New directions in endocrine therapy: renewed interest in the androgen receptor

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Androgen ablation is the mainstay treatment for non-organ-confined prostate cancer. This treatment is only palliative and patients may present with disease progression because of changes in androgen signaling and other pathways. The androgen receptor (AR) could be activated in a ligand-dependent and -independent manner. Small concentrations of dihydrotestosterone present in tissues from patients with advanced prostate cancer are sufficient to activate the AR. Ligand-independent activation of the AR has been described for various cellular regulators. However, its significance for tumor progression in vivo has yet to be completely clarified. During androgen ablation, there is an increase in expression of several coactivators that contribute to AR hypersensitivity. In the majority of prostate cancers, the fusion of the TMPRSS2 gene and a member of the transcription factor ETS family were detected. The main problem associated with the development of therapy resistance in prostate cancer is the inability to develop therapies that block proliferation and anti-apoptotic function in contrast to the prodifferentiation function of the AR.

Current discussions on prostate cancer research and treatment are focused on issues such as the impact of screening on incidence of the disease and optimization of the treatment of tumor in the early stages. In fact, there are different policies and practices on that subject. Patients who present with metastatic disease and those who suffer biochemical relapse will receive endocrine therapy and initially benefit from it. This therapy, established in the middle of the 20th century, is one of the most effective treatments available for patients with metastatic solid tumors. It seems, however, that the issue of improvement of endocrine therapy is still of major interest to researchers, physicians, urologists, oncologists and radiologists, who are responsible for prostate cancer patients. Due to an increase in life expectancy in industrialized countries, one could predict that there will be a considerable percentage of prostate cancer patients who will not undergo radical treatment because of their age and possible diagnosis of other chronic diseases. Therefore, results of the most recent basic studies that led to establishment of new concepts will be presented and discussed in this review. It will focus on, among others, different approaches to address androgen receptor (AR) hypersensitivity, critical coactivators and corepressors in prostate cancer research, the possibility to induce the expression of AR downstream genes in cells with absent AR expression, the relationship between hypoxia and AR activity and chemotherapy and androgen ablation.

Androgen receptor hypersensitivity & therapeutic implications

The concept of hypersensitivity of AR was initially established in studies in which LNCaP cells were subjected to long-term steroid ablation in vitro [1]. It was demonstrated that AR expression and transcriptional activity increase under these conditions. Thus, low concentrations of androgen can elicit a stronger transcriptional response and induce proliferation of prostate cancer cells or expression of prostate-specific genes. These findings were confirmed and it became clear that AR expression and activity may increase in patients during androgen ablation therapy [2]. Mutated AR was detected in sublines of LNCaP cells subjected to prolonged treatment with bicalutamide. Such mutations were not commonly detected in prostate cancer, but they can contribute to the hypersensitive response [3]. However, the significance of many in vitro findings for the clinical situation remains unclear. The concept of hypersensitivity is also related to ligand-independent activation of the AR that is demonstrated in many experimental systems in vitro. Although it is well known that AR activity is regulated by compounds that increase intracellular cAMP levels, growth factors, growth factor receptors and cytokines, the implications of those findings for the in vivo situation are less clear [4–7]. In the LAPC4 xenograft, AR activation by ErbB2 facilitated tumor progression as indicated by shortened...
latency for tumor formation and a higher tumor volume [8]. It was demonstrated that AR tyrosine phosphorylation is important for receptor translocation, recruitment to the chromatin and regulation of growth [9]. However, analysis of ErbB2 expression in prostate cancer clinical specimens by different techniques revealed that its expression does not increase in advanced tumors [10]. On the other hand, Cdc42-associated kinase Ack 1, whose expression is increased in therapy-resistant prostate cancer activated by HER2, promotes prostate cancer progression via AR tyrosine phosphorylation [11]. It seems that HER2 signaling is required for androgenic regulation of cell growth and secretion [12]. Insulin-like growth factor-1 and EGF cause a ligand-independent or synergistic effect on AR activity depending on concentrations and cell type [5]. There are also other interactions between signaling pathways of steroids and growth factors. Androgen or estrogen receptors make an assembly with Src and EGF receptor. Therefore, it is not surprising that administration of the anti-androgen bicalutamide blocks action of epidermal growth factor in prostate cancer cells [13]. Androgen requirement for LNCaP in regulation of growth in vitro and in vivo and stimulation of AR downstream genes are reduced if the signaling pathway of Ras/mitogen-activated protein kinase is hyperactive [14]. This pathway is implicated in growth-factor signaling, and increased expression of mitogen-activated protein kinase was documented in advanced prostate tumors [15].

Other interesting analysis of genes activated in a ligand-dependent and -independent manner were carried out after stimulation by androgen and forskolin or bombesin, respectively [16,17]. Interestingly, only a limited number of genes were found to be induced by both androgen and forskolin. Genes of the kallikrein family belong to this group. The significance of those findings has to be studied in the future. A total of 72 genes were found to be similarly regulated by bombesin and androgen, whereas bombesin alone exhibited an effect on regulation of 62 genes and androgen regulated expression of more than 100 genes. Bombesin effects on
growth of AR-positive prostate cancer cells in culture were blocked by bicalutamide. Another compound that causes a ligand-independent activation of the AR is interleukin-6 (IL-6) [7]. IL-6 is a multifunctional cytokine and its effects on prostate tumor cells vary; they include inhibition of proliferation and a pro-survival effect [18]. IL-6 and its receptor are widely expressed in prostate cancer tissues and their expression may increase during androgen ablation therapy or due to the loss of expression of the tumor suppressor retinoblastoma [19]. In cells subjected to prolonged treatment with the cytokine, IL-6 acts as an autocrine stimulator of tumor cell growth, rather than as a paracrine cytokine, IL-6 acts as an autocrine stimulator of transcriptional activity by the cytokine [25]. It is and colleagues observed inhibition of transcription of AR and target genes is heterogenous in prostate tumor cells is rather heterogenous. Several AR downstream genes were found to be downregulated in tissue specimens obtained from individuals with a higher incidence of metastases or in xenografts representing advanced stage of disease even in the presence of the AR [26]. Silencing of AR target genes may occur because of epigenetic changes, such as hypermethylation, in the promoter of the AR gene or those genes themselves. Aberrant AR gene methylation of the 5’CpG island was first described by Jarrard and colleagues in AR-negative cell lines and metastatic tissue specimens [27]. Taken together, all the findings, including those from patients’ autopsies and indicate that expression of AR and target genes is heterogenous in prostate cancer [28]. For this reason, appropriate targeting of multiple metastatic lesions in prostate cancer is still a problem.

One of the most important recent discoveries in the field of androgenic signaling is that of the fusion of the 5’untranslated region of the TMPRSS2 gene and either ERG or ETV1 ETS transcription factor member in the majority (>50%) of prostate cancer samples [29]. The association between this fusion and prostate cancer progression is, however, not completely understood. Petrovits and colleagues reported that higher expression of ERG is associated with better clinical outcome, whereas Perner and colleagues observed a trend towards biochemical recurrence in patients with the fusion [30,31]. The TMPRSS2–ERG fusion may be present in the absence of gene expression in late-stage prostate cancer, in which there is no AR expression [32]. On the other hand, it seems that the presence of the fusion is associated with an increase in death rate in patients in a watchful waiting cohort [33]. Thus, detection of the fusion may be important to discriminate between the patients who will need radical therapy from those with an indolent disease. To date, some papers support association of the TMPRSS2–ETS transcription factor fusion with morbidity, but the question of its relationship with the disease stage, grade and prognosis is not completely answered.

Residual androgen receptor ligands in recurrent prostate cancer

Analysis of expression of androgens by liquid chromatography/electrospray tandem mass spectrometry revealed that dihydrotestosterone levels present in the tissue are still sufficient for AR activation [34]. Thus, dihydrotestosterone decreases by more than 90% in prostate specimens, but the signaling through the AR pathways is preserved. Adaptation of prostate cancer cells to androgen-deprivation therapy could also be explained by enhanced expression of various genes that mediate androgen metabolism, such as aldo-keto reductase family 1 member C3 [35]. Consistent with measurements of androgen level in tissues from patients with advanced prostate cancer, major transcripts of androgen-regulated genes were detected in progressive prostate cancer [35,36]. Strongly diminished expression of dihydrotestosterone could be attributed to reduced activity of 5α-reductase that is also seen in metastatic lesions of prostate cancer [37].
knowledge on expression of adrenal androgens in prostate cancer in individual cases is important because of the prediction of responsiveness to drugs such as ketoconazole [38]. It is used as an inhibitor of adrenal androgen synthesis and it was demonstrated that higher levels of adrostenedione, an adrenal androgen, predict a favorable response to ketoconazole.

Identification of androgen receptor coactivators & corepressors of importance for prostate cancer progression

Progress in AR-coregulator research has been achieved because of availability of antibodies and application of siRNA technology. Interestingly, androgen-induced and -repressed coactivators have been identified in human prostate cancer. The fact that androgen ablation leads to an increase in expression of several coactivators arises questions about timing and duration of the treatment. Nuclear accumulation of the Tip60 coactivator was observed in biopsies obtained from patients with therapy-resistant prostate cancer [39]. In vitro experiments revealed androgenic repression of Tip60. Importantly, Tip60 could be recruited to the PSA promoter in androgen-independent prostate cancer cells in the absence of androgen. CBP and p300 are the two cofactors that are also upregulated by androgen ablation [40,41]. Those findings are of clinical relevance because of increased activation of the AR by the antiandrogen hydroxyflutamide in cells in which CBP is overexpressed [42]. Gelsolin is another AR coactivator that is regulated in a similar way, which is overexpressed in patients’ specimens following androgen ablation. It potentiates agonistic effects of hydroxyflutamide [43]. PSA could be induced in cells in which AR expression is not detectable if p300 is overexpressed [44]. p300 is increasingly expressed in biopsies from prostate cancer with larger tumor volumes, extraprostatic extension and seminal vesicle involvement [45]. Whether this mechanism may lead to upregulation of other AR target molecules by various coactivators remains to be determined. In concordance with findings obtained with Tip60, CBP and p300, Agoulnik and colleagues found that androgens directly repress expression of the transcription intermediary factor 2 [46]. In vivo, transcription intermediary factor 2 expression is high in patients who present with relapsed prostate cancer after endocrine therapy. It seems that selective upregulation of AR coactivators substantially contributes to enhanced activation of the androgen signaling pathway in prostate carcinoma.

The question whether inhibition of one or more AR coactivators provides a therapeutic benefit in prostate cancer is a complex one. Administration of specific antisense oligonucleotides or siRNA in vitro will in most cases cause a partial inhibition of proliferation or PSA expression, but the effect may be compensated by several other coregulatory proteins. In this context, it is important to note that several cofactors have biological effects on other signaling pathways. SRC-3 (RAC3), an AR cofactor whose expression correlates with tumor grade and stage and poorer disease-specific survival, is implicated in regulation of cell death through the Akt pathway [47,48]. An important role of SRC-3 in prostate cancer in vivo was confirmed in experiments in nude mice in which its inhibition by the short-hairpin approach caused decreased growth of an AR-negative xenograft, associated with decreased proliferating-cell nuclear antigen and Bcl-2 expression [49]. SRC-3 is recruited to promoters of insulin-like growth factor-1 and insulin receptor substrate-2, thus having a critical role in the inhibition of apoptosis through the Akt pathway [50]. These findings indicate that therapeutic inhibition of SRC-3 may have implications on diverse signaling pathways and could therefore be justified. In contrast, inhibition of the SRC-1 coactivator affected growth of LNCaP cells and their androgen-independent derivative, but not that of AR-negative cells PC-3 and DU-145 [51].

Most studies that examined the role of corepressors of the AR were focused on NCoR and SMRT. Although there are a small number of studies in which their expression has been investigated in prostate cancer, the question whether these corepressors are required for activity of anti-androgens is a matter of debate. In experiments in which the levels of these corepressors were downregulated by siRNA, there was no indication for agonistic activity of bicalutamide under those conditions [52]. Bicalutamide acted as an AR agonist in cells in which AR hypersensitivity was observed after chronic androgen ablation or after long-term treatment with the antiandrogen [2,3]. Interestingly, SMRT levels are increased in prostate cancer cells and this increase may be responsible for acquisition of
therapeutic resistance to vitamin D [53]. Thus, the presence of corepressors in prostate cancer tissue is not necessarily a predictor of a more favorable disease outcome. Cyclin D1 is another corepressor of the AR and its action is similar to that of a dominant-negative receptor mutant [54]. However, if cyclin D1 is mutated in prostate cancer, its effect on inhibition of AR activity is abolished [55]. An agonistic role of AR antagonists may be observed in conditions in which the stress kinase pathway is activated by IL-1. This may result in the dismissal of the corepressor NCoR from the nucleus, thus allowing AR antagonists to act as agonists [56].

Prostate cancer stem cells, androgen receptor & endocrine therapy

Increasing interest in studying the origin and phenotype of prostate cancer stem cells also generated a discussion about the appropriateness of current therapies for prostate cancer. Prostate tumor stem cells are rare and possess a capacity for self-renewal. Cancer stem cells that express CD44, CD133 and integrin (0.1% of cells in the tumor) are AR-negative [57–59]. It is therefore obvious that current therapies that either decrease the levels of circulating androgen or block transcription activation function of the AR do not have an effect on stem cells. In recent studies, it was demonstrated that prostate cancer cells with stem cell characteristics reconstitute the human tumor in vivo [59]. Those findings may have implications on endocrine therapy for prostate carcinoma. It is clear that this therapy will not eliminate the cells with stem-cell like properties and, therefore, novel approaches have to be considered. Such therapies may represent combinations of agents that target AR-positive and AR-negative tumor cells. One of the priorities in prostate cancer research is improvement of understanding of processes that led to the development of cancer stem cells versus normal stem cells, so that selected targeting could be achieved.

A modest prolongation of the lifetime of patients with advanced prostate cancer has been reported following use of docetaxel, a microtubuli inhibitor. Docetaxel has been approved in clinics and there are experimental attempts to optimize its combination with androgen ablation therapy. The strongest inhibition of tumor growth was achieved in experiments in which docetaxel was administered before castration [60]. Interestingly, lower doses of androgen that are known to cause a mitogenic response were found to potentiate cell death caused by taxane [61].

Therapies aimed to downregulate androgen receptor expression

Several groups demonstrated that inhibition of AR expression in LNCaP cells or derivatives that grow in an androgen-independent manner leads to inhibition of tumor growth in vitro and in vivo. In particular, LNCaP sublines that express high levels of the AR LNCaP-abl derived after continuous androgen ablation and C4–2 that are established after co-culture with stromal cells and grow independently of androgen are retarded in their proliferation after administration of antisense oligonucleotides or specific antibodies [62,63]. AR N-terminal decoy molecule when over-expressed in vivo yielded decreased tumor incidence and reduced tumor volume [64]. In a subline of CWR22 cells, cell-cycle progression is strictly dependent on the presence of the AR [65]. Knockdown of AR expression led to increased expression of the tumor suppressor p27 and reduced phosphorylation of retinoblastoma. Interestingly, it seems that inhibition of ligand-independent activation of the AR is more efficient by the shRNA approach than by bicalutamide [66].

It is also well-known that numerous agents that are proposed to be used for chemoprevention of prostate cancer downregulate AR expression. For example, downregulation of PSA expression by selenium occurs through reduction of AR expression and binding to respective response elements [67]. Some of them are also inhibitors of the signaling pathway of nuclear factor κB whose overexpression is associated with increased angiogenesis, invasion and metastasis. Nuclear factor κB also regulates the expression of proinflammatory cytokines that are elevated in prostate cancer tissue, but may also be responsible for early events in prostate carcinogenesis.

Activity of the AR is also increased under hypoxic conditions [68]. Experimental therapies aimed to interfere with androgenic regulation of pro-angiogenic events should consider the fact that dihydrotestosterone is a potent regulator of vascular endothelial growth factor and hypoxia-inducible factor 1 [69,70]. The latter effect is mediated through activation of the phosphotidylinositol 3-kinase/Akt pathway. It was shown that androgen withdrawal in vivo
reduces hypoxia and renders the cells more sensitive to radiation therapy [71]. Another potential approach to inhibit prostate cancer with a natural compound that does not cause systemic cytotoxicity, such as emodin, is decreasing association with heat-shock proteins and promoting AR degradation by proteasome complex [72]. A possible advantage of using a chaperone inhibitor such as 17-AAG in cancer therapy is that it acts not only through inhibition of the AR, but it also degrades ErbB2 and Akt [73]. Histone deacetylase inhibitors are considered for prostate cancer experimental therapy because of their low toxicity [74]. Their mechanism of action involves induction of cell death in AR-positive cell lines through inhibition of AR expression.

Conclusions & future directions
The major problem in development of more rational therapy for prostate cancer is the fact that we are still not able to selectively block proliferative and anti-apoptotic versus pro-differentiation effects mediated by the AR. In human prostate epithelial cells, introduction of the AR induces changes similar to those found in organ-confined tumors and tumorigenicity if injected orthotopically [75]. On the one hand, increase in AR expression was the most consistent change in prostate cancer xenografts associated with progression, alterations in coactivator:corepressor ratio and acquisition of agonistic activities of anti-androgens [76]. However, it is obvious that compounds that upregulate AR activity in a ligand-independent manner, such as forskolin or IL-6 may display both growth-stimulatory and -inhibitory effects [77–80]. Moreover, AR transcription function and PSA expression are upregulated by the prodifferentiation agent phenylbutyrate [81]. Forced expression of the AR in PC-3 cells resulted in a less malignant phenotype, and androgen also inhibited growth of a cell line derived from metastatic ascites [82,83]. PC-3 cells that are stably transfected with AR cDNA show decreased tumorigenicity, reduced expression of RelA, a subunit of nuclear factor xB, Bcl-2 and IL-6 [84]. Thus, in that cellular model AR acts as a proapoptotic and anti-angiogenic molecule. Biphasic regulation of cell growth in LNCaP cells by androgen and environmental estrogen bisphenol A, is dependent on AR status [85]. In AR-negative cells, an inhibitory effect of higher concentrations of bisphenol A was not seen. It is assumed that environmental estrogens are present in amounts sufficient to facilitate prostate cancer development. It is also not clear whether amplification of the AR gene is in some way beneficial to patients who receive endocrine treatment; in a study by Palmberg and colleagues it was demonstrated that there are more responders to complete androgen blockade in a collective of patients with AR amplification [86]. Interestingly, in a recent report it was shown that the pure antiestrogen fulvestrant (ICI 182, 780) exerts its inhibitory effect in prostate cancer through inhibition of AR mRNA and protein expression and activity [87]. Use of that compound may be of interest in patients who failed anti-androgen therapy, especially if the AR contains mutation(s) that increase their sensitivity to conventional anti-androgens.

Resistance to androgen ablation therapy certainly involves an AR-unrelated mechanism. In sublines of LNCaP cells developed during intermittent androgen ablation in vitro, it was demonstrated that changes in AR levels cannot re-establish androgenic control of cell growth [88]. Molecular mechanisms leading to therapy resistance in that system are not well understood, but it could be hypothesized that inhibitors of apoptosis such as survivin are upregulated in those sublines. Survivin is induced by androgen and suppressed by flutamide [89]. Heparin-binding EGF, a compound that inhibits AR expression, is a positive growth factor for tumor cells [90]. Again, this is another example showing that AR expression does not necessarily correlate with tumor cell proliferation or inhibition of cell death.

To date, single novel therapies for prostate cancer were shown to have little effect. Although our knowledge on AR-dependent and -independent mechanisms underlying prostate cancer progression has considerably increased in the last decade, key issues regarding AR control of proliferation need to be resolved as a condition for improvement of endocrine therapy.

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### Executive summary

**Androgen receptor hypersensitivity & therapeutic implications**
- Different mechanisms are responsible for androgen receptor (AR) hypersensitivity in prostate cancer.
- AR mutations are not very common in the early stages, but are increasingly detected during endocrine therapy.
- ErbB2 activation of the androgen receptor contributes to tumor progression in a prostate cancer model; in vivo significance is not clear.
- IL-6 activation of the androgen receptor promotes differentiation of LNCaP cells.
- TMPRSS2 and ETS transcription factor fusion occurs in the majority of prostate cancer samples.

**Residual androgen receptor ligands in recurrent prostate cancer**
- The levels of dihydrotestosterone in advanced prostate cancer are sufficient to activate the AR.
- Genes that mediate androgen metabolism (aldo-keto reductase family 1 member C3) are increasingly expressed in prostate cancer.

**Identification of androgen receptor coactivators & corepressors are of importance for prostate cancer progression**
- Coactivator expression or interaction with the AR is elevated in some cases in prostate cancer.
- Androgen ablation therapy leads to increase in the expression of selected coactivators (Tip60, CBP and p300).
- There is no conclusive evidence that corepressors are required for the inhibitory effect of anti-androgens.

**Therapies aimed to downregulate androgen receptor expression**
- AR downregulation was achieved by antisense oligonucleotides and siRNA.
- AR inhibition has an effect on androgen receptor-independent cell lines.
- There is still no possibility to block androgen receptor proliferative/antiapoptotic versus prodifferentiation effects.

### Bibliography

Papers of special note have been highlighted as of interest (*) or of considerable interest (**) to readers.

   - Multifunctional cytokine that regulates inflammation promotes differentiation through androgen receptor (AR) activation.
   - Mechanism of AR ligand-independent activation relevant to tumor progression.
Pathway involved in AR activation is clinically highly relevant.

Residual androgens in advanced prostate cancer activate the AR.


Fusion of TMPRSS2 and transcription factor is an important event in the majority of prostate cancers.


Residual androgens in advanced prostate cancer activate the AR.


Comuzzi B, Nemes C, Schmidt S et al.: The androgen receptor co-activator CBP is up-regulated following androgen withdrawal and is highly expressed in advanced prostate cancer. J. Pathol. 204, 159–166 (2004).


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52. Ting HJ, Bao BY, Reeder JE, Messing EM, Gu G, Yuan J, Wills M, Kasper S: Prostate cancer cells with stem cell characteristics reconstitute the original human tumor in vivo.


73. Gu G, Yuan J, Wills M, Kasper S: Prostate cancer cells with stem cell characteristics reconstitute the original human tumor in vivo.


