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Activation of the androgen receptor by polypeptide growth factors and cellular regulators

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Summary. The polypeptide growth factors insulin-like growth factor I (IGF-I), epidermal growth factor (EGF), and transforming growth factor-α (TGF-α); second-messenger cyclic adenosine monophosphate (cAMP); protein kinase activators; and neurotransmitters were found to activate the estrogen (ER), progesterone (PR), and glucocorticoid receptor (GR) either in the absence of their natural ligands or synergistically with the respective hormone. There is now evidence of coupling of signaling pathways involving the androgen receptor (AR). Three polypeptide growth factors, IGF-I, keratinocyte growth factor (KGF), and EGF, stimulated AR-mediated reporter-gene transcription in the absence of androgen in DU-145 cells, which were cotransfected with the reporter gene and an AR expression vector. IGF-I effects were observed irrespective of the promoter driving the reporter gene. This growth factor increased the prostate-specific antigen (PSA) level in LNCaP cells, which contain endogenous AR. In CV-1 cells, which transiently express the AR, second-messenger cAMP potentiated effects of testosterone in stimulation of AR-mediated reporter-gene activity. Inhibition of androgen-stimulated chloramphenicol acetyltransferase (CAT) activity in the LNCaP cell line was achieved with retinoic acid. Stimulation and inhibition of prostatic carcinoma cell growth by polypeptide growth factors and cellular regulators may depend on the presence of the AR in an androgen-depleted environment.

It has long been believed that only steroid hormones activate their respective receptors to initiate transcription of steroid-responsive genes. A number of recent studies, however, have provided evidence for coupling of the signaling pathways of various cellular regulators and steroid hormone receptors. This paper summarizes current data on nonsteroidal activation of steroid receptors with emphasis on androgen receptor (AR) activation (Table 1). In addition, possible pathophysiological and clinical implications of these novel signaling pathways are discussed.

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Estrogen, progesterone, and glucocorticoid receptor activation in response to polypeptide growth factors, second-messenger cyclic adenosine monophosphate, protein kinase activators, and dopamine

In various estrogen-sensitive tissues and cell lines the effects of estrogenic hormones on cell growth and differentiation and gene expression are mimicked by the polypeptide growth factors insulin-like growth factor I (IGF-I) and epidermal growth factor (EGF). For example, EGF given in vivo promotes uterine and vaginal growth in ovariectomized mice and induces expression of the estrogen-regulated gene lactoferrin in the uterine epithelium [27]. EGF behaves similarly as estrogen in enhancing nuclear localization of the estrogen receptor (ER) [14]. Growth of the estrogen-responsive cell line MCF-7 is stimulated by EGF and IGF-I [12]. IGFs were recently shown to control the growth and differentiation of human neuroblastoma cell line SK-ER3 stably transfected with the ER [24]. Another characteristic effect mediated by the ER is up-regulation of progesterone receptor (PR) expression. Not only estradiol, but also IGF-I (at physiological concentrations) and insulin (at pharmacological concentrations) were found to increase the PR level in MCF-7 breast cancer cells [19]. In primary cultures of uterine cells the PR is up-regulated by estradiol, IGF-I, and 8-Br-cAMP [2]. Estradiol indirectly potentiates the effects of IGF-I by stimulation of its secretion [26].

These observations have raised questions about the molecular mechanisms that are responsible for the estrogen effects of IGF-I and EGF. It had been supposed that the action of polypeptide growth factors was mediated by the ER. To prove this hypothesis, experiments were performed in which uterine cells were transfected with an estrogen-responsive reporter gene and treated with either estrogen or IGF-I or 8-Br-cAMP [3]. IGF-I and cAMP were found to be as effective as estradiol in stimulating ER-mediated chloramphenicol acetyltransferase (CAT) reporter-gene expression. Their effects were blocked by the pure antiestrogen ICI 164,384 [3]. Newton et al. [29] also observed stimulation of ER-mediated reporter-gene activity by IGF-I in the pituitary tumor cell line GH_3.

Table 1. Activation of steroid receptors by polypeptide growth factors and various cellular regulators

Receptor	Activation in the absence of ligand	Potentiation of hormone signal
ER	IGF-I, EGF, TGF-α, cAMP	Protein kinase A and C activators
PR	Dopamine, cAMP, gonadotropin-releasing hormone	EGF, TGF-α, cAMP
GR	–	cAMP, protein kinase A and C activators, EGF
AR	IGF-I, KGF, EGF	cAMP, protein kinase A and C activators

Ignar-Trowbridge et al. [15] have reported that EGF and transforming growth factor-α (TGF-α) elicit transcriptional activation of an estrogen-responsive element. Both growth factors were effective in a strain of Ishikawa human endometrial carcinoma cells, which were cotransfected with an ER-expression vector and an estrogen-responsive reporter gene, and in BG-1 human ovarian adenocarcinoma cells, which contain endogenous steroid receptors. When applied simultaneously with physiological concentrations of estrogen, either EGF or TGF-α acted in a synergistic manner and increased reporter-gene activity. Pretreatment of Ishikawa cells with neutralizing antibodies to the EGF receptor resulted in decreased CAT activity in response to EGF. This suggests that EGF activates the tyrosine kinase of its receptor, which directly or indirectly interacts with the ER. Analysis of ER deletion mutants has revealed that amino acids in the NH_2-terminus of the ER may be more important for ER activation by EGF than amino acids at the COOH-terminus [15]. Taken together, all these results provide conclusive evidence for estrogen-independent activation of the ER by growth factors. These substances may act in a synergistic manner with estrogens or they may replace estrogen activation.

An involvement of protein kinases in nonsteroidal ER activation is further supported by experiments showing a synergistic action of estrogen and protein kinase A or C activators in the induction of ER-mediated reporter-gene expression [5]. Several studies were focused on alternative mechanisms of activation of the PR. They showed that depending on cell and promoter context, various cellular regulators stimulate transcription of progesterone-responsive genes. The PR is activated by the neurotransmitter dopamine in the absence of progesterone [32]. EGF and TGF-α enhance the effects of the synthetic progestin R 5020 on PR-mediated reporter-gene expression in the human mammary carcinoma cell line T47D [22]. This potentiation of progesterone action is not accompanied by changes in the DNA-binding activity of the PR [22]. Activation of the PR in the absence of progesterone was achieved with gonadotropin-releasing hormone, which increases the intracellular cAMP level, and with the synthetic cAMP analogue 8-Br-cAMP. The antiprogesterone RU 486 inhibited PR activation of both gonadotropin-releasing hormone and 8-Br-cAMP [40]. Studies of the functional properties of the PR are complicated because of the existence of two isoforms of this receptor: the full-length B receptor and the A form truncated at the N-terminus. In the presence of elevated levels of cAMP, some antiprogestins display agonistic properties when interacting with the B receptor but not with the A receptor [37].

For the glucocorticoid receptor (GR), potentiation of the dexamethasone signal by 8-Br-cAMP and by activators of protein kinase A has been described [30]. The outcome of response of the GR to dexamethasone applied with either substances that increase the level of cAMP by inhibiting its turnover or with the protein kinase C activator phorbol ester was cell-type- and promoter-dependent [30].

Polypeptide growth factors and AR activation

The AR is a ligand-induced transcription factor that regulates the growth and differentiation of androgen-dependent tissues such as the prostate gland. The majority of prostatic carcinomas in a late stage is androgen-independent. It has been postulated that the loss of androgen dependence may be associated with alterations in the androgen-signaling chain. AR expression and function in human and rat prostatic tumors has been studied extensively. It has been shown that the majority of prostatic carcinomas in advanced stages do not lack the AR [23, 36]. Although the presence of the AR in tumor tissue does not necessarily imply that the receptor is functionally activated, it may reflect a growth advantage for androgen-independent cells. One of the mechanisms that contribute to tumor progression may be nonsteroidal activation of the AR in the situation of androgen deprivation. We performed experiments to investigate activation of the AR by substances other than androgens. DU-145 cancer cells, which do not contain endogenous steroid receptors, were cotransfected with an androgen-inducible reporter CAT gene and an AR expression vector, and the ability of polypeptide growth factors to activate AR-mediated reporter-gene expression was tested [11]. At a concentration of 50 ng/ml, IGF-I activated AR-mediated transcription in experiments in which the reporter CAT gene was driven either by artificial promoters consisting of one or two androgen-responsive elements in front of a TATA box or by the naturally occurring androgen-inducible promoter of the prostate-specific antigen (PSA) gene. Less potent stimulatory effects on CAT activity were observed with keratinocyte growth factor (KGF) and EGF. These growth factors were effective only in experiments in which the reporter gene was driven by a synthetic promoter consisting of two responsive elements in front of a TATA box. In all cases the nonsteroidal antiandrogen casodex antagonized growth factor activation of the AR, showing that these growth factor effects are indeed mediated by the AR.

In the prostatic tumor cell line LNCaP, which contains endogenous AR, the effects of growth factors on the expression of an endogenous androgen-regulated gene were evaluated by measurement of PSA secretion. In LNCaP cells, which contain the mutated AR with a broad steroid specificity, expression of the PSA gene is positively regulated by androgens and also by progestagenic and estro-

genic steroids [25, 35]. At a concentration of 50 ng/ml, IGF-I increased the PSA level in the supernatant of LNCaP cells 5-fold and was only slightly less efficient than the synthetic androgen methyltrienolone, which caused an 8-fold increase. Again, casodex suppressed the stimulatory effects of the synthetic androgen and IGF-I on PSA secretion [11]. These results indicate a novel pathway of AR activation that is probably important in the situation of androgen deprivation in prostatic carcinoma.

Further experiments should address the question as to whether IGF-I acts in a synergistic manner together with very low doses of androgen in the activation of AR-mediated effects. The response of the AR to low doses of androgen and a growth factor is of special interest because in castrated prostate cancer patients the adrenal glands continue to provide a low level of androgens. One can expect that androgens together with growth factors enable tumor cells to activate the androgen-signaling pathway despite androgen ablation therapy, offering a possible mechanism contributing to the progression to androgen independence. In addition, IGF-I effects on the AR are to be tested in conditions in which AR protein expression increases due to long-term androgen depletion. There are experimental data describing an increased sensitivity to lower concentrations of the synthetic androgen methyltrienolone in an LNCaP subline established after long-term growth in steroid-deprived serum [20].

In the prostatic tumor cell line LNCaP and in tissue specimens, mutant ARs have been detected [10, 28, 38, 39, 41]. Generally, these mutant receptors have a broadened activation spectrum with increased specificity toward other steroid hormones and nonsteroidal antiandrogens. Some of the mutants may be activated more efficiently by polypeptide growth factors than the wild-type receptor.

IGF-I and KGF action in the prostate: possible implications of AR activation

IGF-I, which is synthesized in the liver, is a polypeptide consisting of 70 amino acids with about 70% sequence homology to IGF-II and 50% homology to insulin. It is a potent mitogen of many malignant epithelial tumors. The effects of IGF-I on the proliferation of prostatic cells have been studied in primary cultures of prostatic cells and in cancer cell lines. Primary cultures of prostatic epithelial cells contain IGF receptor type I, and IGF-I is a mitogen for these cells [6]. It is more potent than IGF-II and insulin [6]. Whether this pronounced stimulatory effect of IGF-I has something in common with its ability to activate the AR requires further investigation.

Secretion of IGF-I and stimulation of the growth of prostatic tumor cell lines by IGF-I is a matter of ongoing study and, at present, there are no unequivocal data in this respect [9, 17, 31]. Results obtained by Iwamura et al. [17] and Connolly and Rose [9] support the view that prostatic carcinoma cells respond to exogenous IGF-I but do not secrete IGF-I into conditioned media. These investigators detected IGF-I receptors in three prostatic carcinoma cell lines and measured no IGF-I in the cell supernatants. In their measurements the androgen-independent cell lines PC-3 and DU-145 had higher IGF-I receptor contents than did the androgen-sensitive cell line LNCaP [17]. IGF-I significantly stimulated thymidine uptake in the androgen-independent cell lines, whereas in the LNCaP cells it stimulated DNA synthesis only in the presence of dihydrotestosterone. Since the androgen did not enhance the expression of the IGF receptor, one may speculate that potentiation of the androgen signal by IGF-I accounts for this phenomenon. In contrast to these findings, Pietrzkowski et al. [31] measured significant amounts of immunoreactive IGF-I in conditioned medium of all three human prostatic cell lines. According to these authors, prostatic tumor cell lines do not need exogenous IGF-I for proliferation. Reiter et al. [33] found IGF-I mRNA in rat prostatic mesenchymal cells and not in the epithelium. Thus, the issue as to whether IGF-I acts as an autocrine or a paracrine factor in prostatic carcinoma cell lines has not yet been resolved.

During prostatic carcinoma progression, bones are most frequently affected by metastases. It is assumed that bone cells support the growth of prostate cancer cells by producing paracrine stimulators such as growth factors. IGF-I, of which high amounts have been found in bone cells, seems to be one of the important factors [4]. One can assume that AR activation by IGF-I is involved in metastatic spread to the bone. The activity of IGF-I is regulated by its association and dissociation with binding proteins. The most important binding protein is IGF-I-binding protein-3, which is the major serum carrier for IGFs. Connolly and Rose [9] have found that DU-145 cells secrete a specific IGF-binding protein, but not IGF-I, into conditioned medium. Most interestingly, the proteolytic activity of PSA cleaves IGF-binding proteins [7]. In concordance with that observation, IGF-I-binding protein-3 levels are decreased in patients with metastatic disease, whereas IGF-I-binding protein-2 levels are increased [8, 18]. Thus, access of IGF-I to cancer cells is facilitated and all effects of this growth factor during tumor progression may be potentiated by PSA. Another mechanism of IGF-I recruitment is elicited by EGF, which is known to suppress IGF-I-binding protein-3 levels in cancer cells [13]. Indeed, EGF potentiates IGF-I effects on DU-145 prostatic carcinoma cells, which express significant levels of IGF-I-binding proteins [9].

Growth hormone, whose effects are mediated by IGF-I, was shown to increase the mRNA mean levels of the C3 subunit of probasin [34]. Probasin is an androgen-regulated protein in the rat ventral prostate. Since this effect of growth hormone occurred in the absence of androgen, the underlying mechanism may also involve AR activation by IGF-I.

KGF also activated the AR in cotransfection-transactivation experiments, but it was less effective than IGF-I. KGF is thought to be a mediator in signal transmission between the stromal and epithelial compartments of the prostate [42]. KGF was detected in the prostatic stromal cells and its receptor in the epithelium. In the Dunning tumor system, KGF receptor expression decreases with the progression toward androgen insensitivity [42].

Potentiation of androgen action by cAMP and protein kinase activators

Ikonen et al. [16] have investigated the effects of cAMP on AR-mediated reporter-gene transcription in CV-1 cells under transient expression conditions. In these cells, the second-messenger cAMP alone displayed weak stimulatory effects on reporter-gene activity, but in combination with testosterone it acted in a synergistic manner. The synergism of androgen and cAMP was antagonized by the nonsteroidal antiandrogen casodex. Analysis of AR deletion mutants revealed that intact DNA- and hormone-binding domains are mandatory for this synergism. The potentiation of the androgen signal by cAMP did not result from changes in the cellular AR content, as analyzed by immunoblot and ligand-binding assays, or from qualitative and quantitative changes in AR/androgen-responsive-element interaction, as revealed by electrophoretic mobility shift assay [16]. Since cAMP is a mediator of the action of several polypeptide hormones, e.g., gonado-tropins, its cooperation with low doses of androgens may be one of the mechanisms of the AR activation by growth factors that enables tumor cells to overcome androgen blockade.

Forskolin, an activator of protein kinase A, and phorbol 12-myristate 13-acetate (PMA), an activator of protein kinase C, also displayed similar synergistic effects with testosterone in cotransfected CV-1 cells. In the androgen-sensitive LNCaP cells, phorbol ester stimulated proliferation, but it decreased the expression of androgen-regulated PSA protein, suggesting different mechanisms in mitogenic effects and AR-mediated gene expression [1]. Insensitivity to the protein kinase C activator phorbol ester may be a characteristic feature of androgen-insensitive prostatic carcinoma cells [21].

Retinoic acid antagonizes androgen effects

It has been thought that only steroidal (cyproterone acetate) and nonsteroidal (hydroxyflutamide, casodex) antiandrogens are capable of repressing androgen-induced effects. A study of Young et al. [43], focused on the possible therapeutic effects of retinoic acid for prostatic carcinoma, showed that retinoic acid at concentrations of 1–10 μM antagonized the proliferative effects of androgens on LNCaP cells and on induction of the PSA gene and the human glandular kallikrein gene. Retinoic acid decreases androgen-binding activity by about 40% in the LNCaP cells [43]. It completely suppressed the induction of an androgen-inducible reporter gene stimulated by the synthetic androgen mibolerone [43]. These experiments clearly demonstrated antagonistic properties of retinoic acid on the AR level.

In conclusion, it has become evident that the AR is activated not only by androgens but also by growth factors and other cellular regulators and that it participates in different signaling pathways. Further characterization of nonandrogenic stimulatory and inhibitory effects on AR-mediated gene transcription will certainly provide information that is necessary for a better understanding of the molecular basis of prostatic carcinoma progression.

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