c-fos Promoter Insensitivity to Phorbol Ester and Possible Role of Protein Kinase C in Androgen-independent Cancer Cells

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Introduction

Biological and genetic response to hormone depends on expression and structural integrity of the hormone receptor. In some cases, however, biological and genetic response to hormone may depend on more than the hormone receptor. For example, response to androgen along the proximal distal axis of the prostatic duct is qualitatively variable (1), even though androgen receptor is uniformly expressed (2). Similarly, in cancer, androgen-dependent growth may evolve to androgen-independent growth without change in androgen receptor expression (3). Because biological responses require integration of diverse signals, full understanding of the biological determinants of androgen action may depend on understanding the interaction of androgen signals with other signals. We have observed that the androgen-independent prostate cancer cell line PPC-1, in contrast to the androgen-dependent cell line LNCaP, grows well in low serum and does not induce Fos protein with phorbol esters. This observation is potentially relevant to our understanding of androgen independence in that Fos levels may critically determine genetic response to androgen (4). We have found that phorbol ester insensitivity is a feature common to five androgen-independent epithelial cancer lines, which are associated with biological sensitivity to PKC inhibitor. We have developed a model of androgen independence in which PKC activity may not only explain phorbol ester insensitivity but also represent cellular signaling activity, which is necessary for viability of androgen-independent cancer cells.

Materials and Methods

Cell Culture. All cell lines were cultured in 5% CO2/humidified air at 37°C and routinely incubated in RPMI 1640 containing 10% fetal bovine serum (media culture facility, Sylvester Comprehensive Cancer Center). In some cases, however, biological and genetic response to hormone may depend on more than the hormone receptor. For example, response to androgen along the proximal distal axis of the prostatic duct is qualitatively variable (1), even though androgen receptor is uniformly expressed (2). Similarly, in cancer, androgen-dependent growth may evolve to androgen-independent growth without change in androgen receptor expression (3). Because biological responses require integration of diverse signals, full understanding of the biological determinants of androgen action may depend on understanding the interaction of androgen signals with other signals. We have observed that the androgen-independent prostate cancer cell line PPC-1, in contrast to the androgen-dependent cell line LNCaP, grows well in low serum and does not induce Fos protein with phorbol esters. This observation is potentially relevant to our understanding of androgen independence in that Fos levels may critically determine genetic response to androgen (4). We have found that phorbol ester insensitivity is a feature common to five androgen-independent epithelial cancer lines, which are associated with biological sensitivity to PKC inhibitor. We have developed a model of androgen independence in which PKC activity may not only explain phorbol ester insensitivity but also represent cellular signaling activity, which is necessary for viability of androgen-independent cancer cells.
Phorbol esters regulate c-fos partly through transactivation of the c-fos promoter (12). To examine the possibility of a transactivation defect as a basis for insensitivity of Fos to phorbol esters in PPC-1 and the question whether phorbol ester insensitivity is a common feature of androgen-independent cancer cells, we undertook transient transfection of a luciferase reporter construct containing a "full-length" c-fos promoter. Basal expression was 47- to 35-, 4-, 8-, and 17-fold higher in the androgen-independent TAC (androgen-independent LNCaP subline), PPC-1, DU145, ALVA-31, and MFM-M, respectively, than in the androgen-dependent LNCaP cells (Fig. 1A, inset). Increased basal expression was associated with reduced sensitivity to phorbol ester. In the androgen-dependent LNCaP cells, c-fos promoter was inducible 256-fold by the phorbol ester PMA (Fig. 1A). By contrast, in the androgen-independent cells TAC, PPC-1, DU145, ALVA-31, and MFM-M the induction with phorbol 12-myristate 13-acetate 13-acetate was 1.8-, 1.8-, 2.9-, 1.7-, and 1.6-fold, respectively. Thus, failure of phorbol esters to transactivate the c-fos promoter appears to be a common feature of androgen-independent cancer cells.

The SRE genetic cis element is necessary for activation of c-fos by phorbol esters (12). We tested involvement of the SRE in phorbol ester insensitivity of the c-fos promoter by transient reporter assays. As with the c-fos promoter, basal SRE levels were 12-, 3.7-, 3-, 106-, and 13-fold higher in TAC, PPC-1, DU145, ALVA-31, and MFM-M, respectively, than in LNCaP (Fig. 1B, inset). This increase was also associated with reduced sensitivity to phorbol ester. In the androgen-dependent line LNCaP, SRE was inducible 39-fold, whereas in the androgen-independent cell lines TAC, PPC-1, DU145, ALVA-31, and MFM-M it was inducible 3.8-, 2.8-, 1.7-, 1.1-, and 0.7-fold, respectively (Fig. 1B). Thus, phorbol ester insensitivity of c-fos promoter in androgen-independent cells may be due to a failure of phorbol esters to induce the SRE.

It is commonly believed that phorbol esters act through PKC phosphorylation pathways. Thus, the phorbol ester insensitivity seen in androgen independence could involve PKC. Unfortunately, the existence of many PKC isoforms complicates the interpretation of isoform nongenomic PKC activity measurements, which are the only ones now available. We therefore tested a possible causal relationship between PKC isoform activation and phorbol ester insensitivity through transient transfection. It had been shown previously that direct PKC activation through overexpression or mutation may cause biological phorbol ester or growth factor desensitization and raise basal levels of AP-1-responsive genetic elements (13). However, this finding has not previously been associated with desensitization of AP-1-responsive-genetic elements to phorbol esters (13). We selected a PKC expression plasmid (mPKC) in which the coding sequences were mutated to raise enzymatic activity (14). PKC belongs to the novel class of PKC isoforms and is independent of calcium, minimizing the possibility that negative results could be due to suboptimal calcium concentrations. We also tested a possible causal relationship between activation of SRF, a DNA-binding protein mediating the phosphorylation-target promoter. V-SRF, is a chimeric construct in which the phosphorylation-dependent transcriptional domain was replaced by the constitutively active VP16 viral transactivation domain (15).

Expression of mPKC or V-SRF in androgen-dependent LNCaP cells elevated basal c-fos promoter levels 21- and 18-fold, respectively (Fig. 2a, inset). Surprisingly, this elevation was associated with highly significant noninduction by phorbol esters (P < 0.02), with reduction from 181- to 3.2- and 2.3-fold induction, respectively (Fig. 2a). SRE basal levels were elevated 1.7- and 6.5-fold with mPKC and V-SRF, respectively (Fig. 2b, inset) and were also associated with highly significant noninduction by phorbol esters (P < 0.02), with reduction from 77- to 3.5- and 1.6-fold induction, respectively (Fig. 2b). These results affirm a possible causal relationship between activation of PKC and/or SRF and phorbol ester insensitivity.

Because mPKC or V-SRF was sufficient to cause noninduction by phorbol ester as seen in androgen-independent cells, we tested their effect on a partial rat probasin promoter in response to the synthetic androgen methylone. The probasin gene codes an androgen-dependent, prostate-specific secretory product, the promoter of which contains well-characterized androgen-responsive sequences. As seen with effects on c-fos promoter or SRE, basal expression of probasin promoter was elevated 3.7- and 6.4-fold with mPKC and VP16-SRF, respectively (Fig. 3, inset). mPKC or V-SRF significantly (P < 0.01) inhibited induction of probasin promoter by androgen, from 38- to 4.8- and 5.8-fold, respectively (Fig. 3). Thus, activation of PKC or SRF causes relative androgen noninduction of at least one androgen target promoter.
Which Signal Modulators Can Explain c-fos Insensitivity to Phorbol Esters? While c-fos noninduction has been described in nonproliferating senescent cells, noninduction of c-fos in cancer cells seems paradoxical, given that c-fos expression is often associated with cellular proliferation. At the same time, malignant transformation by the ras oncogene can also be associated with c-fos noninduction (17); ras transformation can also be associated with repression of the SRE-like CARG cis elements of the α-actin gene. Repression of the CARG elements can be reversed through overexpression of the SRF, in association with reversion of the transformed phenotype (18). These observations support a model of malignant transformation in which loss of c-fos sensitivity to phorbol esters can be caused by ras repression of the SRE. Whether ras is involved in c-fos insensitivity to phorbol esters in androgen-independent cancer cells is unknown. Since c-fos and SRE insensitivity to phorbol esters was associated with androgen independence, it may be that in some cancers, such as prostate cancer, loss of phorbol ester sensitivity is a marker not of the transformed phenotype but of specific phenotypic features such as hormone independence.

The ability of a PKC isoform to cause c-fos noninduction was somewhat surprising, in light of previous work that showed no interference with phorbol ester signals. PKC activation has been linked, however, to the activity of other powerful signal modulators. Thus, concurrent and/or separate activation of many signal modulators such as PKC or ras could theoretically cause c-fos insensitivity to phorbol esters as seen in androgen-independent cells.

Can c-fos Insensitivity to Phorbol Esters Be Explained by a Single Transcriptional Regulatory Mechanism? We showed that c-fos insensitivity to phorbol ester was associated in every cell line with insensitivity of the SRE to phorbol esters. Also, mPKC and V-SRF were both able to desensitize the c-fos promoter and the SRE...
Fig. 4. Androgen-independent cells selectively killed by the PKC inhibitor chelerythrine. Cells were plated at subconfluence in 1% dextran-charcoal-stripped serum with or without 5 μM chelerythrine in dimethyl sulfoxide. Control dishes received dimethyl sulfoxide only. On day 5, dishes were visually inspected for morphological changes in cells growing in chelerythrine. Representative fields are shown. × 120. In no case were viable androgen-independent cells seen in dishes containing chelerythrine. ZR-75-1 reacted the same as LNCaP, whereas DU145, ALVA-31, and MFM-M reacted the same as TAC and PPC-1 (not shown).

to phorbol esters. However, while mPKC and V-SRF had virtually identical effects on the c-fos promoter, their effects on the SRE were different: mPKC had a negligible effect on SRE basal activity, while V-SRF elevated SRE basal activity. Either construct caused phorbol ester insensitivity of the SRE. This phenomenon might be explained by coregulatory squelching (19). In squelching, expression or activation of transcription factors acting on separate response elements. Expression of SRF, which activates the SRE genetic cis element, may interfere with transcription factors acting on separate response elements. Expression of SRF, which activates the SRE genetic cis element, may interfere with activity of CREB transcription factor, which activates the CRE genetic cis element and vice versa. Thus, activation of different transcription factors can lead to insensitivity of the c-fos promoter through activation of different cis elements, even though in every case the SRE may be insensitive to phorbol ester.

Is PKC, Which Can Cause Phorbol Ester Insensitivity of c-fos, Biologically Relevant in All Cells in Which c-fos Phorbol Ester Insensitivity Is Seen? Since we had shown that PKC activation could result in c-fos insensitivity to phorbol esters, we selected a PKC inhibitor to test the biological role of PKC activity in cells in which phorbol ester insensitivity was seen, i.e., in androgen-independent cells. It is not yet technically feasible to measure isoform-specific PKC activity; thus it is not yet feasible to implicate specific isoforms in phorbol ester insensitivity. The difference in response between cells in which c-fos was sensitive or insensitive to phorbol esters was dramatic, with resultant rounding and sloughing of the androgen-independent cells only. The granululation and general appearance of the androgen-independent cells suggested that they are dying in response to chelerythrine, consistent with previous observations that chelerythrine can induce apoptotic cell death (20). This finding suggests that regardless of which signal modulator or which gene-regulatory mechanism underlies phorbol ester insensitivity in androgen-independent cancer cells, PKC may be biologically relevant and, in fact, may be required for cell viability.

How Might Sensitivity of Androgen-independent Cells to PKC Inhibitor Fit with Current Concepts of Prostate Epithelial Growth Regulation by Androgen? Androgens may induce prostate epithelial growth through induced expression of paracrine growth factors from adjacent nonepithelial cells (21). It is widely accepted that growth factors activate PKC. Under conditions of low paracrine growth factors (low serum in monolayer culture as in the present study or in androgen deprivation in prostate cancer), androgen-dependent cells would have low growth-factor-dependent PKC activity and would stop growing. These cells would not be susceptible to the biological actions of PKC inhibition. By contrast, androgen-independent cells (those that grow despite reduction of androgen-dependent paracrine growth factors) would have growth factor-independent PKC activity and would not stop growing. These cells would be susceptible to the biological actions of PKC inhibition. Our observations of the effects of chelerythrine on cells grown in low serum (low growth factor concentration) fit well with a model in which the intracellular growth-promoting signals, e.g., PKC, of androgen-independent cells are rendered independent of androgen-induced paracrine growth factors.

Since growth factors or phorbol esters may antagonize androgen action (22), evolution of androgen-dependent cancers to androgen independence may not be a passive consequence of a general transfer to a more malignant state. Instead, evolution to androgen independence may be an active process in which abnormal PKC-dependent signaling confers specific disruption of the genetic response to androgens, in association with reduced cellular dependence on paracrine growth factors. We are currently investigating which PKC isoforms may be necessary for androgen-independent prostate cancer cell growth.

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References

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