Epidermal Growth Factor (EGF) Receptor Blockade Inhibits the Action of EGF, Insulin-like Growth Factor I, and a Protein Kinase A Activator on the Mitogen-activated Protein Kinase Pathway in Prostate Cancer Cell Lines


ABSTRACT

Epidermal growth factor (EGF) and insulin-like growth factor I (IGF-I) are potent mitogens that regulate proliferation of prostate cancer cells via autocrine and paracrine loops and promote tumor metastasis. They exert their action through binding to the corresponding cell surface receptors that initiate an intracellular phosphorylation cascade, leading to the activation of mitogen-activated protein kinases (MAPKs), which recruit transcription factors. We have studied the effects of EGF, IGF-I, and the protein kinase A (PKA) activator forskolin on the activation of p42/p44 MAPK-specific antibody. EGF, IGF-I, and forskolin-induced PKA activity stimulate intracellular signaling pathways converging at the level of p42/ERK2. In the androgen-insensitive DU145 cell line, there is a constitutive basal p42/ERK2 activity that is not present in androgen-sensitive LNCaP cells. Constitutive p42/ERK2 activity is abrogated by blockade of the EGFR receptor. Hence, it is obviously caused by an autocrine loop involving this receptor. The effects of EGF on p42/ERK2 are potentiated by forskolin in both cell lines. The blockade of PKA by the specific inhibitor H89 attenuates this synergism. This finding is in contrast to those obtained in several other systems studied thus far, in which PKA activators inhibited MAPKs. p42/ERK2 in DU145 cells is highly responsive to IGF-I stimulation, whereas no effect of IGF-I on p42/ERK2 can be measured in LNCaP cells. Moreover, our results demonstrate that selective blockade of the PKA activity in prostate cancer cells does not only inhibit the action of EGF, but also IGF-I-induced activation of the MAPK pathway and the interaction with the PKA pathway. In conclusion, these findings offer new possibilities for a therapeutic intervention in prostate cancer by targeting signaling pathways of growth factors and PKA.

INTRODUCTION

Androgen ablation, the standard treatment for nonorgan-confined prostate cancer, intends to block the hormonal signaling cascade leading to the activation of the androgen receptor. In the hormone-responsive stage of the tumor, this treatment causes down-regulation of androgen-responsive genes and leads to apoptosis. Although androgen ablation is initially successful, tumors overcome androgen blockade and develop a hormone-unresponsive phenotype with resistance to therapy. Progression to therapy-refractory prostate cancer can be, in part, explained by the concept of hypersensitive tumor cells (1). These cells are highly responsive to residual circulating androgens, growth factors, and other cellular regulators.

In this respect, the mitogenic effects of growth factors are of utmost significance (2, 3). EGF (3) and IGF-I are potent mitogens that play a regulatory role in proliferation of prostate cancer cells. It has been demonstrated that stromal prostate tissue supports the growth of cancer cells predominantly by secreting growth factors. Moreover, various autocrine loops have been postulated in prostate cancer cells. Additionally, EGF and IGF-I activate the androgen receptor in prostate cancer cells in the absence of androgen (4), and IGF-I has been suggested to promote prostate cell metastasis (5). In concordance with that observation, high serum levels of IGF-I have recently been shown to be associated with an increased risk for prostate cancer (6).

The androgen-independent human prostate cancer cell line DU145 and the androgen-sensitive prostate cancer cell line LNCaP are responsive to stimulation with EGF and IGF-I (3). These growth factors exert their effects through the corresponding receptors expressed in both cell lines. Ligand binding to the cell surface receptor initiates an intracellular phosphorylation cascade resulting in the activation of MAPKs, which recruit transcription factors and, thus, control transcriptional activity.

Among the subgroups of MAPKs (7–9), the ERKs function as key mediators of the mitogenic potential of growth factors. In general, the Ras/Raf/ERK cascade is associated with proliferative effects. For LNCaP cells, it has been reported that overexpression of a mutated Ras results in increased growth. The chemotherapeutic agent phenylacetate has reduced the phosphorylated forms of p42/ERK2 via the Ras pathway and has inhibited cell proliferation (10). An antisense oligonucleotide directed against Raf-1 has also demonstrated inhibitory effects in LNCaP cells (11).

The cAMP-inducible PKA (12) interacts with growth factor signaling. Inhibitory links of cAMP and the PKA pathway to the ERK cascade have been described in many systems (13–17). However, cAMP does not always attenuate MAPK action (18–22). The implications of these second messenger pathways on the ERK cascade in prostate cancer cells are unclear. For LNCaP cells, it has been shown that second messengers including cAMP mediate proliferative effects of the neuropeptide calcitonin (23). In ALVA-4I prostate cancer cells, a cAMP analogue has increased growth rate (24), whereas high cAMP levels have retarded the prostate cancer cell line PC-3 (25).

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2 To whom requests for reprints should be addressed, at Department of Urology, Anichstrasse 35, A-6020 Innsbruck, Austria. Fax: 43-512-504-4817 or 43-512-504-4873; E-mail: Helmut.Klocker@uibk.ac.at.

3 The abbreviations used are: EGF, epidermal growth factor; IGF-I, insulin-like growth factor I; PKA, protein kinase A; MAPK, mitogen-activated protein kinase; ERK, extracellular signal-regulated kinase; cAMP, cyclic adenosine 3’,5’-monophosphate; GST, glutathione S-transferase; HRP, horseradish peroxidase; TBS, Tris-buffered saline; ECL, enhanced chemiluminescence; TBST, Tris-buffered saline Tween 20.

References

[1] The abbreviations used are: EGF, epidermal growth factor; IGF-I, insulin-like growth factor I; PKA, protein kinase A; MAPK, mitogen-activated protein kinase; ERK, extracellular signal-regulated kinase; cAMP, cyclic adenosine 3’,5’-monophosphate; GST, glutathione S-transferase; HRP, horseradish peroxidase; TBS, Tris-buffered saline; ECL, enhanced chemiluminescence; TBST, Tris-buffered saline Tween 20.
metastasis, displays properties of prostate cancer early in development (28, 29).

We show that forskolin-induced PKA activity and the putative mitogens EGF and IGF-I activate intracellular signaling pathways converging at the level of MAPK p42/ERK2. The basal activity of p42/ERK2 is constitutively elevated in the DU145 cell line. The effects of exogenously added EGF can be potentiated by forskolin in both cell lines. Moreover, our results demonstrate that blockade of the EGF receptor in prostate cancer cells attenuates not only the actions of EGF, but also IGF-I-induced activation of the MAPK pathway and the interaction with the PKA pathway.

MATERIALS AND METHODS

Cell Culture

LNCaP and DU145 cell lines were obtained from the American Type Culture Collection. Cells were maintained in RPMI 1640 (Hyclone, Logan, UT) with 10% FCS (Hyclone), 100 units/ml penicillin, and 0.1 mg/ml streptomycin (PAK Laboratory, Linz, Austria) at 37°C and 5% CO2. Cells were routinely tested for mycoplasma by using a PCR ELISA kit (Boehringer Mannheim, Vienna, Austria). Before any experiment, cells were trypsinized and plated in 6-well plates (Falcon; Becton Dickinson, Lincoln Park, NJ; Costar, Cambridge, MA) in RPMI 1640 with 1% FCS and antibiotics. After serum starvation for 24 h, nearly confluent cells were incubated with growth factors, forskolin, and inhibitors. Treatment with EGF (Stratham Biotech, Hannover, Germany), IGF-I (Bionol, Hamburg, Germany), forskolin (Sigma Chemical Co., St. Louis, MO), MAH-EGFR-528 (Santa Cruz Biotechnology, Santa Cruz, CA), Tyrphostin AG 1478 (Alexis, San Diego, CA), and H89 (Calbiochem, La Jolla, CA) was followed by subsequent MAPK assays.

MAPK Assays

Immune Complex Kinase Assays for p42/ERK2.

After growth factor stimulation, cells were lysed in ice-cold buffer containing 50 mM Tris-HCl (pH 7.3), 5 mM EDTA, 50 mM NaCl, 5 mM Na3PO4 X 10 H2O, 5 mM NaF, 5 mM Na2VO4, 2% Triton X-100, 6 mg/ml aprotinin, and 6 mg/ml leupeptin. Lysates were clarified by centrifugation at 13,000 rpm for 10 min at 4°C. The supernatant was preclarified with 20 µl of Pansorbin-cells (Calbiochem) for 1 h on a shaker at 4°C. After removing Pansorbin-cells by centrifugation (3 min, 8000 rpm, 4°C), immune precipitation using an anti-p42/ERK2 rabbit polyclonal Ig (Santa Cruz Biotechnology) was performed overnight on a shaker at 4°C. Then, 20 µl of Pansorbin-cells were added and agitated for 1 h at 4°C. Immune complexes were collected by centrifugation (3 min, 8000 rpm, 4°C) and washed three times in lysis buffer and once in kinase buffer containing 25 mM Hepes (pH 7.5), 2 mM MnCl2, and 20 mM MgCl2.

The kinase reaction was performed by resuspending immune complexes in 20 µl of kinase buffer supplemented with 0.45 mg/ml GST-Elk1, 10 µM ATP, and 1 µCi/ml ATP32 (New England Nuclear, Dreieichenhain, Germany). After a 30-min incubation at 30°C, the reaction was stopped by adding electrophoresis sample buffer. Before electrophoresis, samples were boiled for 10 min and separated on a 14% SDS-polyacrylamide gel. Proteins were transferred to Immobilon-P membranes (Millipore, Bedford, MA) for 5 min at 300 mA in Towbin buffer containing 25 mM Tris, 192 mM Glycin, 20% methanol, and 3.5 mM SDS (pH 8.3). Then, membranes were exposed to autoradiography films (Amersham, Buckinghamshire, England) for 24 h.

After autoradiography, membranes were probed for detection of phosphorylated p42/ERK2 protein levels. Herefore, membranes were soaked to determine the corresponding protein levels. Herefore, membranes were soaked in methanol and washed in TBS buffer containing 50 mM Tris-HCl (pH 7.5) and 150 mM NaCl for 5 min. Then they were incubated with 62.5 mM Tris-HCl (pH 6.8), 100 mM mercaptoethanol, and 2% SDS for 30 min at 60°C. After washing five times for 5 min in TBS, membranes were soaked in methanol and washed in TBS containing 1% nonfat dry milk for 1 h and incubated for 2 h with anti-p42/ERK2 rabbit polyclonal IgG (dilution, 1:666; Santa Cruz Biotechnology) in TBS containing 1% nonfat dry milk. After washing five times for 10 min in TBS, membranes were probed for 1 h with an HRP-conjugated antirabbit antibody (dilution, 1:2000; Santa Cruz Biotechnology) in TBS containing 1% nonfat dry milk. Proteins were visualized by the ECL detection reagents after washing five times in TBS and once in TBS.

Immunoblot of Phosphorylated p42/ERK2 and p44/ERK1.

This assay uses a monoclonal antibody specific for activated p42/ERK2 and p44/ERK1. Cells were lysed in electrophoresis sample buffer and boiled for 5 min. After separation on 12% SDS-polyacrylamide gels, proteins were transferred to Immobilon-P membranes as described above. Membranes were washed in TBS buffer containing 50 mM Tris-HCl (pH 7.5) and 150 mM NaCl for 5 min. After washing three times for 5 min in TBS containing 50 mM Tris-HCl (pH 7.5), 150 mM NaCl, and 0.05% Tween 20, membranes were blocked with TBS containing 1% nonfat dry milk for 1 h. Membranes were incubated for 2 h with antibodies (Phospho-p44/p42 MAPK monoclonal antibody; dilution, 1:666; New England Biolabs, Beverly, MA) in TBS containing 1% nonfat dry milk. After washing five times for 10 min in TBS, membranes were probed for 1 h with an HRP-conjugated antimouse antibody (dilution 1:2000; Amersham) in TBS containing 1% nonfat dry milk. After washing five times in TBS and once in TBS, phosphorylated p42/ERK2 and p44/ERK1 proteins were visualized by the ECL detection reagents. In any experiment performed, there was no significant difference between the two assays (i.e., autophosphorylation status of p42/ERK2 and the kinase activity toward GST-Elk1).

After immunoblotting with phospho-p44/p42 MAPK monoclonal antibodies, membranes were stripped to determine the corresponding protein levels. Herefore, membranes were soaked in methanol and washed in TBS buffer containing 50 mM Tris-HCl (pH 7.5) and 150 mM NaCl for 5 min. Then they were incubated with 62.5 mM Tris-HCl (pH 6.8), 100 mM mercaptoethanol, and 2% SDS for 30 min at 60°C. After washing five times for 5 min in TBS, membranes were blocked with TBS containing 1% nonfat dry milk for 1 h and incubated for 2 h with anti-phospho/p42/ERK2 rabbit polyclonal IgG (dilution, 1:666; Santa Cruz Biotechnology) in TBS containing 1% nonfat dry milk. After washing five times for 10 min in TBS, membranes were probed for 1 h with an HRP-conjugated antirabbit antibody (dilution, 1:2000; Santa Cruz Biotechnology) in TBS containing 1% nonfat dry milk. Proteins were visualized by the ECL detection reagents after washing five times in TBS and once in TBS.

RESULTS

Basal Activity of p42/ERK2 Is Elevated in DU145 Cells.

DU145 and LNCaP cells are model systems with different growth characteristics and phenotypes (26–29), reflecting the heterogeneity of human prostate carcinomas. We addressed the question whether these distinct properties also are evident at the level of the mitotic ERK signaling pathway. Herefore, we investigated the activity of the MAPK p42/ERK2. The basal phosphorylation status of p42/ERK2 in human prostate cancer cell lines was determined after starvation for 24 h in RPMI containing 1% FCS. Activity of immunoprecipitated p42/ERK2 was then measured by phosphorylation of the transcription factor Elk1, which was expressed as a recombinant GST-fusion protein. Alternatively, the activation status of p42/ERK2 and p44/ERK1 was determined by Western blotting of the cell lysate with phosphospecific antibodies that detect p42/ERK2 and p44/ERK1 phosphorylated at threonine 202 and tyrosine 204. DU145 cells displayed elevated activity of p42/ERK2 when compared with LNCaP cells (Fig. 1). This finding clearly indicates a constitutively active MAPK in DU145 cells.

Inhibition of the EGF Receptor Blocks Constitutive Activation of p42/ERK2 in DU145 Cells.

Exogenously added EGF enhanced activity of p42/ERK2 in LNCaP and DU145 cells (Fig. 2). We asked whether the constitutively active p42/ERK2 in DU145 cells could be associated with autocrine stimulation of the EGF pathway. For this purpose, we examined possibilities to down-regulate ERK activity by blocking the EGF receptor. Preincubation of DU145 cells with Tyrophostin AG 1478, a selective inhibitor of the tyrosine kinase of the EGF receptor, for 1 h resulted in decreased constitutive activity of p42/ERK2. This effect was concentration-dependent, showing a weak inhibition of p42/ERK2 phosphorylation with 3 mM Tyrophostin AG 1478 (data not shown). Treatment with 30 mM Tyrophostin AG 1478...
resulted in a complete inhibition of constitutive p42/ERK2 activity (Fig. 3). Treatment of DU145 cells with 3 nM of the monoclonal antibody MAb-EGFR-528 for 1 h also abrogated constitutive p42/ERK2 activity. This antibody binds to a cell surface epitope of the EGF receptor and inhibits binding of EGF, thus, antagonizing EGF-stimulated tyrosine kinase activity. Taken together, these results provide evidence for an extracellular effector of the EGF receptor that stimulates the downstream kinase activity leading to a constitutive p42/ERK2 signal in DU145 cells, rather than a dominant positive signaling protein upstream of this MAPK.

The PKA Activator Forskolin and EGF Act Synergistically to Induce p42/ERK2 Activity in Prostate Cancer Cells. The intracellular signaling network of MAPKs is affected by various modulators. We focused on the regulatory links of the PKA activator forskolin to the ERK cascade. Fig. 4 shows a densitometric analysis of the time course of p42/ERK2 activity in response to EGF and forskolin. For these experiments, EGF concentrations that allowed the measurement of fine nuances in p42/ERK2 activities were determined. EGF doses were 2.5 ng/ml for DU145 and 10 ng/ml for LNCaP cells, respectively. Exogenously added EGF increased the phosphorylation of p42/ERK2 in both cell lines, whereas forskolin (20 μM), in the absence of any other supplement, displayed a weak stimulatory effect on p42/ERK2 activity in DU145 cells. No influence of forskolin on p42/ERK2 phosphorylation could be detected in LNCaP cells, even at higher concentrations (40 μM). However, incubation of cells with EGF and forskolin potentiated the effects of EGF on p42/ERK2 activity. The cooperative action of simultaneously added EGF and forskolin in DU145 cells was transient and less pronounced than in LNCaP cells and could be achieved predominantly in the early phase of p42/ERK2 induction. The low intensity of this p42/ERK2 costimulation and the failure to induce a costimulative activation of p42/ERK2 when DU145 cells were treated with forskolin before the EGF addition (data not shown) suggests a short-term additive effect, but not exclusively the involvement of PKA. The cooperative effect of EGF and forskolin was more evident in LNCaP cells. In this cell line, synergism also could be detected after 20 min of preincubation with forskolin (data not shown). Pretreatment for 30 min with H89 (10–25 μM), an inhibitor of PKA, abrogated the synergism of EGF and forskolin in LNCaP cells (Fig. 5), which indicates a PKA-dependent cooperative activation of p42/ERK2.

Different Effects of IGF-I on p42/ERK2 Activation in Androgen-insensitive and Androgen-sensitive cells. There is increasing evidence that IGF-I is an important growth factor in the pathogenesis of prostate cancer (30). IGF-I signaling shares both common and distinct pathways with EGF (31). Therefore, we were interested whether both growth factors activate p42/ERK2 in prostate cells in a similar manner. Fig. 6 demonstrates a response of p42/ERK2 in serum-starved DU145 cells after stimulation with 100 ng/ml IGF-I. We observed that IGF-I-induced p42/ERK2 kinetics was similar to that of EGF. In contrast to the findings in DU145 cells, no influence on p42/ERK2 activity could be measured in serum-starved LNCaP cells in a time range of 60 min with IGF-I concentrations from 50–200 ng/ml (data not shown). Fig. 6 shows a representative experiment in which LNCaP cells were treated with 100 ng/ml IGF-I. Our results with EGF in LNCaP cells demonstrated that, within 60 min,
p42/ERK2 activation can be achieved. Consequently, IGF-I and EGF-activated pathways are either different or IGF-I receptor stimulation in LNCaP cells is insufficient for activating p42/ERK2.

The Blockade of the EGF Receptor in Prostate Cancer Cells Attenuates not only the Action of EGF, but also IGF-I-induced Activation of the ERK Pathway and Interaction with the PKA Pathway. The effective blockade of the constitutive activation of p42/ERK2 in DU145 cells (Fig. 3) supports the view of the EGFR as a central component in the activation of MAPKs. To address the question whether EGFR and IGF-I pathways interact with each other, we investigated the effects of MAb-EGFR-528 on growth factor stimulation. As expected, preincubation of DU145 cells for 1 h with MAb-EGFR-528 (3–20 nm) abrogated the activity of p42/ERK2 induced by EGF (2.5 ng/ml; Fig. 7). Surprisingly, MAb-EGFR-528 also inhibited the IGF-I-induced (25 ng/ml) p42/ERK2 signal in DU145 cells (Fig. 7). Similar results were achieved when the selective inhibitor Tyrphostin AG 1478 (3–300 nm) was used to block the tyrosine kinase of the EGFR receptor (data not shown). This result clearly indicates that EGFR and IGF-I activate the ERK pathway via the involvement of the EGF receptor in DU145 cells.

Fig. 8 demonstrates that the EGF receptor blockade abrogates EGF-induced p42/ERK2 activation in LNCaP cells. This is in line with the finding that the blockade of the EGF receptor in DU145 cells inhibited forskolin-mediated enhancement of basal p42/ERK2 activity (Fig. 8). Obviously, in this case, forskolin action on p42/ERK2 depends on autocrine growth factor loops.

In conclusion, EGF receptor blockade does not only inhibit EGF, but also IGF-I-initiated mitogenic action and the synergistic effects of the PKA pathway on p42/ERK2. These findings offer new possibilities for therapeutical intervention in prostate cancer.

DISCUSSION

In the present study, we have investigated the activity of p42/ERK2 and its response to stimulation with growth factors and a PKA activator in prostate cancer cell lines. Previous studies have demonstrated the impact of growth factors on prostate cancer cell growth. For the androgen-independent prostate cancer cell line DU145, it has been shown that EGF stimulates thymidine incorporation and proliferation (32–34). High levels of EGF receptor expression (32, 35–39) and autocrine secretion of its ligands EGF and transforming growth factor α have been reported for this cell line (33, 34, 40, 41). Antibodies directed against the EGF receptor have been shown to decrease the growth rate of DU145 cells (38, 42) and to reduce autophosphorylation of the EGF receptor (38, 39, 43). Peng et al. (44) have induced G1 cell cycle arrest of DU145 cells with EGF receptor blockade. Our finding that EGF receptor blockade inhibits constitutive p42/ERK2 activity is consistent with previous studies and suggests an up-regulated ERK activity due to autocrine growth factor loops in androgen-independent prostate cancer cells. It also supports the hypothesis that autocrine growth regulation via the EGF receptor offers...
Fig. 7. EGF receptor blockade inhibits EGF and IGF-I-induced activation of p42/ERK2 in DU145 cells. Preincubation of DU145 cells for 1 h with the indicated concentrations of MAb-EGFR-528 was carried out before stimulation with IGF-I (25 ng/ml) or EGF (2.5 ng/ml) for 15 min. Phosphorylation of p42/ERK2 was determined by immunoblotting with a phospho-p44/p42 MAPK monoclonal antibody (top). The loading control was carried out with an anti-p42/ERK2 rabbit polyclonal antibody.

Fig. 8. EGF receptor blockade inhibits forskolin-induced activation of p42/ERK2 in DU145 cells and EGF-induced activation of p42/ERK2 in LNCaP cells. Preincubation of cells for 1 h with the indicated concentrations of MAb-EGFR-528 was carried out before stimulation with 10 ng/ml EGF or 40 μM forskolin for 15 min. Phosphorylation of p42/ERK2 was determined by immunoblotting with a phospho-p44/p42 MAPK monoclonal antibody (top). The loading control was carried out with an anti-p42/ERK2 rabbit polyclonal antibody (bottom).

a possibility to bypass the need for normal levels of androgens in advanced tumors.

In androgen-sensitive LNCaP cells, p42/ERK2 is not constitutively activated, although autocrine phosphorylation of the EGF receptor has been reported for these cells (39, 45). This may be due to the expression of lower amounts of EGF and transforming growth factor α (40) and fewer EGF receptors (32, 35, 36, 39) than in DU145 cells. However, p42/ERK2 in LNCaP cells is activated by EGF. Therefore, our results support the concept that the reported growth-promoting effects of EGF in LNCaP cells (46, 47) are mediated, at least in part, by p42/ERK2.

The role of the PKA pathway in the regulation of p42/ERK2 has not been investigated in prostate cancer cells before our study. For various other cell lines, it has been demonstrated that second messenger pathways provide regulatory links to the Ras/Raf/ERK cascade, resulting in a subsequent reduction of MAPK activities (13–17). However, cAMP-raising substances do not always counteract ERK action. cAMP-mediated stimulation of ERK activities (18–22), as well as a lack of effects of forskolin on growth factor- or serum-induced MAPK activities (48, 49) has been reported. The influence of PKA on Ras/Raf interaction is regarded as a key regulatory step in integrating cAMP and growth factor signals into the ERK cascade. The underlying mechanism is cell type-specific and dependent on isotype expression of the signaling molecules involved. Differential regulation of Raf isotypes by cAMP (50, 51) has been reported. Additionally, phosphorylation of Raf-1 by PKA modulates its interaction with different members of the Ras family (52). Nevertheless, growth factor signaling via Ras/Raf-independent pathways should be taken into account (19, 53). PKA isotype expression also plays a role in the interaction with EGF receptor-mediated signal transduction pathways. In fact, a linkage of PKA-I to the adaptor protein Grb2 has been demonstrated (54) in mammary epithelial cells, which offers the possibility that PKA-I mediates mitogenic signaling of EGF and related growth factors.

Despite unresolved questions concerning the appropriate PKA and Ras/Raf/ERK interaction, we demonstrate that forskolin supports the EGF-induced p42/ERK2 activity in prostate cancer cell lines. This result provides evidence that cAMP-raising substances vigorously increase the mitogenicity of the ERK pathway at least in androgen-sensitive LNCaP cells.

Activation of p42/ERK2 in response to EGF stimulation is a common feature of the tested cell lines. In contrast, different effects of IGF-I can be detected in LNCaP and DU145 cells. Both cell lines have been shown to express the IGF-I receptor (55–59). mRNA levels for the IGF-I receptor have been reported to be higher in DU145 cells than in LNCaP cells (59), which is in line with higher IGF-I receptor concentrations in DU145 cells calculated in ligand binding studies (58). Previous studies concerning responses to IGF-I in prostate cells are, in part, contradictory. IGF-I has been demonstrated to stimulate thymidine uptake in DU145 (57, 58) but not in LNCaP cells (58), whereas IGF-I-induced growth stimulation in both DU145 (5, 56) and LNCaP cells (5) has been reported. Peptide analogues of IGF-I that compete with IGF-I for binding and an antisense oligonucleotide to IGF-I receptor have inhibited the growth of DU145 and LNCaP cells in serum-free medium. Detection of autophosphorylated IGF-I receptors and IGF-I secretion has indicated the existence of an autocrine loop in these cells. In that study, no further increase in proliferation could have been measured in response to exogenously added IGF-I (59). Our results demonstrate that IGF-I is a potent activator of the ERK cascade in DU145 cells. Although the mitogenicity of IGF-I has been suggested, no corresponding p42/ERK2 activation can be measured in LNCaP cells as a consequence of IGF-I stimulation. The reasons for this unexpected result are unclear. A possible explanation for our finding is that IGF-I uses different mitogenic signaling pathways involving an ERK-independent mechanism in LNCaP cells. Moreover, it can be hypothesized that IGF-I receptor expression in LNCaP is not sufficient for inducing a measurable short-term down-stream MAPK signal. In respect to the complexity of IGF-I and EGF actions, the interplay between the autocrine growth factor loops and receptor interactions could be of profound importance. This view is supported by our finding that blockade of the up-regulated ERK pathway evoked by MAb-EGFR-528 also abrogates IGF-I-induced p42/ERK2 activation in DU145 cells. Similar results have been reported by Connolly and Rose (57), who have demonstrated that the interruption of the ERG autocrine loop by an anti-ERG receptor antibody in DU145 cells results in a complete loss of IGF-I responsiveness.

Taken together, we demonstrate that the mitogenetic effects of EGF, IGF-I, and forskolin-induced PKA in prostate cancer cells converge at the level of the MAPK p42/ERK2. Blockade of the EGF receptor was sufficient to abrogate autocrine effects, as well as the mitogenic action of exogenous growth factors. The fact that the inhibition of signal
transduction at the receptor level does not only abrogate EGF-induced p42/ERK2 activity but also IGF-I and forskolin-induced PKA action emphasizes the significance of the EGF receptor as a central component in MAPK signaling in prostate cancer cells and suggests possibilities for therapeutic intervention. EGF receptor-blocking antibodies, alone or in combination with PKA inhibitors, have been demonstrated to display antitumor activity in various human cancer cell lines in vitro and in vivo. Furthermore, EGF receptor-blocking antibodies and PKA inhibitors have already entered human clinical trials (60). In conclusion, the observations in our model system suggest that this therapeutical strategy should be further evaluated in prostate cancer treatment.

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REFERENCES


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