene of this cell line contains one mutation at codon 877 (Thr to Ala). The growth of LNCaP cells is stimulated *in vitro* by androgens, estrogens, progestogens and several anti-androgens, indicating a widely responsive property of the LNCaP cell.

Mutations in the AR gene have been detected in about 10–20% of prostate cancer specimens. The frequency of mutation generally appears higher in hormone-refractory, metastatic tumors compared with untreated lower-grade primary tumors (Suzuki *et al*. 1993, 1996, Gaddipati *et al*. 1994, Taplin *et al*. 1995, 1999, Marcelli *et al*. 2000). Since the growth of early-stage prostate cancer appears to be mediated by wild-type ARs, receptor mutation appears to function in conferring a growth advantage on cells during progression. Functional analyses of several AR mutations detected in hormone-refractory cancers have revealed the same response variety as those of LNCaP cells (Table 1).

AR gene mutations have been identified in five of 16 patients treated by MAB with flutamide, which powerfully stimulates these mutant ARs (Taplin *et al*. 1999). In contrast, AR mutants detected in 17 patients treated by androgen-ablation monotherapy were not stimulated by flutamide. These findings, together with the clinical history of the patients, indicated that the AR mutations arose in response to strong selective pressure exerted by flutamide. Thus, AR gene mutation might be one molecular mechanism through which prostate cancer loses androgen-dependence.

Given that certain mutations can alter AR ligand specificity, AR mutation might play a key role in 'anti-androgen withdrawal syndrome' (Kelly & Scher 1993, Suzuki *et al*. 1996). This phenomenon occurs in a subset of patients who experience a relapse of tumor growth, and it is characterized by an increasing serum PSA concentration after long-term anti-androgen treatment. The cessation of anti-androgen medication improves symptoms and serum PSA levels decrease, suggesting that the anti-androgen acts agonistically in the tumor cells to promote growth. Our previous study found that in two of four patients who experienced improvement after anti-androgen withdrawal (out of 22 total prostate cancer patients), AR mutations occurred during anti-androgen treatment (Suzuki *et al*. 1996). These mutations were identical to that in LNCaP cells (T877A), and were undetected in untreated tumors. This finding together with the reports of others (Taplin *et al*. 1999, Balk 2002), indicates that 'anti-androgen withdrawal syndrome' is caused by AR 'hyperactivated' mutation.

Down-regulation of ARs in endocrine therapy-resistant prostate cancers – hypermethylation of AR promoter region

Immunohistochemical studies of ARs in prostate cancer have revealed heterogeneous expression of the AR, as it is a feature of the normal prostate, benign prostatic hypertrophy and of prostate cancers including those that are resistant to endocrine therapy (Masai *et al*. 1990, Takeda *et al*. 1996). The ratio of AR-positive cells was negatively related to histological grade (Gleason score) in prostate cancer specimens. In addition, comparisons of AR status before and after endocrine therapy within the same patient showed that the AR was down-regulated during the loss of androgen responsiveness. The exact mechanism for this process remains obscure, but the following pathway could be one explanation.

Many types of malignancies are associated with the DNA hypermethylation of some tumor suppressor genes (i.e. VHL, RB, p16/MTS1/CDK4I etc.) (Baylin *et al*. 1998, Nakayama *et al*. 2001). The main target of regional hypermethylation is normally unmethylated CpG islands located in gene promoter regions. This hypermethylation correlates with transcriptional repression that can serve as an alternative to coding-region mutations to inactivate the genes. Hypermethylated CpG islands are located at the estrogen receptor promoter in breast cancer tissues. Our recent study (Nakayama *et al*. 2000) showed that *in vitro* DNA methylation of the AR promoter in CpG islands is associated with a loss of AR expression in human prostate cancer cells and tissues (Fig. 2). The AR was more frequently hypermethylated in hormone-refractory prostate cancer tissues (29%) compared with untreated primary tissues (10%). These results showed that hypermethylation of the AR promoter region down-regulates AR expression, suggesting one mechanism for the loss of androgen-responsiveness. In this context, our previous study

Table 1 Hyperactivated AR mutations in hormone-refractory prostate cancers

	DHT	Estradiol	Progesterone	Niltamide/flutamide
Wild-type	+ + +	–	–	+ +
V715M*	+	–	+ +	+ +
A721T	+	–	–	+
H874Y*	+	+ +	+ +	+ +
T877A#	+	+ +	+ +	+ +
T877S*	+	+ +	+ +	+ +
Q902R	±	–	–	–

*These mutated ARs were found in the patients who had been treated with flutamide.
#This mutation is identical to that found in LNCaP cell line.

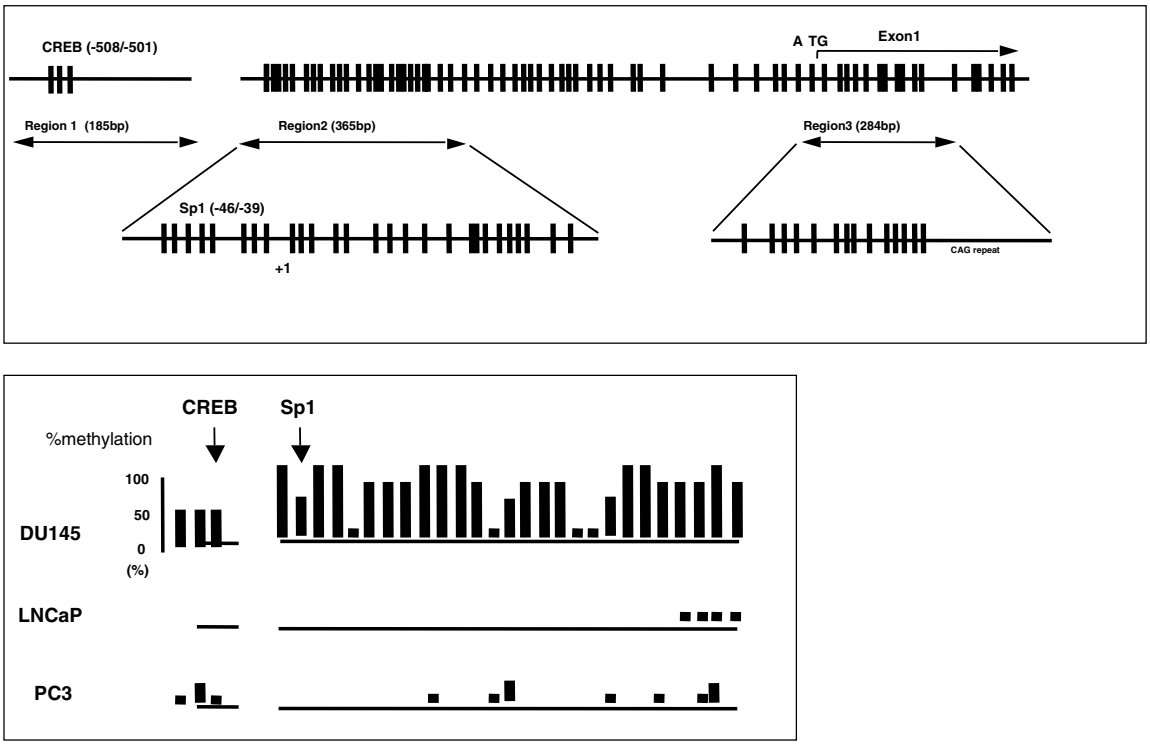

Figure 2 Hypermethylation analysis of AR promoter. Upper panel: AR promoter was divided into three regions and methylation was analyzed. Lower panel: AR promoter is highly methylated in the DU145 prostate cancer cell line. No CpG islands are methylated in control cells. DU145 and PC3 are AR-negative prostate cancer cell lines. LNCaP cell line is AR-positive. CREB, cAMP-response element-binding protein; Sp1, specificity protein-1.

demonstrated that resumption of androgen-dependent processes (i.e. introduction of AR into AR-negative cells) reduces the growth rate, accompanied by changes in phenotype, suggesting that AR activity is a tumor suppressor (Suzuki *et al*. 1994).

Androgen-independent activation of AR activation by interleukin-6 (IL-6)

Recent studies have postulated that androgen-independent activation of the AR mediates the androgen-independent progression of prostate cancer in the absence of androgen. Ligand-independent activation of the AR was first demonstrated in DU145 prostate cancer cells treated with growth factors such as epidermal growth factor (EGF), keratinocyte growth factor and insulin-like growth factor-I (Culig *et al*. 1994). Sadar (1999) has shown that the activator of the protein kinase A pathway, forskolin, up-regulates the transcriptional activity of the AR in a ligand-independent manner. The EGF receptor-related protein HER-2/neu also activates the AR in the absence of ligand (Craft *et al*. 1999). Moreover, some groups have shown that butyrate, luteinizing-hormone releasing hormone, caveolin, thyroid hormone and IL-6 ligand-independently activate AR.

Several clinical investigations have shown that serum levels of IL-6 are significantly elevated in patients with hormone-refractory disease (Drachenberg *et al*. 1999, Nakashima *et al*. 2000). These findings indicate that IL-6 is involved in the androgen-independent progression of prostate cancer. IL-6 receptor is also expressed in both prostate cancer tissues and prostate cancer cell lines. IL-6 is a multifunctional cytokine that plays an important role in the regulation of hematopoiesis, the immune response, inflammation, bone metabolism and neural development and it can increase the proliferation of some types of cancer cells, including prostate cancer. Therefore, IL-6 is considered to be an autocrine or a paracrine growth factor (Okamoto *et al*. 1997). IL-6 binding to its receptor activates signal transducers and activators of transcription (STAT3), as well as mitogen-activated protein kinase (MAPK) in LNCaP cells. These two components mediate signaling cross-talk between steroid nuclear receptors and other signaling pathways. MAPK is involved in estrogen-independent activation by EGF. STAT3 can co-activate glucocorticoid-bound glucocorticoid receptor and lead to its synergistic activation in combination with IL-6 and glucocorticoid. Ueda *et al*. (2002) have reported that IL-6 increases MAPK phosphorylation and activates the AR NTD. Direct interaction between amino acids 234–558 of the AR

NTD and STAT3 in IL-6 treated LNCaP cells has been shown. Figure 3 shows that IL-6 activates human AR NTD through a mechanism that is dependent upon MAPK and STAT3 signal transduction pathways in LNCaP prostate cancer cells. These results suggest that IL-6 is involved in the androgen-independent progression of prostate cancer.

Involvement of co-regulators (co-factors)

Several co-regulators of AR and transcriptional complex have been cloned. Yeh & Chang (1996) have cloned ARA70 as a specific co-regulator for AR and demonstrated that ARA70 functions as a transcriptional activator in DU145, which is an AR-negative prostate cancer cell line, in the presence of androgen. Figure 4 shows AR-interacting proteins, some of which are involved in the progression of prostate cancers. For example, transcriptional intermediary factor 2 and steroid receptor co-activator 1 (SRC-1) are up-regulated in hormone-refractory prostate cancers.

SRC-1 was the first member of the co-activator family to be identified (Onate *et al*. 1995). SRC-1 protein enhances transcriptional activity through intrinsic histone acetyltransferase activity. SRC-1 up-regulates the transcriptional activity of the AR in a ligand-dependent manner and this process is regulated through phosphorylation by MAPK. However, the role of SRC-1 in androgen-independent prostate cancer is not clear. One mechanism of ligand-independent AR activation might include the increased expression of AR co-activators such as SRC-1. Recent studies have shown that SRC-1 expression is increased in many recurrent prostate cancer tissues (Gregory *et al*. 2001). Some groups have shown that SRC-1 interacts with the nuclear receptors in a ligand-independent manner. SRC-1 enhances the activation function-1 activity of the ER (in the presence of both estradiol and tamoxifen (Webb *et al*. 1998)). SRC-1 co-activates ligand-independent activation of the chicken progesterone receptor (cPR). The extracellular signal-regulated kinase kinase inhibitor, U0126, inhibits the 8-bromo-cAMP-dependent and progesterone-dependent activation of cPR (Rowan *et al*. 2000). SRC-1 interacts directly with the AR NTD via a conserved glutamine-rich region between residues 1053 and 1123 and enhances IL-6-induced ligand-independent activation of the AR NTD via a MAPK-dependent pathway in LNCaP human prostate cancer cells. Inhibition of the phosphorylation of either SRC-1 or the AR NTD does not prevent protein–protein interactions, but rather transactivation. This implies that protein–protein interaction between SRC-1 and the AR NTD is independent of MAPK activity. A mechanism additional to MAPK phosphorylation of SRC-1 may be required for ligand-independent activation of the AR (Ueda *et al*. 2002). These results suggested that interactions between the AR and co-activators represent

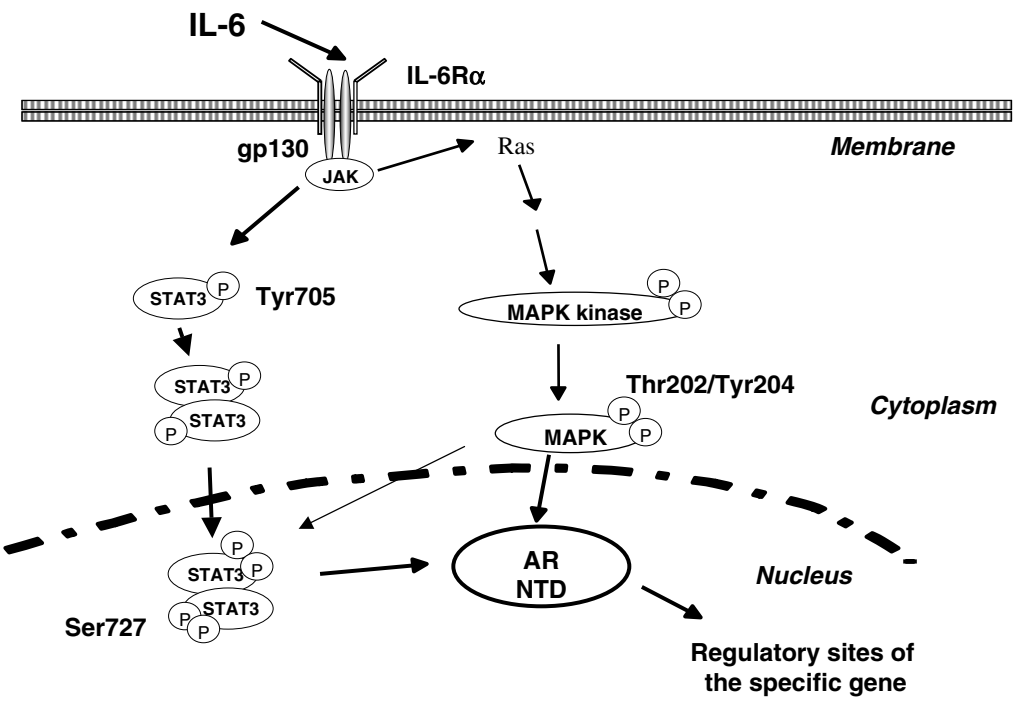

Figure 3 Hypothetical model of signal transduction pathways leading to ligand-independent activation of AR by IL-6 in LNCaP cells. Signals are IL-6-mediated. The IL-6 signaling cascade induces up-regulation of AR-regulated genes such as PSA by ligand-independent activation of AR, which is stimulated by both phosphorylation of STAT3 and MAPK.

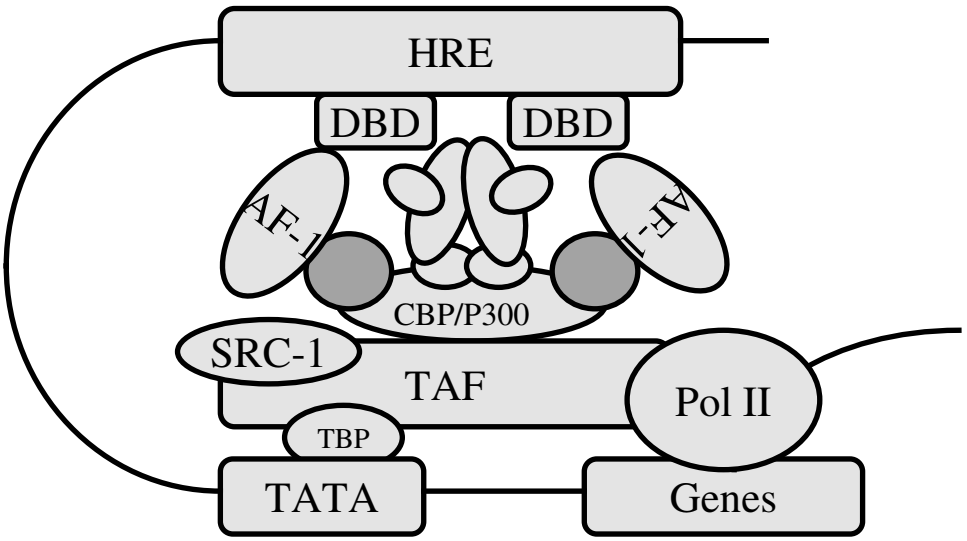

Figure 4 Co-factors (regulators) between AR and transcriptional complex. HRE, hormone-responsive element; DBD, DNA-binding element; TAF, transcription activation function; AF-1, activation function-1; CBP, CREB-binding protein.

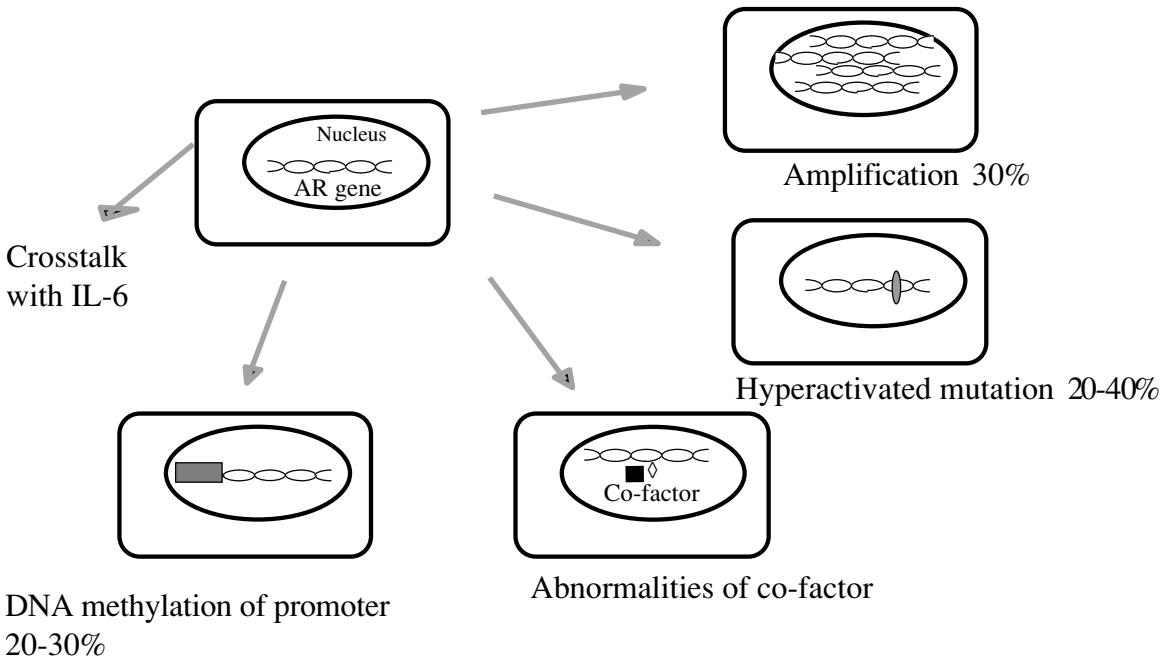

Figure 5 Summary of AR involvement in progression of prostate cancer.

another therapeutic target of treatment for androgen-independent prostate cancer.

Short CAG repeats in the NTD of AR

The incidence of prostate cancer is highly variable among races. The incidence is highest among people of African origin and lowest among Asians. Several reports have shown that shorter polyglutamine and polyglycine repeats correlate with a higher transactivational function or expression level of ARs, which is associated with an increased risk of prostate cancer (Montgomery *et al*. 2001). Hardy *et al*. (1996) found a significant correlation between reduced CAG repeat length and age at onset of prostate cancer, suggesting that CAG

repeat length impinges on mechanisms involved in tumor initiation but not in the progression of localized to advanced cancer. We recently found that shorter CAG repeats can predict the marker response of Japanese patients with metastatic prostate cancers (Suzuki *et al*. 2002). Thus, polymorphisms of endocrine-related genes may have the potential to predict responses to hormonal treatment and patient prognosis.

Conclusions

Androgen-ablation therapy has been an important modality in the treatment of disseminating prostate cancer for nearly 60 years. However, loss of androgen-dependence in prostate cancer remains a key dilemma in treating this malignancy. Since prostate cancer is highly heterogeneous (Suzuki *et al*. 1998), several mechanisms may simultaneously contribute to the loss of androgen-dependence within the same patient.

Acknowledgements

This paper is supported in part by Grant-in Aid for Scientific Research from the Ministry of Education, Science, Technology and Culture, Japan (13671635) and the Ministry of Health, Labour and Welfare, Japan (Aid for Cancer Research) and Japanese Society of Strategies for Cancer Research and Treatment (2001).

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