Protein kinase C-α mediates epidermal growth factor receptor transactivation in human prostate cancer cells

Jubilee R. Stewart and Catherine A. O’Brian

Department of Cancer Biology, The University of Texas M.D. Anderson Cancer Center, Houston, Texas

Abstract

Progression of human prostate cancer to a malignancy that is refractory to androgen-ablation therapy renders the disease resistant to available treatment options and accounts for the high prostate cancer mortality rate. Epidermal growth factor receptor (EGFR) expression in human prostate cancer specimens increases with disease progression to androgen-refractory prostate cancer, and experimental models implicate EGFR-dependent signaling to Erk1/2 activation in the androgen-refractory prostate cancer phenotype. 12-O-Tetradecanoylphorbol-13-acetate (TPA)-induced Erk1/2 activation in human prostate cancer PC-3 cells is a paradigm of diacylglycerol-induced EGFR transactivation in androgen-independent prostate cancer. In this report, we establish an obligatory role for TPA-induced protein kinase C (PKC)-α activation in EGFR transactivation and signaling to Erk1/2 activation in PC-3 cells. TPA-regulated molecules include PKCs, PKDs, and Ras guanyl nucleotide-releasing proteins. The PKC-selective inhibitors GF109203X and Gö6983 each blocked TPA-induced EGFR transactivation, indicating a requirement for PKC. PC-3 cells express four PKC isozymes. Prolonged bryostatin 1 treatment abrogated PKCα expression without altering levels of the other PKC isozymes. Pharmacologic PKCα ‘knockdown’ abrogated TPA-induced Erk1/2 activation without affecting the EGF/EGFR-induced response, indicating that PKCα was required for EGFR transactivation but dispensable for signaling of ligand-activated EGFR to Erk1/2 activation. We corroborated this by showing that Gö6976, which is a PKCα-selective inhibitor in PC-3 cells, likewise abolished TPA-induced Erk1/2 activation and did not inhibit EGF/EGFR-induced Erk1/2 activation. Gö6976 had similar effects in DU145 cells, providing evidence for a common PKCα-dependent Erk1/2 activation mechanism in androgen-independent human prostate cancer cells of distinct genetic origin. These results constitute a rational basis for selective PKCα inhibition as a modality of prostate cancer therapy.

Introduction

Targeted therapeutics that disable signaling molecules upon which in vivo growth and survival advantages of malignant cells pivot, illustrated by Gleevec inhibition of Bcr-Abl, are expected to spawn a new generation of cancer therapeutics for use alone or in conjunction with cytotoxic anticancer drugs such as taxol. Optimism that epidermal growth factor receptor (EGFR)-targeted therapeutics may be honed into an effective weapon against prostatic malignancies is predicated on the expression of EGFR and EGFR ligands in human prostate carcinoma tissue specimens and cell lines, and compelling evidence that EGFR-dependent autocrine signaling contributes to prostate cancer progression to hormone-refractory disseminated disease.

Analysis of human prostate epithelial tissue specimens by immunohistochemistry has revealed that expression levels of EGFR and the EGFR ligands, epidermal growth factor (EGF) and transforming growth factor-α, are significantly increased in neoplastic prostate epithelia compared with the normal epithelium (1). Furthermore, a similar analysis of human prostate cancer tumors corresponding to different clinical disease stages established that EGFR expression significantly increases in neoplastic epithelial cells with disease progression and is predictive of relapse following radical prostatectomy (2). The importance of EGFR-dependent autocrine signaling to prostate cancer cell growth and survival is reflected in the negligible growth stimulation achieved by treatment of cultured human prostate cancer cells (androgen-dependent LNCaP and androgen-independent PC-3 and DU145 cells) with EGFR ligands despite the pronounced growth suppression achieved with the selective EGFR-inhibitor Iressa (gefitinib), which arrested the cells in G1 (3). Likewise, Iressa suppressed the development of androgen-dependent human CWR22 prostate cancer tumors in nude mice and was even more effective against tumors formed by CWR22 sublines with attenuated androgen dependence (4). Interestingly, Iressa and an antisense oligonucleotide targeted to MDM-2, a p53 degradation-inducing oncogene, synergistically inhibited anchorage-independent PC-3 and DU145 cell growth in vitro and cooperatively inhibited PC-3 and DU145 tumor development in nude mice (5).
The EGFR effector pathway Raf→MEK1/2→Erk1/2 is implicated in the contribution of EGFR signaling to prostate cancer disease progression. Immunohistochemical analysis assessed activated Erk1/2 as absent in normal human prostate epithelial tissue, abundant in benign prostatic hyperplasia, and significantly increased in neoplastic prostate epithelial tissue (6), consistent with the hypothesis that EGFR-dependent autocrine signaling elevates the baseline level of activated Erk1/2 in prostate cancer cells. The functional significance of this is likely to involve enhancement of benign prostatic hyperplasia and prostate cancer cell proliferation rates by the activated canonical mitogenic pathway (6). Furthermore, both EGFR and Erk1/2 are implicated in androgen receptor transactivation in prostate cancer, which plays a critical role in the development of androgen-refractory disease. Androgen receptor transactivation by forced growth-factor receptor expression in LNCaP cells is abrogated by suppression of the Raf/Erk1/2 pathway (7), and EGF induces androgen receptor transactivation in human CWR-R1 prostate cancer cells through Erk1/2-dependent effects on the androgen receptor coactivators TIF2/GRIP1 (8). Thus, disruption of the EGFR→Erk1/2 autocrine circuit is a rational modality for use in combination therapy approaches to the treatment of prostate cancer.

EGFR transactivation is a prominent mode of EGFR-dependent signaling that involves cross-talk with G protein–coupled receptors, with EGFR as the G protein–coupled receptor effector (9, 10). This entails G protein–coupled receptor activation of a metalloproteinase of the ADAM family; the ADAM metalloproteinase structure is plasma membrane–spanning with an extracellular catalytic domain (11, 12). ADAM-catalyzed proteolysis of membrane-bound EGFR ligand precursors at the cell surface produces soluble, active ligand, resulting in ligand-induced EGFR activation (9, 11). Theoretically, to the extent that EGFR signaling may in turn stimulate the G protein–coupled receptor partner through positive feedback, the autocrine circuit thus formed could elevate the basal level of EGFR-dependent signaling and enhance cell proliferation as a self-perpetuating low-intensity signal. Studies of EGFR transactivation in human prostate cancer have focused primarily on PC-3 cells, and key features of the transactivation mechanism have been elucidated in this cell line. Thus, bombesin triggers EGFR transactivation in PC-3 cells in a protein kinase C (PKC)–independent manner by interaction with its cognate G protein–coupled receptor (13), and TPA transactivates EGFR by a mechanism presumed to be PKC-dependent (9). Both bombesin– and TPA--triggered modes of EGFR transactivation in PC-3 cells are mediated by hithetastat-sensitive metalloproteinase activity (identified as ADAM10 in the case of bombesin; refs. 9, 12) and they have been shown to be abrogated by heparin-binding (HB)-EGF sequestering agents, i.e., mutant diphtheria-toxin CRM197 in the case of bombesin and neutralizing HB-EGF antibodies in the case of TPA (12, 14), pointing to HB-EGF as the metalloproteinase-generated ligand. HB-EGF signals to Erk1/2 activation in PC-3 cells through EGFR, which is the sole HB-EGF cognate receptor expressed in the cells (15). The significance of the HB-EGF-generating EGFR transactivation mechanisms in PC-3 cells to prostate cancer pathobiology is suggested by the ability of ectopic expression of secreted HB-EGF in LNCaP cells to produce a hormone-refractory malignant phenotype in mice (16).

In this report, we establish that PKCα plays an essential role in EGFR transactivation by TPA in PC-3 cells. Our results identify PKCα as a rational target for the design of prostate cancer therapeutics that thwart EGFR-dependent mechanisms of prostate cancer progression.

Materials and Methods

Cell Culture

Human prostate cancer PC-3 and DU145 cells were from the American Type Culture Collection (Rockville, MD). The cells were maintained in RPMI 1640 supplemented with 10% fetal bovine serum and penicillin-streptomycin in a humidified atmosphere of 5% CO2 at 37°C. RPMI 1640 and other cell culture reagents were from Invitrogen (Carlsbad, CA). The cell treatment reagents Go6976, GFI09203X, Go6983, and AG1478 were purchased from Calbiochem (San Diego, CA), and TPA, bryostatin 1, EGF, and CRM197 were from Sigma (St. Louis, MO). The cells were serum-starved for 16 hours prior to treatment with the indicated reagent at 70% to 85% confluence.

Antibodies

PKCα, PKCζ, and PKCμ mAbs were from BD Transduction Labs (Lexington, KY), polyclonal PKCζ antibody was from Santa Cruz (Santa Cruz, CA). PKD, Erk1/2, activated Erk1/2 (P-Thr202, P-Tyr204), and EGFR polyclonal antibodies, and phospho-tyrosine mAb (PY-100) were from Cell Signaling (Beverly, MA). Horseradish peroxidase–linked secondary antibodies and enhanced chemiluminescence reagent were from Amersham Pharmacia Biotech. Western analysis was done by standard methods using an enhanced chemiluminescence detection system.

Cell Treatment and Kinase Analysis

Serum-starved cells were treated for 2 hours at 37°C with the indicated PKC or EGFR inhibitor or CRM197, and then cotreated with the inhibitor and either 100 nmol/L TPA or vehicle control (0.01% DMSO). TPA treatment was terminated at 15 minutes for analysis of Erk1/2 activation and at 3 minutes for EGFR transactivation analysis. In some Erk1/2 activation analyses, cells were treated with 10 ng/mL EGF for 10 minutes in lieu of TPA. At the end of the treatment period, cells were rinsed with PBS, harvested with lysis buffer, and stirred for 1 hour at 4°C. Lysis buffer contains 1.0% Triton X-100 in 20 mmol/L Tris-HCl (pH 7.5), 10 mmol/L EGTA, 10 mmol/L EDTA, 0.1 mg/mL leupeptin, 5.0 μmol/L phenylmethylsulfonyl fluoride, 100 μmol/L microcystin, 1.0 mmol/L Na3VO4, 10 mmol/L NaF, 15 mmol/L β-mercaptoethanol. Lysates were briefly centrifuged to clear the supernatants, and supernatants were processed for Western analysis (75 μg cell lysate protein per lane for Erk1/2 and 40–75 μg cell lysate protein per lane for PKC isozymes) or EGFR immunoprecipitation.
To measure Erk1/2 activation, blots were probed for activated Erk1/2, and then reprobed for total Erk1/2 (14). EGFR activation was measured as reported in ref. (9) by Western analysis of the immunoprecipitated receptor; the blots were probed with P-Y mAb to detect tyrosine phosphorylation of EGFR and reprobed for total EGFR.

To analyze bryostatin 1–induced PKC isozyme down-regulation in PC-3 cells and attendant effects on TPA- and EGF-induced Erk1/2 activation responses, the cells were exposed to 50 nmol/L bryostatin 1 or 0.01% DMSO for 18 hours at 37°C under serum starvation conditions, followed by treatment with TPA, EGF, and kinase inhibitors and processing for Western analysis of cell lysates, as specified in the above-described cell treatment protocol, except that a 10-minute treatment period was used for both TPA and EGF. Lysates were probed for PKC isozymes, activated Erk1/2, and total Erk1/2.

**Results**

Evidence that TPA induces Erk1/2 activation in human prostate cancer PC-3 cells through a HB-EGF ectodomain-shedding mechanism of EGFR transactivation consists of observations that neutralizing EGFR and HB-EGF mAbs each inhibit the response (9, 14). To validate the use of TPA-induced Erk1/2 activation in PC-3 cells as an in vitro model of EGFR transactivation by HB-EGF shedding in human prostate cancer in this study, we tested the effects of the selective EGFR inhibitor AG1478 (17) and the HB-EGF-sequestering agent CRM197 (9) on the response. AG1478 abrogated TPA-induced Erk1/2 activation in PC-3 cells (Fig. 1) with a concentration dependence that closely resembled its potency against EGF-induced Erk1/2 activation in a control analysis done in parallel (data not shown), corroborating the previous observation with 5 μg/mL neutralizing EGFR mAb (14) that TPA induces Erk1/2 activation in PC-3 cells by EGFR transactivation. Furthermore, 10 μg/mL CRM197, which was sufficient to suppress TPA-induced EGFR transactivation by HB-EGF shedding in Cos-7 and HEK293 transfectants in ref. (9), blocked TPA-induced Erk1/2 activation in PC-3 cells (Fig. 1), corroborating the previous finding with 10 μg/mL neutralizing HB-EGF mAb (14) that the mechanism of EGFR transactivation by TPA in PC-3 cells is HB-EGF shedding.

Although TPA is commonly described as a specific PKC activator, the phorbol ester is also a high-affinity ligand of other proteins involved in growth factor signaling, e.g., PKD isozymes (former nomenclature, PKCθι), which are distant from PKC in the Ser/Thr protein kinase superfamily, and the Ras guanyl nucleotide-releasing protein family of guanine-nucleotide exchange factors. Thus, TPA may also influence growth factor signaling through effects on non-PKC targets (18). To determine whether TPA induces EGFR transactivation in PC-3 cells by a PKC-dependent mechanism, we investigated the effects of the PKC inhibitors GF109203X and Gö6983 on the response. GF109203X and Gö6983 selectively inhibit PKC and are 100- to 1,000-fold less effective against PKD (19–21).

Figure 2 shows that 100 nmol/L TPA induced tyrosine phosphorylation of EGFR in PC-3 cells in an AG1478-sensitive manner, indicative of EGFR transactivation. At 1 μmol/L, GF109203X and Gö6983 each abrogated the TPA-induced EGFR transactivation response, demonstrating a PKC-dependent mechanism.

PC-3 cells express four PKC isozymes, the TPA-responsive isozymes PKCα and PKCζ and the TPA-unresponsive isozymes PKCs and PKCγ (22). Prolonged treatment with phorbol esters or other PKC activators of nanomolar affinity, such as the macrocyclic lactone bryostatin 1, is a pharmacologic approach to posttranslationally attenuate

---

**Figure 1.** TPA induces Erk1/2 activation in human prostate cancer PC-3 cells by EGFR transactivation. A, Western analysis shows the concentration-dependent inhibition of TPA-induced Erk1/2 activation in PC-3 cells by the selective EGFR inhibitor AG1478. Cells were preincubated with AG1478 for 2 h and then cotreated with 100 nmol/L TPA and AG1478 for 15 min at 37°C. The upper blot (P-Erk1/2) is the Western analysis of AG1478 (nM) and an independent analysis that is not shown. The densitometric value corresponding to TPA-induced Erk1/2 activation at 0 nmol/L AG1478 is designated as 100%. B, graph is the averaged densitometric analysis of the Western analysis (top) and an independent analysis that is not shown. The densitometric value corresponding to TPA-induced Erk1/2 activation at 0 nmol/L AG1478 is designated as 100%. C, Western analysis shows the inhibition of TPA-induced Erk1/2 activation in PC-3 cells by the HB-EGF-sequestering agent CRM197. Cells were preincubated with 10 μg/mL CRM197 for 2 h and then cotreated with 100 nmol/L TPA and 10 μg/mL CRM197 for 15 min at 37°C. The results shown were reproduced in an independent analysis.
Figure 2. EGFR transactivation is suppressed by selective PKC inhibitors in PC-3 cells. Western blot analyses of immunoprecipitated EGFR show that the selective PKC inhibitors GF109203X (1 μmol/L; A) and Gö6983 (1 μmol/L; B), which are ineffective against PKD, abrogate EGFR transactivation by TPA in PC-3 cells. Top blot (P-Y) is a Western analysis of the phosphotyrosine content of EGFR, and the bottom blot corresponds to total EGFR. The AG1478-sensitivity of TPA-induced EGFR tyrosine phosphorylation (P-Y) verifies that EGFR P-Y reflects EGFR transactivation in each experiment. PC-3 cells were preincubated with the kinase inhibitor specified (AG1478, GF109203X, or Gö6983) at the concentration shown for 2 h at 37°C and then cotreated with 100 nmol/L TPA and the kinase inhibitor for 3 min at 37°C. The results shown were reproduced in separate experiments.

Although only PKCα expression was altered by bryostatin 1 in Fig. 3A, TPA also induced partial down-regulation of PKCα (Fig. 3B). These results indicate that the GF109203X-sensitive protein kinase mediating EGF-induced Erk1/2 activation in PC-3 cells is not subject to pharmacologic knockdown by TPA (Fig. 3B). The GF109203X-sensitive kinase may be a TPA-insensitive PKC isozyme (PKCζ or PKCδ), PKCe, or another PKC isozyme that is weakly expressed in the cells.

As an independent test of a requirement for PKCs in TPA-induced EGFR transactivation in PC-3 cells, we investigated the effects of Gö6976, which is a selective inhibitor of Ca2+-responsive PKC isozymes (21) and thus PKCα-selective in PC-3 cells. Figure 4 (top) shows that 1 μmol/L Gö6976 abrogated TPA-induced Erk1/2 activation in PC-3 cells (lane 3 versus lane 6) and did not affect EGF-induced Erk1/2 activation (lane 2 versus lane 5). Furthermore, 1 μmol/L Gö6976 abrogated TPA-induced tyrosine phosphorylation of EGFR, in experiments done in parallel with the analyses of GF109203X and Gö6983 in Fig. 2 (data not shown). These results corroborate the finding in Fig. 3 that PKCα is required for EGFR transactivation by TPA but is dispensable for EGF/EGF\(\rightarrow\)signaling to Erk1/2 activation in PC-3 cells. Androgen-independent human prostate cancer DU145 cells express the same PKC isozymes as PC-3 cells (22) and respond to EGF with Erk1/2 activation (24). A similar pattern of PKC isozyme expression, including strong PKCα expression, has been established for early stage prostate cancer by immunohistochemical analysis of clinical specimens (25), indicating the relevance of PC-3 and DU145 cells as models of PKC signaling in human prostate cancer. Figure 4 (bottom) shows that 1 μmol/L Gö6976 inhibited TPA but not EGF-induced Erk1/2 activation in DU145 cells, suggestive of a common PKCα-dependent mechanism of phorbol ester--induced Erk1/2 activation in PC-3 and DU145 cells. The baseline Erk1/2 activation response of the serum-starved DU145 cells, which reflects autocrine signaling (24), was not inhibited by 1 μmol/L Gö6976 (lane 1 versus lane 4), indicative of PKCα independence.

The GF109203X-insensitivity of EGF-induced Erk1/2 activation in DU145 cells (Fig. 5) provides evidence that the response is PKC-independent. Thus, PC-3 and DU145 cells may share a PKCα-dependent mechanism of EGFR transactivation (Fig. 4), but their mechanisms of Erk1/2 activation downstream of ligand-induced EGFR activation are distinct (Figs. 3 and 5). In addition, the lack of effect of GF109203X on EGF/EGF\(\rightarrow\)signaling in DU145 cells (Fig. 5) clarifies that the effects of GF109203X on EGF\(\rightarrow\)dependent signaling in this report do not involve direct inhibition of EGFR.

**Discussion**

Progression of human prostate cancer to a malignancy that is refractory to androgen-ablation therapy, i.e., androgen-independent, renders the disease resistant to available treatment options and accounts for the high prostate cancer...
mortality rate (26, 27). EGFR expression levels in the neoplastic epithelium of clinical prostate cancer positively correlate with progression to androgen-refractory, untreatable malignant disease (2, 28). The significance of this association is strongly suggested by experimental models of human prostate cancer that implicate EGFR-dependent signaling to Erk1/2 activation in the androgen-independent phenotype, at least in part as a consequence of androgen receptor transactivation mechanisms (3–5, 7, 8).

In this report, we establish that PKCα plays an obligatory role in EGFR transactivation and signaling to Erk1/2 activation induced by phorbol esters in androgen-independent human prostate cancer PC-3 cells. We also provide evidence for a similar requirement for PKCα in the phorbol ester–induced Erk1/2 activation response of an unrelated androgen-independent human prostate cancer cell line, DU145, which expresses the same PKC isozymes as PC-3 and is also Iressa-sensitive (3, 22). sn-1,2-diacylglycerol, the endogenous PKC-activating homologue of TPA, is present at elevated levels in transformed cells (29, 30), and may serve as the pathophysiologic counterpart of acute TPA treatment by sustaining a moderate level of PKCα stimulation that in turn sustains EGFR transactivation in prostate cancer cells in vivo. Taken together with the frequent expression of PKCα and EGFR in clinical prostate cancer specimens (2, 25), our results support the notion that PKCα-dependent EGFR transactivation may contribute to the development and help maintain the androgen-refractory phenotype of advanced prostate cancer. Our results offer a rationale for application of PKCα-inhibitory therapeutics, which are currently under development (31), in the management of advanced prostate cancer. Interestingly, the conclusions of this report are congruous with our recent observation that suppression of TPA-induced Erk1/2 activation in PC-3 cells by resveratrol, a dietary phytoalexin with anticancer activity, was associated with isozyme-selective PKCα inhibition (22).

Our results also provide evidence that a GF109203X-sensitive PKC isozyme distinct from PKCα plays a role in the EGF/EGFR-induced Erk1/2 activation response in PC-3 cells. Furthermore, prolonged TPA treatment did not affect Erk1/2 activation by EGF/EGFR in PC-3 cells; this implicates a TPA-insensitive PKC species in the GF109203X-sensitive response. In contrast, EGF/EGFR-induced Erk1/2 activation in DU145 cells was unaffected by GF109203X. This indicates that PKC-dependent Erk1/2 activation is not a general feature of EGF/EGFR signaling.
shown were reproduced in independent analyses. Of these, PC-3 and DU145 cells express only PKCα. G66976 (1 μmol/L) blocked TPA but not EGF induction of Erk1/2 activation in PC-3 (A) and DU145 cells (B), indicating a common requirement for PKCα at a point upstream of EGFR in the TPA-induced responses of PC-3 and DU145 cells. Cells were preincubated with 1 μmol/L G66976 for 2 h and then cotreated with 1 μmol/L G66976 and 100 nmol/L TPA for 15 min or 10 ng/mL EGF for 10 min at 37°C. The results shown were reproduced in independent analyses.

Figure 4. TPA-induced Erk1/2 activation is blocked by selective PKCα inhibition in PC-3 and DU145 cells. G66976 selectively inhibits the PKC isoforms PKCα, PKCγ, and PKCδ. Of these, PC-3 and DU145 cells express only PKCα. G66976 (1 μmol/L) blocked TPA but not EGF induction of Erk1/2 activation in PC-3 (A) and DU145 cells (B), indicating a common requirement for PKCα at a point upstream of EGFR in the TPA-induced responses of PC-3 and DU145 cells. Cells were preincubated with 1 μmol/L G66976 for 2 h and then cotreated with 1 μmol/L G66976 and 100 nmol/L TPA for 15 min or 10 ng/mL EGF for 10 min at 37°C. The results shown were reproduced in independent analyses.

A sustained, moderate elevation of baseline Erk1/2 activation in prostate cancer cells by EGFR transactivation, similar to the low-intensity autocrine-signaling to Erk1/2 activation characteristic of serum-starved DU145 cells (24) and evident in Fig. 4, may foster an androgen-refractory malignant phenotype through Erk1/2-dependent mechanisms of androgen receptor transactivation (7, 8) and the canonical, mitogenic Erk1/2 pathway. EGFR signaling is linked to p21cip1/Waf1-mediated growth arrest in cancer cells that express the receptor at very high levels (32, 33). This suggests that prostate cancer cells with high levels of EGFR-dependent autocrine signaling may be selected against in vivo due to autonomous growth arrest, so that the expanding tumor cell population would more likely reflect moderate EGFR transactivation in vivo. This paradigm of mitogenic EGFR signaling in prostate cancer in vivo is consistent with the potent antitumor effects of Iressa against androgen-refractory human prostate cancer tumor xenografts in nude mice (4, 5).

The rationale offered by our findings for PKCα inhibition as a modality of prostate cancer therapy is compatible with the results of other recent studies which suggest stabilization of PKCδ expression in prostate cancer cells as a logical approach to reverse drug resistance in prostate cancer. Although PC-3 and DU145 cells do not apoptosis in response to TPA, the phorbol ester potently induces apoptosis of androgen-sensitive human prostate cancer LNCaP cells and the androgen-hypersensitive subline C4-2 (34, 35). Partial knockdown of PKCδ in C4-2 cells by RNA interference is sufficient to abolish the TPA-induced apoptosis response, and indicates an absolute requirement for PKCδ in TPA-induced C4-2 apoptosis (35). In an interesting twist, PKCδ-dependent prostate cancer cell apoptosis has been linked to expression of neutral endopeptidase (36), which is a cell surface protease that is expressed at diminished levels in androgen-refractory human prostate cancer specimens compared with androgen-sensitive counterparts (37). Similarly, LNCaP cells express neutral endopeptidase, whereas PC-3 and other androgen-independent human prostate cancer cell lines do not (37). Sumimoto et al. (36) recently discovered that a specific neutral endopeptidase inhibitor induced loss of PKCδ expression in LNCaP cells by promoting proteolytic degradation of the isozyme, and that treatment of PC-3 cells with recombinant neutral endopeptidase up-regulated PKCδ expression from a negligible level to a level similar to LNCaP cells. Furthermore, recombinant neutral endopeptidase–treated PC-3 cells underwent PKCδ-dependent apoptosis in response to etoposide, whereas the PC-3 cells were etoposide-resistant in the absence of recombinant neutral endopeptidase. PKCδ expression is weak or absent in human prostate cancer specimens (25). The complementary findings in refs. (35, 36)

in androgen-independent human prostate cancer cells. Studies in other human prostate cancer cell lines will be necessary to gauge the importance of this PKC-dependent signaling mechanism in prostate cancer. Identification of the PKC isoform mediating EGFR-EGFR-induced Erk1/2 activation in PC-3 cells will require RNA interference or dominant-negative strategies of isozyme-selective PKC targeting. Likewise, these molecular strategies will be useful to confirm the importance of PKCα to the TPA-induced EGFR transactivation mechanism in human prostate cancer cells.

A sustained, moderate elevation of baseline Erk1/2 activation in prostate cancer cells by EGFR transactivation, similar to the low-intensity autocrine-signaling to Erk1/2 activation characteristic of serum-starved DU145 cells (24) and evident in Fig. 4, may foster an androgen-refractory malignant phenotype through Erk1/2-dependent mechanisms of androgen receptor transactivation (7, 8) and the canonical, mitogenic Erk1/2 pathway. EGFR signaling is linked to p21cip1/Waf1-mediated growth arrest in cancer cells that express the receptor at very high levels (32, 33). This suggests that prostate cancer cells with high levels of EGFR-dependent autocrine signaling may be selected against in vivo due to autonomous growth arrest, so that the expanding tumor cell population would more likely reflect moderate EGFR transactivation in vivo. This paradigm of mitogenic EGFR signaling in prostate cancer in vivo is consistent with the potent antitumor effects of Iressa against androgen-refractory human prostate cancer tumor xenografts in nude mice (4, 5).

The rationale offered by our findings for PKCα inhibition as a modality of prostate cancer therapy is compatible with the results of other recent studies which suggest stabilization of PKCδ expression in prostate cancer cells as a logical approach to reverse drug resistance in prostate cancer. Although PC-3 and DU145 cells do not apoptosis in response to TPA, the phorbol ester potently induces apoptosis of androgen-sensitive human prostate cancer LNCaP cells and the androgen-hypersensitive subline C4-2 (34, 35). Partial knockdown of PKCδ in C4-2 cells by RNA interference is sufficient to abolish the TPA-induced apoptosis response, and indicates an absolute requirement for PKCδ in TPA-induced C4-2 apoptosis (35). In an interesting twist, PKCδ-dependent prostate cancer cell apoptosis has been linked to expression of neutral endopeptidase (36), which is a cell surface protease that is expressed at diminished levels in androgen-refractory human prostate cancer specimens compared with androgen-sensitive counterparts (37). Similarly, LNCaP cells express neutral endopeptidase, whereas PC-3 and other androgen-independent human prostate cancer cell lines do not (37). Sumimoto et al. (36) recently discovered that a specific neutral endopeptidase inhibitor induced loss of PKCδ expression in LNCaP cells by promoting proteolytic degradation of the isozyme, and that treatment of PC-3 cells with recombinant neutral endopeptidase up-regulated PKCδ expression from a negligible level to a level similar to LNCaP cells. Furthermore, recombinant neutral endopeptidase–treated PC-3 cells underwent PKCδ-dependent apoptosis in response to etoposide, whereas the PC-3 cells were etoposide-resistant in the absence of recombinant neutral endopeptidase. PKCδ expression is weak or absent in human prostate cancer specimens (25). The complementary findings in refs. (35, 36)
suggest potential for development of therapeutic strategies that overcome prostate cancer resistance to chemotherapy agents by up-regulating PKCα expression in prostate cancer cells.

Acknowledgments

We thank Dr. Curtis A. Pettaway (M.D.A.C.C.) for constructive discussions.

References

Molecular Cancer Therapeutics

Protein kinase C-α mediates epidermal growth factor receptor transactivation in human prostate cancer cells

Jubilee R. Stewart and Catherine A. O’Brien


Updated version
Access the most recent version of this article at:
http://mct.aacrjournals.org/content/4/5/726

Cited articles
This article cites 37 articles, 20 of which you can access for free at:
http://mct.aacrjournals.org/content/4/5/726.full#ref-list-1

Citing articles
This article has been cited by 9 HighWire-hosted articles. Access the articles at:
http://mct.aacrjournals.org/content/4/5/726.full#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.