Invited Review

Hyperactive androgen receptor in prostate cancer: what does it mean for new therapy concepts?

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Summary. Investigations on androgen signaling alterations in the late stages of prostate cancer revealed new molecular mechanisms that may be in part responsible for failure of endocrine therapy. Both primary and metastatic lesions from prostate cancer express androgen receptor protein. Amplification of androgen receptor gene occurs in a subset of prostate cancer patients. Several point mutations of androgen receptor gene have been described; they generate receptors which are functionally activated by androgens, other steroids, and even by antihormones. The frequency of androgen receptor mutations may be high in tumor metastases. Functional activity of androgen receptor is influenced by nonsteroidal factors, such as peptide growth factors and second messengers. Thus, prostate cancer cells adapt to low androgen environment by various mechanisms utilizing androgen receptor. Therefore, new strategies for switching off the androgen receptor are needed.

Key words: Steroids, Antiandrogens, Prostate tumor metastases, Androgen receptor activation

Introduction

Carcinoma of the prostate is the most commonly diagnosed malignant neoplasm and the second leading cause of tumor-related death in the male population in the Western world (Borring et al., 1993). The crucial role of androgens in promoting growth of the normal prostate and prostatic tumors in the early stages is well-established (Huggins and Stevens, 1940). Several functions of the prostate depend on the presence of the androgen receptor (AR), a transcriptional regulator that is activated by androgens and modulates expression of specific genes (Rundlett et al., 1990). Androgen deprivation results in the programmed death of prostatic glandular cells (Kyprianou and Isaacs, 1988). This is the basis for endocrine therapy for prostatic carcinoma which decreases the level of circulating androgens and/or blocks the transcriptional regulatory function of the AR. However, it is only temporarily effective and virtually all tumors progress into the therapy-refractory stage (Crawford et al., 1990). It was believed for a long time that the androgen signal transduction cascade is inactivated in endocrine therapy-resistant prostatic carcinoma.

Recent findings obtained in studies on AR expression and alterations of the androgen signal transduction pathway in advanced prostatic cancer provided new insight into the mechanisms enabling tumor cells to adapt to low concentrations of androgen. These results have several implications for endocrine therapy. Therefore, a new concept for so-called "androgen-independent" carcinoma of the prostate is needed and this paper is focused on issues which we consider essential for a better understanding of resistance to endocrine therapy, like AR structural alterations, its up-regulation following long-term androgen ablation, and the modulation of its activity by nonsteroidal factors.

Androgen receptor expression in late stages of prostate cancer

Early data on AR expression in prostate cancer were collected in studies on rat and human tumor cell lines. In the rat Dunning tumor system, progression is accompanied by a decline in AR levels, as revealed by ligand binding, Northern blot analysis and immunohistochemical studies (Quarmby et al., 1990). In vivo AR-negative sublines were found to exhibit aggressive behavior (Isaacs et al., 1986; Steiner and Barrack, 1992). The same correlation was found in three unrelated cell lines obtained from patients with metastatic lesions. Cells expressing very low levels of AR (PC-3) and those which are devoid of AR (DU-145) are also more aggressive than AR-positive LNCaP cells (Kozlowski et al., 1984; Tilley et al., 1990; Gleave et al., 1991; Culig et al., 1993a,b). As a consequence of these findings one would expect that in vivo the AR is lacking in the majority or, at least, a considerable percentage of resistant prostate cancers. However, the results of two
independent studies published in 1991 are in contrast to this view (Sadi et al., 1991; van der Kwast et al., 1991). In fact, as shown by immunohistochemistry, nearly all primary prostatic carcinomas, even those which recurred after ablation therapy, contained the AR. These observations were confirmed by other groups, including those who used computer-assisted video image analysis to assess AR concentration per cell to permit better prediction of tumor behavior (Sadi and Barrack, 1993; Ruizeveld de Winter et al., 1994; Tilley et al., 1994). AR expression in therapy-refractory tumors is obviously accompanied by an amplification of the AR gene. Visakorpi and colleagues screened samples obtained from patients who underwent endocrine therapy for genetic aberrations by comparative genomic hybridization and fluorescence in situ hybridization and found AR gene amplification in about 30% of recurrent tumors (Visakorpi et al., 1995). Amplification occurred during therapy; none of the specimens obtained from these patients prior to treatment had an amplified AR gene. One must assume that these genetic alterations result in an increased AR level and reflect an adaptation to conditions of low androgen concentrations. Consistent with these observations in vivo, Kokontis et al. showed that LNCaP cells cultured in androgen-depleted medium for about 60 passages up-regulate AR levels and its transcriptional activity (Kokontis et al., 1994). As a consequence of such changes in AR responsiveness low androgen concentrations like those supplied by the adrenal glands would suffice for triggering tumor growth.

Until recently it was still unknown whether AR protein is expressed in metastatic lesions from prostatic carcinomas. To address this question we performed immunohistochemical studies on samples of lymph node metastases obtained from patients who underwent radical prostatectomy and samples of distant metastases from patients who did not respond to endocrine therapy (Hobisch et al., 1995, 1996). All samples except one lymph node metastasis stained for the AR. These findings were confirmed by Taplin and colleagues who detected high AR mRNA levels in bone marrow metastases from patients presenting tumor progression after endocrine therapy (Taplin et al., 1995). Immunohistochemical data on AR expression in metastatic lesions from prostatic carcinoma were also recently provided by Kleinerman and colleagues (Kleinerman et al., 1996); these authors found that AR-positive nuclei were predominant in 42 bone metastases obtained from 38 patients. Again, there was no tumor metastasis which was AR-negative. Taken together, these new data have clearly shown that the changes in the androgen signaling pathway in human prostatic carcinoma are different to those in the Dunning tumor model.

**Significance of mutant androgen receptors in prostatic tumors for endocrine treatment**

One might argue that the detection of ARs in specimens from patients unresponsive to endocrine therapy does not necessarily imply that these receptors are functional. Performing functional studies on the AR in its natural cellular environment is by no means an easy task. Therefore, many groups were searching for structural alterations of the AR; these studies led to the identification of mutant receptors. The first mutant AR in prostatic carcinoma was detected in LNCaP cells (Harris et al., 1990; Veldscholte et al., 1990); several others were subsequently found in tumor material obtained from prostate cancer patients (Newmark et al., 1992; Culig et al., 1993a,b; Suzuki et al., 1993; Castagnaro et al., 1993; Elo et al., 1995; Taplin et al., 1995; Evans et al., 1996; Sharief et al., 1996; Uchida et al., 1996). In the majority of the reports the frequency of AR mutations in primary tumors of the prostate was found to be low regardless of tumor stage and grade. In contrast, Tilley et al. detected mutations in 11 out of 25 prostate tumor specimens obtained prior to initiation of hormonal therapy (Tilley et al., 1996). These discrepancies in frequency of AR mutations in the primary lesions are currently a subject of debate.

The function of some of the mutant receptors was analyzed in heterologous and prostate cancer cells devoid of endogenous steroid receptors. All mutant ARs that have been functionally characterized are so-called "promiscuous receptors" because they are stimulated not only by synthetic and testicular androgens but also by other ligands such as adrenal androgens, dihydrotestosterone metabolites, estrogenic and progestagenic steroids, and even by nonsteroidal antiandrogens (Culig et al., 1993a,b; Klocker et al., 1994; Elo et al., 1995; Peterziel et al., 1995; Taplin et al., 1995; Bubley et al., 1996; Tan et al., 1996). It is interesting to note that there are obvious differences between the action of hydroxyflutamide and casodex with mutant receptors; agonistic properties have been frequently reported for hydroxyflutamide but not for casodex (Veldscholte et al., 1990; Klocker et al., 1994; Peterziel et al., 1995; Bubley et al., 1996). Enhanced activation of a mutant AR by androgen precursors and metabolites as well as by antiandrogens is one possibility whereby prostatic tumor cells adapt to low androgen levels.

According to Taplin et al., mutant ARs are present far more frequently in bone marrow metastases from prostatic cancer than in primary tumors (Taplin et al., 1995). In that study mutant ARs were identified in the metastatic lesions in half of the 10 patients investigated. Two of the mutant receptors were functionally characterized and their promiscuous properties were confirmed. Thus, a variety of aberrant responses to circulating steroids and administrated antiandrogens seem to occur in metastatic lesions. So far there is no evidence that AR mutations in prostate cancer cause inactivation of the receptor in clinically manifest tumors. Frequent inactivating AR mutations have been recently described in latent carcinomas of the prostate in Japanese males (Takahashi et al., 1995). If confirmed, these findings may provide at least a partial explanation.
for marked racial differences in the incidence of prostate cancer.

In the past few years, several clinical studies have been published on the antiandrogen withdrawal syndrome (summarized by Moul et al., 1995). In some patients with progression of the disease, discontinuation of nonsteroidal and steroidal antiandrogens led to temporary improvement of their condition and to a decrease in the prostate-specific antigen levels. This phenomenon was first observed upon flutamide withdrawal, but more recently also after casodex and megestrol acetate withdrawal (Small and Carroll, 1994; Dawson and McLeod, 1995). Structural changes of the AR causing an agonistic action of antiandrogens is one possible explanation for this paradoxical response. However, conclusive evidence is still lacking. Interestingly, tumor tissue from one patient who showed this response to flutamide withdrawal contained a mixture of two ARs; one with 18 and the other one with 24 CAG repeats which encoded the poly-glutamine region in the N-terminus of the receptor. Adjacent normal tissue contained the AR with 24 CAG repeats (Schoenberg et al., 1994). Reduction of the CAG repeats in the AR molecule is associated with increased transactivation activity of the receptor (Chamberlain et al., 1994). The prevalence of short CAG microsatellites was highest in African-American males with the highest risk for prostate cancer, intermediate in non-Hispanic whites, and lowest in Asians. Compared to the distribution of the size of CAG repeats in a population there was a shift towards shorter repeats in those individuals with prostate cancer. Based on these results it was speculated that AR gene polymorphisms may be in part responsible for racial differences in prostate cancer incidence.

Extended concept of hypersensitivity in prostate cancer cells

All findings obtained in recent studies on androgen responsiveness in advanced prostatic carcinoma indicate development of a hypersensitive tumor cell during endocrine therapy. This enables the tumor to activate the androgen signaling cascade despite low androgen supply. A conception of hypersensitive cells was first introduced by Labrie and his group. They studied the response to androgen of Shionogi mouse mammary carcinoma cells in culture (Labrie and Veilleux, 1986). Some subclones obtained from this tumor displayed hypersensitivity to dihydrotestosterone and were stimulated at much lower hormone concentrations than the parental cells. We argue for a more extended concept of hypersensitivity in prostatic tumor cells. In our view, it refers to the stimulation of specific cellular events by very low doses of androgen alone, by other steroids or antihormones, by growth factors in the absence of androgen, and by a combination of low doses of androgen and either growth factors, or peptide hormones or other cellular regulators (Fig. 1).

Androgen receptor activity is modulated by nonsteroidal factors

Ligand-independent activation of the AR and modulation of AR activity by regulators of cellular

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**Fig. 1.** The hyperresponsive AR. Selection during endocrine therapy results in tumor cells with the hyperactive androgen signaling cascade. A key element in this process is the AR (N: nucleus). AR in advanced prostatic carcinoma may be activated, in addition to testicular androgens, by other steroid hormones, antiandrogens, growth factors and peptide hormones.
function in cooperation with androgen have recently been described. In DU-145 cells transiently expressing the AR, AR activity was stimulated to different extents by three polypeptide growth factors, namely insulin-like growth factor-I, keratinocyte growth factor and epidermal growth factor (Culig et al., 1994). Consistent with these observations, Reiter and colleagues recently demonstrated that insulin-like growth factor-I stimulates transcription of the androgen-inducible prostatein C3 subunit gene in the rat prostate (Reiter et al., 1995). Furthermore, low doses of androgen and insulin-like growth factor-I acted in a synergistic manner to increase the transcriptional activity of the AR (Culig et al., 1995). A synergism between androgen and a growth factor, in this case epidermal growth factor, was also observed in PC-3 cells stably transfected with AR cDNA (Brass et al., 1995). In addition to growth factors, AR activation by androgens is enhanced by luteinizing hormone-releasing hormone and cyclic adenosine monophosphate, which is a second messenger in signaling pathways of many peptide hormones and cytokines (Ikonen et al., 1994; Culig et al., 1995). These synergisms significantly lower ligand concentrations required for activation of the androgen signaling pathway and thus contribute to AR hypersensitivity. Recent studies have provided evidence that there is a shift towards increased expression and autocrine production of growth factors during progression of prostate cancer (Sherwood and Lee, 1995; Culig et al., 1996). Thus, the basic requirements for increased AR activation by nonsteroidal factors are present and this pathway is probably relevant to the active role of the AR in the progression of prostate cancer.

Conclusions

In summary, the studies described imply that advanced prostate tumor cells are indeed dependent on an active androgen signaling cascade. There is now conclusive evidence that prostate tumors adapt to an environment with low androgen supply by utilizing a hyperactive AR. The mechanisms involved in this adaptation are mutations of the AR generating promiscuous receptors, increased receptor expression and increased activation through other signaling pathways. Therefore, new strategies for switching off the AR are needed in prostate cancer therapy. Investigations in this field should focus on testing candidate substances for new antiandrogens or application of antisense oligonucleotides, or other means for blocking AR function, and therapy schemes that slow-down development of a hyperactive AR, like intermittent androgen suppression. Tumor progression in the in vivo LNCaP model can be delayed approximately 3-fold by the repetitive induction of apoptosis with periodic interruption of androgen blockade compared to continuous androgen ablation (Sato et al., 1996). The variety of AR mutants and modulation of AR activity by nonsteroidal factors will certainly hamper work on new AR antagonists. However, there is a hope that improved understanding of AR-mediated signal transmission will greatly stimulate work on designing new treatment strategies.

Acknowledgements. The authors thank Professor G. Daxenbichler for critical reading of the manuscript and M. Trebo for editorial assistance. Work done in authors' laboratories was supported by grants SFB 002 P203 from the Austrian Research Funds (FWF) and Ca 130/3-1 from the German Science Foundation (DFG).

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