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.

Keywords: androgen receptor, coactivators, dihydrotestosterone, endocrine therapy, fusion, ligand-independent activation, prostate cancer, stem cells

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 (**Figure 1**) [4–7]. In the LAPC4 xenograft, AR activation by ErbB2 facilitated tumor progression as indicated by shortened

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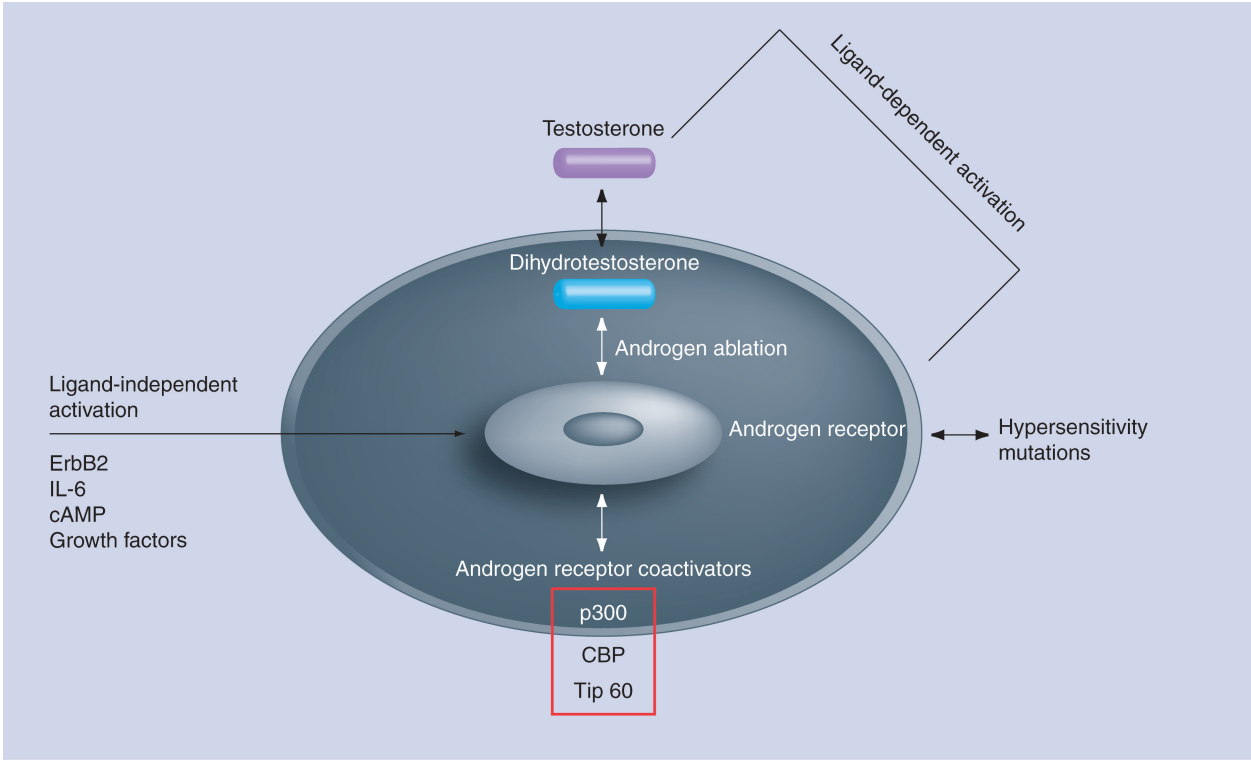
Figure 1. Androgen ablation therapy aims to interfere with ligand-dependent and ligand-independent activation of the androgen receptor.



Therapy resistance develops because of hypersensitivity of the androgen receptor, increased expression of androgen receptor coactivators, point mutations and androgen receptor-independent mechanisms.

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 chromatins 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-I 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 inhibitor [20,21]. In line with all these findings, AR transcriptional activity and prostate-specific antigen (PSA) expression are potentiated by IL-4 [22]. In LNCaP cells, AR activation by IL-6 is associated with inhibition of proliferation and induction of PSA expression [7]. Thus, experimental evidence linking IL-6 and prostate tumor progression is currently missing. For stimulation of PSA expression by IL-4, activation of the Akt pathway is required [22]. This finding is interesting because enhanced Akt phosphorylation in clinical specimens indicates a poor prognosis [23]. It was shown that both pathways of STAT3/MAPK are required for AR activation by IL-6 [24]. In contrast to results showing induction of AR activity by IL-6, Jia and colleagues observed inhibition of transcriptional activity by the cytokine [25]. It is known that different time-dependent interactions of STAT3 in target cells may influence the outcome of these experiments.

However, findings obtained in other experimental models indicate that AR expression 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]. The

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 anti-androgen 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-I 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 anti-androgen [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 α-2-β-1 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 overexpressed *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 prodifferentiation 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 antiandrogens [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 ReIA, a subunit of nuclear factor κB, 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.

Prostate cancer stem cells, androgen receptor and endocrine therapy

- Cells with stem-cell characteristics (i.e., androgen receptor-negative) may reconstitute the human tumor *in vivo*.
- Therapies that target AR-positive and androgen receptor-negative cells in prostate cancer should be developed.

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.

1. Kokontis J, Takakura K, Hay N, Liao S: Increased androgen receptor activity and altered c-myc expression in prostate cancer cells after long-term androgen deprivation. *Cancer Res.* 54, 1566–1573 (1994).
2. Culig Z, Hoffmann J, Erdel M *et al.*: Switch from antagonist to agonist of the androgen receptor blocker bicalutamide is associated with prostate tumour progression in a new model system. *Br. J. Cancer* 81, 242–251 (1999).
3. Hara T, Miyazaki J, Araki H *et al.*: Novel mutations of androgen receptor – a possible mechanism of bicalutamide withdrawal syndrome. *Cancer Res.* 63, 149–153 (2003).
4. Nazareth LV, Weigel NL: Activation of the human androgen receptor through a protein kinase A signaling pathway. *J. Biol. Chem.* 271, 19900–19907 (1996).
5. Culig Z, Hobisch A, Cronauer MV *et al.*: Androgen receptor activation in prostatic tumor cell lines by insulin-like growth factor-I, keratinocyte growth factor, and epidermal growth factor. *Cancer Res.* 54, 5474–5478 (1994).
6. Yeh S, Lin HK, Kang HY, Thin TH, Lin MF, Chang C: From HER2/Neu signal cascade to androgen receptor and its coactivators: a novel pathway by induction of androgen target genes through MAP kinase in prostate cancer cells. *Proc. Natl Acad. Sci. USA* 96, 5458–5463 (1999).
7. Hobisch A, Eder IE, Putz T, Horninger W, Bartsch G, Klocker H, Culig Z: Interleukin-6 regulates prostate-specific protein expression in prostate carcinoma cells by activation of the androgen receptor. *Cancer Res.* 58, 4640–4645 (1998).

• **Multifunctional cytokine that regulates inflammation promotes differentiation through androgen receptor (AR) activation.**

8. Craft N, Shostak Y, Carey M, Sawyers CL: A mechanism for hormone-independent prostate cancer through modulation of androgen receptor signaling by the HER-2/neu tyrosine kinase. *Nat. Med.* 5, 280–285 (1999).

•• **Mechanism of AR ligand-independent activation relevant to tumor progression.**

9. Guo Z, Dai B, Jiang T *et al.*: Regulation of androgen receptor activity by tyrosine phosphorylation. *Cancer Cell* 10, 309–319 (2007).
10. Savinaienen KJ, Saramaki OR, Linja MJ *et al.*: Expression and gene copy number analysis of *ERBB2* oncogene in prostate cancer. *Am. J. Pathol.* 160, 339–345 (2002).
11. Mahajan NP, Liu Y, Majumder S, *et al.*: Activated Cdc42-associated kinase Ack1 promotes prostate cancer progression via androgen receptor tyrosine phosphorylation. *Proc. Natl Acad. Sci. USA* 104, 8438–8443 (2007).
12. Liu Y, Majumder S, McCall W *et al.*: Inhibition of HER-2/neu kinase impairs androgen receptor recruitment to the androgen responsive enhancer. *Cancer Res.* 65, 3404–3409 (2005).
13. Migliaccio A, Di Domenico M, Castoria G *et al.*: Steroid receptor regulation of epidermal growth factor signaling through Src in breast and prostate cancer cells: steroid antagonist action. *Cancer Res.* 65, 10585–10593 (2005).
14. Bakin RE, Gioeli D, Sikes RA, Bissonette EA, Weber MJ: Constitutive activation of the ras/mitogen-activated protein kinase signaling pathway promotes androgen hypersensitivity in LNCaP prostate Cancer Cells. *Cancer Res.* 63, 1981–1989 (2003).
15. Gioeli D, Mandell JW, Petroni GR, Frierson HFJ, Weber MJ: Activation of mitogen-activated protein kinase associated with prostate cancer progression. *Cancer Res.* 59, 279–284 (1999).

16. Wang G, Jones SJ, Marra MA, Sadar MD: Identification of genes targeted by the androgen and PKA signaling pathways in prostate cancer cells. *Oncogene* 25, 7311–7323 (2006).
17. Desai SJ, Ma AH, Tepper CG, Chen HW, Kung HJ: Inappropriate activation of the androgen receptor by nonsteroids: involvement of the Src kinase pathway and its therapeutic implications. *Cancer Res.* 66, 10449–10459 (2006).
18. Culig Z, Steiner H, Bartsch G, Hobisch A: Interleukin-6 regulation of prostate cancer cell growth. *J. Cell. Biochem.* 95, 497–505 (2005).
19. Hobisch A, Rogatsch H, Hittmair A *et al.*: Immunohistochemical localization of interleukin-6 and its receptor in benign, premalignant and malignant prostate tissue. *J. Pathol.* 191, 239–244 (2000).
20. Hobisch A, Ramoner R, Fuchs D *et al.*: Prostate cancer cells (LNCaP) generated after long-term interleukin-6 treatment express interleukin-6 and acquire an interleukin-6-partially resistant phenotype. *Clin. Cancer Res.* 7, 2941–2948 (2001).
21. Lee SQ, Chun JY, Nadiminty N, Lou W, Gao AC: Interleukin-6 undergoes transition from growth inhibitor associated with neuroendocrine differentiation to stimulator accompanied by androgen receptor activation during LNCaP prostate cancer cell progression. *Prostate* 67, 764–773 (2007).
22. Lee SO, Lou W, Hou M, Onate SA, Gao AC: Interleukin-4 enhances prostate-specific antigen expression by activation of the androgen receptor and Akt pathway. *Oncogene* 22, 6037–6044 (2003).
23. Kreisberg JI, Malik SN, Prihoda TJ *et al.*: Phosphorylation of Akt (Ser473) is an excellent predictor of poor clinical outcome in prostate cancer. *Cancer Res.* 64, 5232–5236 (2004).

• **Pathway involved in AR activation is clinically highly relevant.**

24. Yang L, Wang L, Lin HK *et al.*: Interleukin-6 differentially regulates androgen receptor transactivation via PI3K-Akt, STAT3 and MAPK, three distinct signal pathways in prostate cancer cells. *Biochem. Biophys. Res. Commun.* 305, 462–469 (2003).
25. Jia L, Choong CS, Ricciardelli C, Kim J, Tilley WD, Coetzee GA: Androgen receptor signaling: mechanism of interleukin-6 inhibition. *Cancer Res.* 64, 2619–2626 (2004).
26. Hendriksen PJ, Dits NF, Kokame K *et al.*: Evolution of the androgen receptor pathway during progression of prostate cancer. *Cancer Res.* 66, 5012–5020 (2006).
27. Jarrard DF, Kinoshita H, Shi Y *et al.*: Methylation of the androgen receptor promoter CpG island is associated with loss of androgen receptor expression in prostate cancer cells. *Cancer Res.* 58, 5310–5314 (1998).
28. Shah RB, Mehra R, Chinnaiyan AM *et al.*: Androgen-independent prostate cancer is a heterogenous group of diseases: lessons from a rapid autopsy program. *Cancer Res.* 64, 9209–9216 (2004).
29. Tomlins SA, Rhodes DR, Perner S *et al.*: Recurrent fusion of *TMPRSS2* and *ETS* transcription factor genes in prostate cancer. *Science* 310, 644–648 (2005).

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

30. Petrovics G, Liu A, Shaheduzzaman S *et al.*: Frequent overexpression of ETS-related gene-1 (*ERG1*) in prostate cancer transcriptome. *Oncogene* 24, 3847–3852 (2005).
31. Perner S, Demichelis F, Beroukhim R *et al.*: *TMPRSS2*:*ERG* fusion-associated deletions provide insight into the heterogeneity of prostate cancer. *Cancer Res.* 66, 8337–8341 (2006).
32. Hermans KG, van Marion R, van Dekken H *et al.*: *TMPRSS2*:*ERG* fusion by translocation or interstitial deletion is highly relevant in androgen-dependent prostate cancer, but is bypassed in late-stage androgen receptor-negative prostate cancer. *Cancer Res.* 66, 10658–10663 (2006).
33. Demichelis F, Fall K, Perner S *et al.*: *TMPRSS2*:*ERG* gene fusion associated with lethal prostate cancer in a watchful waiting cohort. *Oncogene* 26, 4596–4599 (2007).
34. Titus MA, Schell MJ, Lih FB, Tomer KB, Mohler JL: Testosterone and dihydrotestosterone tissue levels in recurrent prostate cancer. *Clin. Cancer Res.* 11, 4653–4657 (2005).

• **Residual androgens in advanced prostate cancer activate the AR.**

35. Stanbrough M, Bubley GJ, Ross K *et al.*: Increased expression of genes converting adrenal androgens to testosterone in androgen-independent prostate cancer. *Cancer Res.* 66, 2815–2825 (2006).
36. Mohler JL, Gregory CW, Ford OH 3rd *et al.*: The androgen axis in recurrent prostate cancer. *Clin. Cancer Res.* 10, 440–448 (2004).
37. Habib FK, Ross M, Bayne CW, Bollina P, Grigor K, Chapman K: The loss of 5α-reductase type I and type II mRNA expression in metastatic prostate cancer to bone and lymph node metastasis. *Clin. Cancer Res.* 9, 1815–1819 (2003).
38. Ryan CJ, Halabi S, Ou SS, Vogelzang NJ, Kantoff P, Small EJ: Adrenal androgens as predictors of outcome in prostate cancer patients treated with ketoconazole plus antiandrogen withdrawal: results from a cancer and leukemia group B study. *Clin. Cancer Res.* 13, 2030–2037 (2007).
39. Halkidou K, Gnanapragasam VJ, Mehta PB *et al.*: Expression of Tip60, an androgen receptor coactivator, and its role in prostate cancer development. *Oncogene* 22, 2466–2477 (2003).
40. 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).
41. Heemers HV, Sebo TJ, Debes JD *et al.*: Androgen deprivation increases p300 expression in prostate cancer cells. *Cancer Res.* 67, 3422–3430 (2007).
42. Comuzzi B, Lambrinidis L, Rogatsch H *et al.*: The transciptional co-activator cAMP response element-binding protein-binding protein is expressed in prostate cancer and enhances androgen- and anti-androgen-induced androgen receptor function. *Am. J. Pathol.* 162, 233–241 (2003).
43. Nishimura K, Ting HJ, Harada Y *et al.*: Modulation of androgen receptor transactivation by gelsolin: a newly identified androgen receptor coregulator. *Cancer Res.* 63, 4888–4894 (2003).
44. Debes JD, Comuzzi B, Schmidt LJ, Dehm SM, Culig Z, Tindall DJ: p300 regulates androgen receptor-independent expression of prostate-specific antigen in prostate cancer cells treated chronically with interleukin-6. *Cancer Res.* 65, 5965–5973 (2005).
45. Debes J, Sebo TJ, Lohse CM, Murphy LM, Haugen de AL, Tindall DJ: p300 in prostate cancer proliferation and progression. *Cancer Res.* 63, 7638–7640 (2003).
46. Agoulnik IU, Vaid A, Nakka M *et al.*: Androgens modulate expression of transcription intermediary factor 2, an androgen receptor coactivator whose expression level correlates with early biochemical recurrence in prostate cancer. *Cancer Res.* 66, 10594–10602 (2006).

47. Gnanapragasam VJ, Leung HY, Pulimood AS, Neal DE, Robson CE: Expression of RAC3, a steroid hormone receptor co-activator in prostate cancer. *Br. J. Cancer* 85, 1928–1936 (2001).
48. Zhou G, Hashimoto Y, Kwak I, Tsai SY, Tsai MJ: Role of the steroid receptor coactivator SRC-3 in cell growth. *Mol. Cell. Biol.* 23, 7742–7755 (2003).

• **Steroid receptor coactivator may also display AR-independent effects in prostate cancer.**

49. Zhou HJ, Yan J, Luo W *et al.*: SRC-3 is required for prostate cancer proliferation and survival. *Cancer Res.* 65, 7976–7983 (2005).
50. Yan J, Yu CT, Ozen M, Ittmann M, Tsai SY, Tsai MJ: Steroid receptor coactivator-3 and activator protein-1 coordinately regulate the transcription of components of the insulin-like growth factor/Akt signaling pathway. *Cancer Res.* 66, 11039–11046 (2006).
51. Agoulnik IU, Vaid A, Bingman WE 3rd *et al.*: Role of SRC-1 in the promotion of prostate cancer cell growth and tumor progression. *Cancer Res.* 65, 7959–7967 (2005).
52. Hodgson MC, Astapova I, Hollenberg AN, Balk SP: Activity of androgen receptor antagonist bicalutamide in prostate cancer cells is independent of NCoR and SMRT corepressors. *Cancer Res.* 67, 8388–8395 (2007).
53. Ting HJ, Bao BY, Reeder JE, Messing EM, Lee YF: Increased expression of corepressors in aggressive androgen-independent prostate cancer cells results in loss of 1α, 25-dihydroxyvitamin D3 responsiveness. *Mol. Cancer Res.* 5, 967–980 (2007).
54. Petre-Draviam CE, Cook SL, Burd CJ, Marschall TW, Wetherill YB, Knudsen KE: Specificity of cyclin D1 for androgen receptor regulation. *Cancer Res.* 63, 4903–4913 (2003).
55. Burd CJ, Petre CE, Morey LM *et al.*: Cyclin D1b variant influences prostate cancer growth through aberrant androgen receptor regulation. *Proc. Natl Acad. Sci. USA* 103, 2190–2195 (2006).
56. Zhu P, Baek SH, Bourk EM *et al.*: Macrophage/cancer cell interactions mediate hormone resistance by a nuclear receptor derepression pathway. *Cell Biol. Int.* 124, 615–629 (2006).
57. Collins AT, Berry PA, Hyde C, Stower MJ, Maitland NJ: Prospective identification of tumorigenic prostate cancer stem cells. *Cancer Res.* 65, 10946–10951 (2005).

• **Comprehensive characterization of prostate cancer stem cells.**

58. Zhou Z, Flesken-Nikitin A, Nikitin AY: Prostate cancer associated with p53 and Rb deficiency arises from the stem/progenitor cell-enriched proximal region of prostatic ducts. *Cancer Res.* 67, 5683–5690 (2006).
59. Gu G, Yuan J, Wills M, Kasper S: Prostate cancer cells with stem cell characteristics reconstitute the original human tumor *in vivo*. *Cancer Res.* 67, 4630–4637 (2007).
60. Tang Y, Khan MA, Goloubeva O *et al.*: Docetaxel followed by castration improves outcomes in LNCaP prostate cancer-bearing severe combined immunodeficient mice. *Clin. Cancer Res.* 12, 169–174 (2006).
61. Hess-Wilson JK, Daly HK, Zagorski WA, Montville CP, Knudsen KE: Mitogenic action of the androgen receptor sensitizes prostate cancer cells to taxane-based cytotoxic insult. *Cancer Res.* 66, 11998–12008 (2006).
62. Eder IE, Culig Z, Ramoner R *et al.*: Inhibition of LNCaP prostate cancer cells by means of androgen receptor antisense oligonucleotides. *Cancer Gene Ther.* 7, 997–1007 (2000).
63. Zegarra-Moro OL, Schmidt LJ, Huang H, Tindall DJ: Disruption of androgen receptor function inhibits proliferation of androgen-refractory prostate cancer cells. *Cancer Res.* 62, 1008–1013 (2002).
64. Quayle SN, Mawji NR, Wang J, Sadar MD: Androgen receptor decoy molecles block the growth of prostate cancer. *Proc. Natl Acad. Sci. USA* 104, 1331–1336 (2007).
65. Yuan X, Li T, Wang H *et al.*: Androgen receptor remains critical for cell-cycle progression in androgen-independent CWR22 prostate cancer cells. *Am. J. Pathol.* 169, 682–686 (2006).
66. Cheng H, Snoek R, Ghaidi F, Cox ME, Rennie PS: Short hairpin RNA knockdown of the androgen receptor attenuates ligand-independent activation and delays tumor progression. *Cancer Res.* 66, 10613–10620 (2006).
67. Dong Y, Lee SO, Zhang H, Marshall J, Gao AC, Ip C: Prostate specific antigen expression is down-regulated by selenium through disruption of androgen receptor signaling. *Cancer Res.* 64, 19–22 (2004).
68. Park SY, Kim YJ, Gao AC *et al.*: Hypoxia increases androen receptor activity in prostate cancer cells. *Cancer Res.* 66, 5121–5129 (2006).
69. Joseph IB, Nelson JB, Denmeade SR, Isaacs JT: Androgens regulate vascular endothelial growth factor content in normal and malignant prostatic tissue. *Clin. Cancer Res.* 3, 2507–2511 (1997).
70. Mabjeesh NJ, Willard MT, Frederickson CE, Zhong H, Simons JW: Androgens stimulate hypoxia-inducible factor activation via autocrine loop of tyrosine kinase receptor/phosphatidylinositol 3'-kinase/protein kinase B in prostate cancer cells. *Clin. Cancer Res.* 9, 2416–2425 (2003).
71. Milosevic M, Chung P, Parker C *et al.*: Androgen withdrawal in patients reduces prostate cancer hypoxia: implications for disease progression and radiation response. *Cancer Res.* 67, 6022–6025 (2007).
72. Cha TL, Qiu L, Chen CT, Wen Y, Hung MC: Emodin down-regulates androgen receptor and inhibits prostate cancer cell growth. *Cancer Res.* 65, 2287–2295 (2005).
73. Solit DB, Zheng FF, Drobnjak M *et al.*: 17-allylamino-17-demethoxygeeldanamycin induces the degradation of androgen receptor and HER-2/neu and inhibits the growth of prostate cancer xenografts. *Clin. Cancer Res.* 8, 986–993 (2002).
74. Rokhlin OW, Glover RB, Guseva NV, Taghiyev AF, Kohlgraf KG, Cohen MB: Mechanisms of cell death induced by histone deacetylase inhibitors in androgen receptor-positive prostate cancer cells. *Mol. Cancer Res.* 4, 113–123 (2006).
75. Berger R, Febbo PG, Majunder PK *et al.*: Androgen-induced differentiation and tumorigenicity of human prostate epithelial cells. *Cancer Res.* 64, 8867–8875 (2004).
76. Chen CD, Welsbie DS, Tran C *et al.*: Molecular determinants of resistance to antiandrogen therapy. *Nat. Med.* 10, 33–39 (2004).

• **AR downregulation is the most consistent change in various xenografts in prostate cancer progression.**

77. Bang YJ, Kim SJ, Danielpour D *et al.*: Cyclic AMP induces transforming growth factor β 2 gene expression and growth arrest in the human androgen-independent prostate carcinoma cell line PC-3. *Proc. Natl Acad. Sci. USA* 89, 3556–3560 (1992).
78. Farini D, Puglianello A, Mammi C, Siracusa G, Moretti C: Dual effect of pituitary adenylate cyclase activating polypeptide on prostate tumor LNCaP cells: short- and long-term exposure affect proliferation and neuroendocrine differentiation. *Endocrinology* 144, 1631–1643 (2003).
79. Degeorges A, Tatoud R, Fauvel Lafeve F *et al.*: Stromal cells from human benign prostate hyperplasia produce a growth-inhibitory factor for LNCaP prostate cancer cells, identified as interleukin-6. *Int. J. Cancer* 68, 207–214 (1996).

80. Giri D, Ozen M, Ittmann M: Interleukin-6 is an autocrine growth factor in human prostate cancer. *Am. J. Pathol.* 159, 2159–2165 (2001).
81. Sadar MD, Gleave ME: Ligand-independent activation of the androgen receptor by the differentiation agent butyrate in human prostate cancer cells. *Cancer Res.* 60, 5825–5831 (2000).
82. Yuan S, Trachtenberg J, Mills GB, Brown TJ, Xu F, Keating A: Androgen-induced inhibition of cell proliferation in an androgen-insensitive prostate cancer cell line (PC-3) transfected with a human androgen receptor complementary DNA. *Cancer Res.* 53, 1304–1311 (1993).
83. Zhau HY, Chang SM, Chen BQ *et al.*: Androgen-repressed phenotype in human prostate cancer. *Proc. Natl Acad. Sci. USA* 93, 15152–15157 (1996).
84. Nelius T, Filleur S, Yemelyanov A *et al.*: Androgen receptor targets NFκB and TSP1 to suppress prostate tumor growth *in vivo*. *Int. J. Cancer* 121, 999–1008 (2007).
85. Wetherill YB, Fisher NL, Staubach A, Danielsen M, de Vere White RW, Knudsen KE: Xenoestrogen action in prostate cancer: pleiotropic effects dependent on androgen receptor status. *Cancer Res.* 65, 54–65 (2005).
86. Palmberg C, Koivisto P, Kakkola L, Tammela TL, Kallioniemi OP, Visakorpi T: Androgen receptor gene amplification at primary progression predicts response to combined androgen blockade as second line therapy for advanced prostate cancer. *J. Urol.* 164, 1992–1995 (2000).
87. Bhattacharyya RS, Krishnan AV, Swami S, Feldman D: Fulvestrant (ICI 182, 780) down-regulates androgen receptor expression and diminishes androgenic responses in LNCaP human prostate cancer cells. *Mol. Cancer Ther.* 5, 1539–1549 (2006).
88. Hobisch A, Fritzer A, Comuzzi B *et al.*: The androgen receptor pathway is by-passed in prostate cancer cells generated after prolonged treatment with bicalutamide. *Prostate* 66, 413–420 (2006).
89. Zhang M, Latham DE, Delaney MA, Chakravarti A: Survivin mediates resistance to antiandrogen therapy in prostate cancer. *Oncogene* 24, 2474–2482 (2005).
90. Adam RM, Kim J, Lin J, Orsola A, Zhuang L, Rice DC, Freeman MR: Heparin-binding epidermal growth factor-like growth factor stimulates androgen-independent prostate tumor growth and antagonizes androgen receptor function. *Endocrinology* 143, 4599–4608 (2002).