

Androgen Receptor Activation in Prostatic Tumor Cell Lines by Insulin-like Growth Factor-I, Keratinocyte Growth Factor, and Epidermal Growth Factor¹

Zoran Culig, Alfred Hobisch, Marcus V. Cronauer, Christian Radmayr, Jan Trapman, Anton Hittmair, Georg Bartsch, and Helmut Klocker²

Departments of Urology [Z. C., A. Ho., M. V. C., C. R., G. B., H. K.] and Pathology [A. Hi.], University of Innsbruck, Anichstrasse 35, A-6020 Innsbruck, Austria, and Department of Pathology, Erasmus University Rotterdam, P.O. Box 178, 3000 DR Rotterdam, The Netherlands [J. T.]

ABSTRACT

Aberrant activation of the androgen receptor (AR) may be one of the mechanisms which contribute to progression of prostatic carcinoma to an androgen-independent stage. We investigated effects of growth factors on stimulation of the AR-mediated gene transcription in human prostatic tumor cell lines. DU-145 cells, which do not contain endogenous AR, were cotransfected with an androgen-inducible chloramphenicol acetyltransferase (CAT) reporter gene and an AR expression vector. The reporter gene (CAT) was driven either by artificial promoters consisting of one or two androgen-responsive elements in front of a TATA box or by the promoter of the prostate-specific antigen (PSA) gene, a naturally occurring androgen-inducible promoter. Insulin-like growth factor-I (IGF-I), at a concentration of 50 ng/ml, stimulated AR-mediated reporter gene transcription to the same extent as the synthetic androgen methyltrienolone. This growth factor was effective irrespective of the nature of the androgen-inducible promoter. Keratinocyte growth factor (KGF) and epidermal growth factor (EGF), at concentrations of 50 ng/ml, activated CAT reporter gene transcription only in experiments in which the artificial promoter with two androgen-responsive elements was used. Insulin-like growth factor-II and basic fibroblast growth factor displayed no effect on AR-mediated gene transcription. None of the growth factors stimulated reporter gene activity in control experiments when added to cells cotransfected with the CAT gene and an empty expression vector. AR activation by IGF-I, KGF, and EGF was completely inhibited by the pure AR antagonist casodex, showing that these effects are AR mediated. Activation of endogenous AR by growth factors was studied in the LNCaP cell line by determination of PSA secretion. IGF-I, at a concentration of 50 ng/ml, increased the PSA level in the supernatant of this cell line 5-fold. Again, the IGF-I effect on PSA secretion was blocked by casodex. Our results provide evidence that IGF-I, KGF, and EGF directly activate the AR in the absence of androgens, which means that the androgen-signaling chain may be activated by growth factors in an androgen-depleted environment. These findings may have implications for endocrine therapy for metastatic prostatic carcinoma.

INTRODUCTION

Failure of endocrine therapy for metastatic prostatic carcinoma may be accompanied with alterations in transmission of androgen signals. The presence of a mutant AR³ in LNCaP cells (1) and the absence of the AR transcript in PC-3 and DU-145 cells (2) reflect impairments in the androgen-signaling chain in advanced prostatic carcinoma. Moreover, the existence of AR point mutations in primary prostatic tumors (3–5) and tumor metastases (5) and the presence of AR with decreased numbers of CAG repeats in the NH₂ terminus in a specimen derived from a patient showing paradoxical response to the antiandrogen

flutamide (6) support the hypothesis that structural changes of the AR may play a role in prostatic tumor genesis and progression. The two mutant prostatic ARs, Met⁷¹⁵ and Ala⁸⁷⁷, are activated in addition to androgens by a wide spectrum of steroid hormones and nonsteroidal antihormones (1, 4). Because of these findings, the question arises whether another type of aberrant stimulation of the AR exists, namely, receptor activation which is independent of androgens. The ER and the PR, which belong to the same superfamily of ligand-induced transcription factors as the AR, mediate gene transcription not only in response to estradiol and progesterone, respectively, but also in response to IGF-I, EGF, cAMP, protein kinase activators, and dopamine (7–11). Cross-talk between a growth factor and the AR may have several implications on the pathophysiology of malignant and initially hormone-dependent prostatic carcinoma; above all, in a hormone-depleted environment androgen-independent AR activation may activate the androgen-signaling pathway. The aim of this study was to investigate a possible interaction of growth factors with the androgen-signal transduction cascade in prostatic tumor cell lines.

MATERIALS AND METHODS

Cell Lines. DU-145 and LNCaP prostatic carcinoma cell lines were routinely cultured in RPMI supplemented with 5% fetal calf serum and antibiotics at 37°C in an atmosphere of 5% CO₂ in air.

Growth Factors and Hormones. IGF-I, IGF-II, and KGF were purchased from Boehringer Mannheim (Germany), bFGF was purchased from Upstate Biotechnology (Lake Placid, NY), and EGF was purchased from GIBCO BRL (Paisley, United Kingdom). Methyltrienolone was obtained from New England Nuclear (Dreieichenhain, Germany) and casodex was obtained from ICI (Cheshire, United Kingdom).

Cotransfections and CAT Assays. DU-145 cells were grown on 12-well plates. CAT reporter genes were driven either by artificial promoters consisting of one or two AREs in front of a TATA box or by the naturally occurring PSA promoter (12), which contains an ARE at –170 to –156. Reporter plasmids were mixed with AR expression vectors at a ratio of 10:1. Liposome-mediated transfection was performed under serum-free conditions. The DNA solution was mixed with Lipofectamine transfection reagent (GIBCO BRL, Paisley, United Kingdom; 3 μl/μg plasmid DNA) and added to the cells. After an incubation period of about 12 h, the medium was supplemented with 5% charcoal-treated serum. Methyltrienolone or growth factors were added 8 h later. About 32 h after supplementation, the medium was removed and the cells were frozen. CAT activity was assessed as described previously (4). CAT activity in extracts of DU-145 cells treated with 5 nM methyltrienolone has been chosen as a reference (100%), and all other CAT activities are expressed in percentages in relation to this value. Activities were measured in dpm. Depending on the reporter genes used, 100% values ranged from 8,000–16,000 dpm in individual experiments, and background values were approximately 800 dpm. In control experiments, the cells were cotransfected with a reporter plasmid and the empty expression vector. They were treated in the same way as described above. In experiments in which the effects of the nonsteroidal antiandrogen casodex on AR-mediated gene transcription were examined, this AR antagonist was applied simultaneously with either methyltrienolone or growth factors.

PSA Determination in the Supernatant of LNCaP Cells. LNCaP cells were cultured on 24-well plates. After cell attachment they were grown in serum-free RPMI medium containing either methyltrienolone, one of the growth factors tested, or no supplementation (control). After an incubation

Received 5/13/94; accepted 8/17/94.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

¹ Supported by Grant SFB F203 of the Austrian Research Funds.

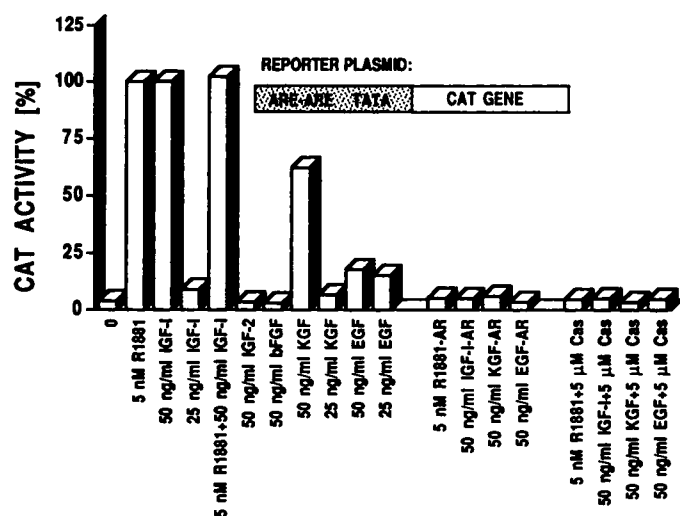
² To whom requests for reprints should be addressed, at Department of Urology, University of Innsbruck, Anichstrasse 35, A-6020 Innsbruck, Austria.

³ The abbreviations used are: AR, androgen receptor; ER, estrogen receptor; PR, progesterone receptor; IGF, insulin-like growth factor; EGF, epidermal growth factor; KGF, keratinocyte growth factor; bFGF, basic fibroblast growth factor; CAT, chloramphenicol acetyltransferase; ARE, androgen-responsive element; PSA, prostate-specific antigen.

period of 72 h, the medium was removed and the PSA level was assessed by an enzyme immunoassay (Abott, Wiesbaden, Germany). PSA levels were corrected with respect to equal cell numbers which were determined by a nonradioactive cell viability assay (Biomedica, Vienna, Austria). In order to evaluate possible effects of treatment on the level of AR expression, we also performed AR-binding assays with radioactively labeled methyltrienolone. The assay procedure was described elsewhere (4).

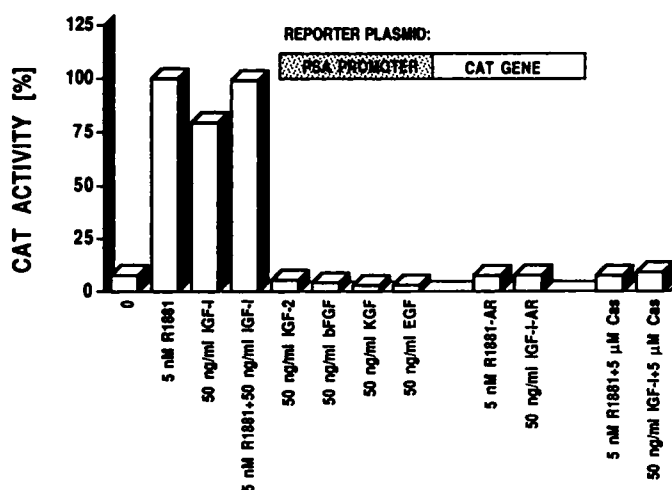
RESULTS

The effects of the synthetic androgen methyltrienolone and of IGF-I, IGF-II, bFGF, KGF, and EGF on AR-mediated transactivation of the androgen-inducible promoter containing two AREs in front of a TATA box were evaluated in DU-145 cells (Fig. 1). These cells were derived from a brain metastasis of a prostate cancer patient and represent a late tumor stage. They do not express endogenous steroid receptors (13). In these cells the reporter CAT gene and the AR expression vector were transiently coexpressed. Maximal stimulation of the AR in cotransfection-transactivation assays by androgen was achieved with 5 nM synthetic androgen methyltrienolone. IGF-I, at a concentration of 50 ng/ml, stimulated AR-mediated CAT activity to the same extent as methyltrienolone. At a concentration of 25 ng/ml, IGF-I displayed only a negligible effect on AR-mediated gene transcription in this experimental system. When 50 ng/ml of KGF were added, CAT activity increased to a level of about two-thirds of that achieved by methyltrienolone or IGF-I. EGF also showed minor stimulatory effects in cotransfection-transactivation assays, whereas IGF-II and bFGF did not cause any increase in CAT activity. In all experiments in which the empty expression vector was cotransfected into DU-145 cells with a reporter gene and androgen or growth factors added in culture media, CAT activity remained at the basal level. The nonsteroidal AR antagonist casodex, at a concentration of 5 μ M, completely inhibited the increase in CAT activity induced by IGF-I, KGF, or EGF (Fig. 1). These results provided evidence that the



SUPPLEMENT

Fig. 1. Induction of CAT activity by a synthetic androgen and growth factors in DU-145 cells transiently cotransfected with an AR expression vector and an androgen-responsive CAT gene, which is driven by a promoter containing two AREs in front of a TATA box. The cells were incubated in the absence (0) or presence of the synthetic androgen methyltrienolone (R 1881) or one of the growth factors IGF-I and -II, KGF, bFGF, and EGF for 32 h. Where indicated the nonsteroidal antiandrogen casodex (Cas) was added. The CAT activity was measured in the cell extracts. A control portion of cells (-AR) was transfected with an empty expression vector and the CAT reporter gene and treated equally as described above. The results are mean values of four independent experiments. Activities were normalized to the CAT activity of samples treated with 5 nM methyltrienolone and are given in percentages.



SUPPLEMENT

Fig. 2. Induction of CAT activity by a synthetic androgen and growth factors in DU-145 cells transiently cotransfected with an AR expression vector and an androgen-responsive CAT gene, which was driven by the PSA promoter. The experiments were performed as described in the legend of Fig. 1. The results are mean values of four to five independent experiments. Cas, casodex.

stimulatory effects of the three polypeptide growth factors on reporter gene transcription are mediated through the AR.

We further investigated whether growth factors had similar effects on AR activation of the PSA promoter, a naturally occurring androgen-regulated promoter, which contains one ARE. IGF-I (50 ng/ml) stimulated AR-mediated PSA gene promoter transcription (Fig. 2). This effect was slightly less pronounced than in the experiments in which the promoter containing two AREs was used. KGF, EGF, IGF-II, and bFGF were ineffective with the PSA promoter. The fact that the nonsteroidal antiandrogen casodex was able to suppress IGF-I-induced CAT activity in experiments with the PSA gene promoter confirms that this effect of IGF-I is mediated by the AR (Fig. 2).

The promoter-dependent differences in activation of reporter gene transcription in response to KGF and EGF prompted us to perform cotransfection experiments in which the CAT reporter gene was driven by an artificial promoter containing one ARE in front of a TATA box. Only IGF-I stimulated CAT activity in this experiment (Fig. 3). Taken together, our data show that IGF-I is able to activate AR-mediated reporter gene transcription irrespectively of the nature of the promoter driving the CAT gene. KGF and EGF, however, were effective only with the promoter containing two AREs in front of a TATA box. In all three experimental systems, 5 nM methyltrienolone and 50 ng/ml of IGF-I were also applied simultaneously to determine whether both AR activators displayed additive effects. There was, however, no increase in reporter gene activity above the maximal level which was achieved with 5 nM methyltrienolone (Figs. 1–3).

In order to confirm results with transiently expressed AR with those obtained with an endogenously expressed AR, we evaluated effects of growth factors on AR-mediated expression in prostatic tumor cells which contain endogenous AR. Among established human prostatic tumor cell lines, only LNCaP contains high amounts of AR. Since our efforts to measure CAT activity in LNCaP cells after introduction of the CAT reporter gene were not successful, we tested AR activation indirectly by measurement of the PSA level after treatment with androgen or one of the growth factors. PSA secretion in the LNCaP cell line has been previously shown to be AR mediated (14). Treatment of LNCaP cells with increasing concentrations of methyltrienolone resulted in a dose-dependent increase in PSA secretion (data not

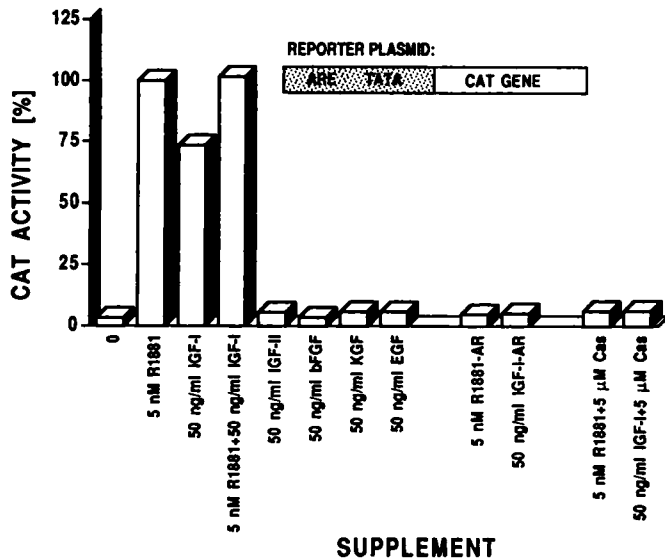


Fig. 3. Stimulation of CAT activity by a synthetic androgen and growth factors in DU-145 cells transiently cotransfected with an AR expression vector and an androgen-responsive CAT gene, which was driven by a promoter containing one ARE in front of a TATA box. The experiments were performed as described in the legend of Fig. 1. The results are mean values of two independent experiments. Cas, casodex.

shown). At a concentration of 5 nM methyltrienolone, PSA secretion was maximally induced. Among growth factors tested, only IGF-I was able to enhance PSA secretion (Fig. 4). The synthetic androgen methyltrienolone, at a concentration of 5 nM, stimulated PSA secretion approximately 8-fold, while IGF-I, at a concentration of 50 ng/ml, increased the PSA level in the supernatant 5-fold. As expected, casodex (5 μM) abolished these stimulatory effects of androgen and IGF-I (Fig. 4). At a concentration of 1 μM, casodex reduced the PSA level to approximately 20% of the maximal value (data not shown). Simultaneous addition of 5 nM methyltrienolone and 50 ng/ml of IGF-I did not increase the PSA level over that achieved by stimulation with 5 nM methyltrienolone alone (Fig. 4). bFGF and EGF exerted slight inhibitory effects on PSA secretion (Fig. 4). The increase in the PSA level achieved by IGF-I cannot be explained by an increase in cell number, since the cell viability assays did not reveal stimulatory effects of this growth factor on LNCaP cell growth during the experiment. We also observed no significant differences in the AR content, as assessed by binding assays (data not shown). Thus, stimulatory effects of IGF-I on PSA secretion cannot be attributed to an increase of the level of AR protein. Taken together, results obtained by measurement of PSA secretion in LNCaP cells are in concordance with the results of the cotransfection-transactivation assays in DU-145 cells. Both kinds of experiments show an activation of the AR by IGF-I.

DISCUSSION

The major finding of this study is that three polypeptide growth factors, namely, IGF-I, KGF, and EGF, can activate the AR directly in the absence of androgen. IGF-I turned out to be more potent than KGF, which, in turn, was more effective than EGF in this respect. IGF-I was effective with all androgen-inducible promoters tested in our experiments, whereas KGF and EGF did not elicit AR-dependent transcriptional activation of promoters containing one ARE. The findings that the pure antiandrogen casodex is capable of reducing the CAT activity induced by IGF-I, KGF, and EGF to the basal level and that no increase in CAT activity was observed in cells in which the empty expression vector was cotransfected with the reporter gene

support the concept of a direct activation of the AR by these three growth factors. At first sight it is surprising that KGF and EGF induce AR-mediated transactivation of a promoter with two AREs in tandem but were ineffective with promoters containing one ARE. With regard to this result one has to bear in mind that AR activation is a multistep process that is not yet understood in detail. We assume an incomplete AR activation by KGF and EGF that allows induction of the promoter with two closely spaced AREs; induction of a promoter with one isolated ARE would need additional activating (co-)signals. The hypothesis that transactivation of the former promoter type needs less AR activation than transactivation of the latter type is supported by the recent characterization of a mutant AR which was present in patients with incomplete androgen insensitivity (15). In cotransfection experiments, mutant AR (Ala⁵⁹⁵→Thr) transactivated the artificial promoter with two AREs as efficiently as the wild-type receptor, whereas it was unable to transactivate a promoter with one ARE.

The molecular mechanism involved in the interaction between growth factors and steroid receptors remains to be elucidated. An increase in overall receptor phosphorylation might be a possible explanation. Analysis of deletion mutants of the PR provided evidence that dopamine effects on PR activation are accompanied with phosphorylation of a specific amino acid in the COOH terminus (11). Estradiol and IGF-I were found to stimulate ER phosphorylation, but the same effect was observed after exposure of the ER to the antiestrogen ICI 164,384 (7). Thus, the relationship between phosphorylation and steroid receptor activation is as yet unclear.

Previous recognition that IGF-I directly activates the ER might provide an explanation for some tumor-promoting effects of this growth factor in breast cancer. IGF-I may stimulate growth of breast cancer cells in the absence of estrogen (16). This growth factor can synergize with estradiol in enhancing PR synthesis in the breast cancer cell line MCF-7 (17). The results presented in this paper suggest a similar interplay between androgen and growth factor-

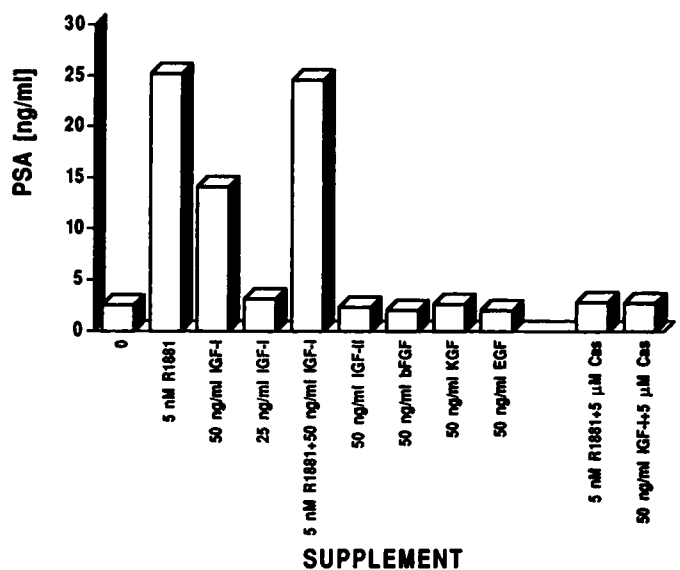


Fig. 4. Induction of PSA secretion in LNCaP cells by a synthetic androgen and growth factors. After attachment, LNCaP cells were cultured under serum-free conditions in the absence (0) or presence of methyltrienolone or growth factors. Where indicated the nonsteroidal antiandrogen casodex (Cas) was added. PSA levels were determined by enzyme immunoassay and normalized according to cell numbers as determined by a nonradioactive cell viability assay. The results are mean values of four independent experiments.

signaling pathways in the prostate. This cross-talk is of particular importance in prostatic carcinoma. It has been shown that lack of AR in advanced tumors is not the reason for failure of endocrine therapy of prostate cancer (18). The majority of tumors, androgen-dependent as well as androgen-independent ones, contain immunoreactive AR protein. Moreover, recent observations with LNCaP cells which were cultured for a long time in androgen-depleted medium even suggest an up-regulation of AR expression by androgen deprivation (19). Under these conditions, aberrant AR activation by growth factors might be facilitated and thus contribute to the failure of androgen ablation therapy. Another possible mechanism was described recently when mutated ARs with a broad hormonal activation spectrum were detected in prostatic carcinoma specimens (3–6).

Effects of IGF-I, which displayed strong stimulation on the AR-mediated reporter gene transcription, on growth of prostatic cells were investigated previously (20–22). IGF-I was found to stimulate proliferation of prostatic epithelial cells in primary cultures more efficiently than IGF-II and far more strongly than insulin (20). In view of our results, it is possible that at least a part of these growth stimulatory effects is due to activation of the AR. Recently, Barni *et al.* (23) provided evidence that IGF-I mRNA is localized in stroma, whereas the IGF-I receptor is detected in the epithelial compartment. These findings imply a paracrine mode of action of IGF-I in the prostate. In contrast to findings in primary cultures, addition of exogenous IGF-I does not stimulate growth of androgen-responsive LNCaP cells (21, 22). In our experiments we also did not see any growth stimulatory effects in LNCaP cells. With androgen-independent prostatic tumor cell lines PC-3 and DU-145, contradictory effects of exogenous IGF-I on growth were reported (21, 22). Whereas Iwamura *et al.* (21) observed stimulation of DNA synthesis by IGF-I, Pietrzowski *et al.* (22) did not report any growth stimulatory effect of this growth factor. Data regarding IGF-I production in human prostatic tumor cell lines are also not unequivocal. Iwamura *et al.* (21) did not detect immunoreactive levels of IGF-I in the conditioned medium of the prostatic tumor cell lines LNCaP, PC-3, and DU-145. By contrast, Pietrzowski *et al.* (22) claimed that, upon elimination of IGF-I-binding proteins, all three prostatic cancer cell lines produce IGF-I. This latter finding suggests that IGF-I is synthesized but is not effective because it is complexed by IGF-I-binding proteins. Several binding proteins form complexes with IGF-I. The IGF-binding protein-3 has a major role in its transport (24). Recent findings suggest that PSA is an IGF-binding protein-3 protease (25). In patients with metastatic prostatic cancer, decreased levels of IGF-binding protein-3 in serum were measured (26). Thus, PSA may enhance release of IGF-I and, indirectly, potentiate its stimulatory effects on the AR.

KGF, which was effective in stimulation of the reporter gene activity in experiments in which the CAT gene was driven by the promoter consisting of two AREs in front of a TATA box, belongs to the family of heparin-binding growth factors. The source of KGF in the prostate gland is the stromal cells, whereas the epithelial cells express specific receptors for this growth factor (27). Expression of KGF receptors correlates inversely with an evolution toward increased malignancy of the Dunning R3327PAP prostatic tumor (27). KGF appears to be an essential element in the transmission of androgen stimuli. The primary target of androgen action in the prostate is the cells in the mesenchymal compartment (28). In cultures, prostatic epithelial cells respond to dihydrotestosterone only in the presence of KGF; therefore, KGF is considered to have properties of a stromal to epithelial cell andromedin (27). Because of our findings the question of whether activation of the AR by KGF is an integral part of this signaling pathway has to be addressed. Unlike KGF, bFGF, which also belongs to the heparin-binding growth factor family, did not exert any stimulatory effect on AR-mediated gene transcription. This can-

not be due to a lack of bFGF receptor expression. LNCaP as well as DU-145 cells were shown to express bFGF receptors and to respond to exogenous bFGF (29).

EGF effects on transcription of the androgen-responsive reporter gene were less pronounced when compared with those of IGF-I and KGF. These results are in concordance with experiments in which EGF stimulation of ER-mediated transcription was studied (8).

Unfortunately, we were not successful in measuring reporter gene activity after addition of growth factors in LNCaP cells which contain endogenous AR. Similar observations regarding transfection of LNCaP cells with a reporter expression vector were previously reported by Riegman *et al.* (12). Therefore, we evaluated AR activation in the LNCaP cell line indirectly, upon measurement of PSA protein secretion. Regulation of PSA glycoprotein is mediated via the AR (14). Previous studies revealed that hormones which activate the AR in LNCaP cells also increase the PSA mRNA level and protein secretion (1, 14, 30). Our results demonstrated an up-regulation of the PSA protein by IGF-I, whereas other growth factors tested did not stimulate PSA secretion. bFGF and EGF were previously found to decrease the PSA level in LNCaP cells slightly below the values measured in a conditioned medium of untreated cells (14). This coincides with our results.

Previous studies of cross-talk between the androgen-signaling cascade and growth factors in the prostate have reported androgen-mediated regulation of growth factors EGF (31), acidic fibroblast growth factor (32), KGF (27), and transforming growth factor- β (33) expression. Our findings provide the first evidence of the existence of a signaling pathway between growth factors and the AR. The results presented in this study may serve as a basis for further experiments dealing with implications of AR activation by IGF-I, KGF, and EGF in an environment of androgen deficiency. This may influence the design of new modalities of endocrine therapy for advanced prostatic carcinoma.

ACKNOWLEDGMENTS

We gratefully acknowledge the expert technical assistance of G. Hölzl, E. Tafatsch, G. Sierek, R. Höllinger, and P. Dertschnig. We are indebted to Dr. A. C. B. Cato for helpful discussions and to Dr. B. Furr (ICI Pharma, Cheshire, United Kingdom) for providing casodex.

REFERENCES

- Veldscholte, J., Ris-Stalpers, C., Kuiper, G. G. J. M., Jenster, G., Berrevoets, C., Claassen, E., van Rooij, H. C. J., Trapman, J., Brinkmann, A. O., and Mulder, E. A mutation in the ligand-binding domain of the androgen receptor of human LNCaP cells affects steroid binding characteristics and response to antiandrogens. *Biochem. Biophys. Res. Commun.*, 173: 534–540
- Tilley, W. D., Wilson, C. M., Marcelli, M., and McPhaul, M. J. Androgen receptor gene expression in human prostate carcinoma cell lines. *Cancer Res.*, 50: 5382–5386, 1990.
- Newmark, J. R., Hardy, D. O., Tonb, D. C., Carter, B. S., Epstein, J. I., Isaacs, W. B., Brown, T. R., and Barrack, E. R. Androgen receptor gene mutations in human prostate cancer. *Proc. Natl. Acad. Sci. USA*, 89: 6319–6323, 1992.
- Culig, Z., Hobisch, A., Cronauer, M. V., Cato, A. C. B., Hittmair, A., Radmayr, C., Eberle, J., Bartsch, G., and Klocker, H. Mutant androgen receptor detected in an advanced-stage prostatic carcinoma is activated by adrenal androgens and progesterone. *Mol. Endocrinol.*, 7: 1541–1550, 1993.
- Suzuki, H., Sato, N., Watabe, Y., Masai, M., Seino, S., and Shimazaki, Y. Androgen receptor gene mutations in human prostate cancer. *J. Steroid Biochem. Mol. Biol.*, 46: 759–765, 1993.
- Schoenberg, M. P., Hakimi, J. M., Wang, S., Bova, G. S., Epstein, J. I., Fischbeck, K. H., Isaacs, W. B., Walsh, P. C., and Barrack, E. R. Microsatellite mutation (CAG_{24–18}) in the androgen receptor gene in human prostate cancer. *Biochem. Biophys. Res. Commun.*, 198: 74–80, 1994.
- Aronica, S. M., and Katzenellenbogen, B. S. Stimulation of estrogen receptor-mediated transcription and alteration in the phosphorylation state of the rat uterine estrogen receptor by estrogen, cyclic adenosine monophosphate, and insulin-like growth factor-I. *Mol. Endocrinol.*, 7: 743–752, 1993.
- Ignar-Trowbridge, D. M., Teng, C. T., Ross, K. A., Parker, M. G., Korach, K. S., and McLachlan, J. A. Peptide growth factors elicit estrogen receptor-dependent transcrip-

- tional activation of an estrogen-responsive element. *Mol. Endocrinol.*, 7: 992–998, 1993.
9. Cho, H., and Katzenellenbogen, B. S. Synergistic activation of estrogen receptor-mediated transcription by estradiol and protein kinase activators. *Mol. Endocrinol.*, 7: 441–452, 1993.
 10. Power, R. F., Lyndon, J. P., Connelly, O. M., and O'Malley, B. W. Dopamine activation of an orphan of the steroid receptor superfamily. *Science (Washington DC)*, 252: 1546–1548, 1991.
 11. Power, R. F., Mani, S. K., Codina, J., Connelly, O. M., and O'Malley, B. W. Dopaminergic and ligand-independent activation of steroid hormone receptors. *Science (Washington DC)*, 254: 1636–1639, 1992.
 12. Riegman, P. H. J., Vlietstra, R. J., van der Korput, J. A. G. M., Brinkmann, A. O., and Trapman, J. The promoter of the prostate-specific antigen gene contains a functional androgen responsive element. *Mol. Endocrinol.*, 5: 1921–1930, 1991.
 13. Stone, K. R., Mickey, D. D., Wunderli, H., Mickey, G. H., and Paulson, D. F. Isolation of a human prostate carcinoma cell line (DU-145). *Int. J. Cancer*, 21: 274–281, 1978.
 14. Montgomery, B. T., Young, C. Y. F., Bilhartz, D. L., Andrews, P. E., Prescott, J. L., Thompson, N. F., and Tindall, D. J. Hormonal regulation of prostate-specific antigen (PSA) glycoprotein in the human prostatic adenocarcinoma cell line, LNCaP. *Prostate*, 21: 63–73, 1992.
 15. Kaspar, F., Klocker, H., Denninger, A., and Cato, A. C. B. A mutant androgen receptor from patients with Reifenstein syndrome: identification of the function of a conserved alanine residue in the D box of steroid receptors. *Mol. Cell. Biol.*, 13: 7850–7858, 1993.
 16. Dickson, R. B., McManaway, M., and Lippman, M. E. Estrogen induced factors of breast cancer cells partially replace estrogen to promote tumor growth. *Science (Washington DC)*, 213: 1540–1543, 1986.
 17. Katzenellenbogen, B. S., and Norman, M. J. Multihormonal regulation of the progesterone receptor in MCF-7 human breast cancer cells: interrelationships among insulin/insulin-like growth factor-I, serum, and estrogen. *Endocrinology*, 126: 891–898, 1990.
 18. Van der Kwast, T. H., Schalken, J., Ruizveld de Vinter, J. A., van Vroonhoven, C. C. J., Mulder, E., Boersma, W., and Trapman, J. Androgen receptors in endocrine-therapy-resistant human prostate cancer. *Int. J. Cancer*, 48: 189–193, 1991.
 19. Kokontis, J., Takakura, K., Hay, N., and 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.
 20. Cohen, P., Peehl, D. M., Lamson, G., and Rosenfeld, R. Insulin-like growth factors (IGFs), IGF receptors, and IGF-binding proteins in primary cultures of prostate epithelial cells. *J. Clin. Endocrinol. Metab.*, 73: 401–407, 1991.
 21. Iwamura, M., Sluss, P. M., Casameto, J. B., and Cockett, A. T. K. Insulin-like growth factor I: action and receptor characterization in human prostate cancer cell lines. *Prostate*, 22: 243–252, 1993.
 22. Pietrzowski, Z., Mulholland, G., Gomella, L., Jameson, B. A., Wernicke, D., and Baserga, R. Inhibition of growth of prostatic cancer cell lines by peptide analogues of insulin-like growth factor I. *Cancer Res.*, 53: 1102–1106, 1993.
 23. Barni, T., Vannelli, B. G., Sadri, R., Pupilli, C., Ghiandi, P., Rizzo, M., Selli, C., Serio, M., and Fiorelli, G. Insulin-like growth factor-I (IGF-I) and its binding protein IGFBP-4 in human prostatic hyperplastic tissue: gene expression and its cellular localization. *J. Clin. Endocrinol. Metab.*, 78: 778–783, 1994.
 24. Czech, M. P. Signal transmission by the insulin-like growth factors. *Cell*, 59: 235–238, 1989.
 25. Cohen, P., Graves, H. C. B., Peehl, D. M., Kamarei, M., Giudice, L. C., and Rosenfeld, R. G. Prostate specific antigen (PSA) is an insulin-like growth binding protein-3 protease found in seminal plasma. *J. Clin. Endocrinol. Metab.*, 75: 1046–1053, 1992.
 26. Kanety, H., Madjar, Y., Dagan, Y., Levi, J., Papa, M. Z., Pariente, C., Goldwasser, B., and Karasik, A. Serum insulin-like growth factor-binding protein-2 (IGFBP-2) is increased and IGFBP-3 is decreased in patients with prostate cancer: correlation with serum prostate-specific antigen. *J. Clin. Endocrinol. Metab.*, 77: 229–233, 1993.
 27. Yan, G., Fukabori, Y., Nikolaropoulos, S., Wang, F., and McKeehan, W. L. Heparin-binding keratinocyte growth factor is a candidate stromal to epithelial cell andromedin. *Mol. Endocrinol.*, 6: 2123–2128, 1992.
 28. Chang, S.-M., and Chung, L. W. K. Interaction between prostatic fibroblast and epithelial cells in culture: role of androgen. *Endocrinology*, 125: 2719–2727, 1989.
 29. Nakamoto, T., Chang, C. S., Li, A. K., and Chodak, G. W. Basic fibroblast growth factor in human prostate cancer cells. *Cancer Res.*, 52: 571–577, 1992.
 30. Young, C. Y. F., Montgomery, B. T., Andrews, P. E., Qui, S., Bilhartz, D. L., and Tindall, D. J. Hormonal regulation of prostate-specific antigen mRNA in a human prostatic adenocarcinoma cell line LNCaP. *Cancer Res.*, 51: 3748–3752, 1991.
 31. Schuurmans, A. L. G., Bolt, J., Voorhorst, M., Blankenstein, R. A., and Mulder, E. Regulation of growth and epidermal growth factor receptor levels of LNCaP prostatic tumor cells by different steroids. *Int. J. Cancer*, 42: 917–922, 1988.
 32. Harris, S. E., Hall, J. A., Rong, Z. X., Chui, I.-M., and Harris, M. Structure and function of the acidic fibroblast growth factor I gene in the androgen-dependent prostate cancer cell line LNCaP and hamster DDT1 cells. *J. Cell. Biochem.* 16D: 97, 1992.
 33. Wilding, G., Mitchen, J., Cheng, A.-L., Ripple, M., Rago, R., Mott, F., and Chang, C. The role of transforming growth factor- β in human prostate cancer. *J. Cell. Biochem.*, 16D: 87, 1992.