Decreased PKC-α Expression Increases Cellular Proliferation, Decreases Differentiation, and Enhances the Transformed Phenotype of CaCo-2 Cells

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ABSTRACT

Previous studies have shown that PKC-α protein expression is decreased in sporadic human colon cancers, as well as in colonic tumors of rats induced by chemical carcinogens. To elucidate the potential role of PKC-α on several phenotypic characteristics of colon cancer cells, we have transfected cDNAs for PKC-α in sense or antisense orientations into CaCo-2 cells, a human colon adenocarcinoma cell line. Transfected clones were isolated that demonstrated ~3-fold increases (sense transfec- tants) and ~95% decreases (antisense transfec- tants) in PKC-α expression with no significant alterations in other PKC isoforms. Transfection of CaCo-2 cells with PKC-α in the antisense orientation resulted in enhanced proliferation and decreased differentiation, as well as in a more aggressive transformed phenotype compared with empty vector-transfected control cells. In contrast, cells transfected with PKC-α cDNA in the sense orientation demonstrated decreased proliferation, enhanced differentiation, and an attenuated tumor phenotype compared with these control cells. These data show that alterations in the expression of PKC-α induce changes in the proliferation, differentiation, and tumorigenicity of CaCo-2 cells. Furthermore, these findings indicate that loss of PKC-α expression in sporadic human and chemically induced colonic cancers may confer a relative growth advantage during colon cancer malignant transformation.

INTRODUCTION

PKC consists of a gene family of serine/threonine kinases that initiate phosphorylation-dependent alterations in a number of proteins and thereby regulate important cellular events including proliferation and differentiation (1). Members of this family include at least 11 isoenzymes that are phospholipid dependent and are classified according to their cofactor requirements. Classical isoforms are regulated by DAG and calcium and include isoforms α, β, βII, and γ. Novel isoforms δ, ε, η, and θ are activated by DAG but are calcium independent. Atypical isoforms are both calcium and DAG independent and include ι, λ, and ζ (2). In addition to differences in their activating cofactor requirements, these isoforms also differ in tissue expression, subcellular localization, and substrate specificity (3, 4), which together appear to account for the wide variety of processes mediated by PKC in a cell-specific, as well as an isoform-specific, manner (5–9).

Alterations in the biochemical activity and expression of a number of isoforms of PKC have been implicated in the malignant transformation process of several organs, including the colon, in both humans and experimental animals. In this regard, a number of studies (10–13), but not all (14–16), have indicated that total PKC activity and/or expression is decreased in human colon carcinomas and adenomas compared with adjacent mucosa or with colonic mucosa from normal controls. More recent studies have also shown that human colonic tumors may have a decrease in the protein expression of several specific PKC isoforms, including PKC-α (11, 17). In agreement with these studies, the expression of PKC-α has been shown to be reduced in colon tumors of animals treated with the known colon carcinogen, 1,2-dimethylyhydrazine (18). Recently, using another closely related experimental model of colon carcinogenesis, the azoxymethane model, our laboratory has demonstrated that PKC-α expression was also decreased in tumors induced by this colon carcinogen (19).

In an attempt to define the functional consequences of alterations of expression of individual isoforms of PKC, investigators have used various techniques, including transfection of sense or antisense constructs of the PKC cDNAs of interest (20–27). Data from these experiments have at times, however, been conflicting, as specific isoforms have been shown to have opposing effects in different cell types. Overexpression of PKC-α in rat fibroblasts, for example, limited their cell growth (26), whereas overexpression of this same isoform in MCF-7 breast cancer cells enhanced their growth phenotype (27). Many of the aforementioned studies in transfected cell lines, moreover, demonstrated alterations in nontargeted PKC isoforms; or investigators have not reported the effects of the transfections on nontargeted isoforms (20–23, 27). In an attempt to characterize phenotypic changes induced by altered expression of the various isoforms of PKC, investigators have also administered phorbol esters at varying doses and time periods to activate PKC (20–22). It bears emphasis, however, that these agents may not only activate, but also subsequently down-regulate, both the endogenous, as well as transfected, phorbol ester-responsive PKC isoforms. These effects of phorbol esters on PKC activity and expression complicate the interpretation of phenotypic changes noted in these studies.

Based on the observations that decreases in the expression of PKC-α exist in both human and experimental colonic tumors, we directly examined the effects of altering PKC-α expression on several phenotypic characteristics of CaCo-2 cells, using stable PKC-α sense or antisense cDNA transfectants. The CaCo-2 cell line is derived from a human colon adenocarcinoma (28) and is commonly used by investigators to study a number of important intestinal epithelial cellular events, including cell growth and differentiation (5, 7, 29–32). CaCo-2 cells are particularly suited for these in vitro studies, because they do not possess mutations of members of the c-ras family (32), upstream regulators of PKC (33). Because the transfected cells in the present studies possessed no significant alterations in nontargeted isoforms of PKC, the functional consequences of amplified or inhibited expression of PKC-α in CaCo-2 cells on cellular proliferation, differentiation, and transformation could be assessed in an unambiguous manner. The results of these studies and a discussion of their significance form the basis for this communication.
MATERIALS AND METHODS

Cell Culture. CaCo-2 cells were cultured at 37°C in an atmosphere of 5% CO2/95% air as described previously by our laboratory (29). Cells from passages 21–40 were maintained in DMEM with 4.5 g/L glucose, 2 mm L-glutamine, 50 units/ml penicillin, 50 μg/ml streptomycin, 2 μg/ml gentamicin, 10 mM HEPES, 1% essential and nonessential amino acids, and 20% FBS, unless otherwise indicated.

Plasmid Construction, Transfection, and Expression of Human Protein Kinase C-α in CaCo-2 Cells. The full-length cDNA encoding human PKC-α in pBluescript was obtained from Dr. Hubert Hug (34). PKC-α was subcloned into the eukaryotic expression vector pRc/CMV (Invitrogen, San Diego, CA). For this orientation, PKC-α was excised by digestion with NotI and Apal and directionally subcloned into the NotI and Apal sites of pRc/CMV. For the antisense orientation, PKC-α was excised by digestion with XhoI and HindIII and directionally subcloned into the XhoI and HindIII sites of pRC/CMV.

Plasmid DNA was purified using Qiagen columns (Qiagen, Inc., Chatsworth, CA) according to the manufacturer's recommendations. CaCo-2 cells (~1.2 × 10⁶) were transfected with 40 μg of the PKC-α constructs or the pRc/CMV vector alone by calcium phosphate coprecipitation for 4 h, followed by treatment with 15% glycerol for 2 min (35). Fresh media was replaced, and 48 h later, the cells were trypsinized and replated in media containing 800 μg/ml G418. Resistant clones were selected using cloning cylinders and were expanded and maintained in media containing 400 μg/ml G418 until 1 week prior to experiments. From the G418-resistant clones of empty vector, sense, and antisense transfections, four to five clones of each transfection type were found to demonstrate minimal alterations in the nontargeted isoforms, as assessed by Western blotting (see below). These clones were expanded for further study.

Northern Blot Analysis of PKC-α mRNA. Total RNA was prepared from parental and transfected CaCo-2 cells at day 8 after plating using a modified guanidinium thiocyanate extraction (36). RNA (20 μg) was electrophoresed through a 1% agarose-2% formaldehyde gel, transferred by capillary action to Maximum Strength Nytran+ nylon membranes overnight (Schleicher and Schuell, Keene, NH), and UV cross-linked. Membranes were hybridized and washed at high stringency as described previously (37), using a 1.3-kb fragment of the PKC-α cDNA and rehybridized with an 18S RNA probe to verify equivalent loading (38). Autoradiograms were obtained with DuPont ReFlection film (DuPont, Wilmington, DE) exposed for the indicated times at −80°C.

Western Blotting. Parental and transfected CaCo-2 cells were lysed by boiling in PBS and 1% SDS containing 100 μg/ml phenylmethanesulfonyl fluoride and 1 mM sodium orthovanadate at the indicated days after plating. Proteins were assayed with bicinchoninic acid (39). Proteins (20 μg) were separated by SDS-PAGE (10% resolving gel) and electrophobded to Immobilon-P membranes (Millipore, Bedford, MA; Ref. 40). Homogenates (10 μg of protein) from rat or human brain were included as positive controls, where appropriate. Blots were stained with 0.1% India ink in 50 mM Tris-HCl (pH 7.4), 150 mM NaCl with 0.05% Tween 20 (TBST). Nonspecific binding of antibodies was blocked by incubating the blots with 5% nonfat dry milk in TBST for 2 h. After blocking, the blots were incubated overnight at 4°C with total protein loaded (5).

Cell Number. Transfected CaCo-2 cells (10⁵ cells plated/well) were grown in six-well multiwell plates (Corning Glass Works, Corning NY) in DMEM media supplemented with 0.25 μg/ml amphotericin B (Sigma Chemical Co., St. Louis, MO). Cells were removed at the indicated days after plating using 0.2% trypsin-EDTA in HBSS. Trypsinization was stopped by the addition of an equal volume of media containing serum, and cells were counted using a Coulter Counter (Coulter Electronics, Miami, FL). Data are reported as the number of cells/well.

Tritiated Thymidine Incorporation. DNA synthesis was determined by incubating cells (10⁶ cells plated/well) in six-well multiwell plates with [3H]thymidine (2 μCi/well) for 4 h prior to harvesting at the indicated days after plating. Cells were washed twice with PBS, fixed with methanol/acetic acid (3:1; v/v) for 20 min, and treated with ice-cold 10% trichloroacetic acid for 10 min. DNA was then solubilized by an overnight incubation in 0.1 N NaOH at room temperature. Contents of the wells were suspended in scintillation fluid and counted in a Packard 1500 Tri-Carb Liquid Scintillation Analyzer (Packard Instrument Co., Downers Grove, IL).

Assessment of Dome Formation. Cells (1 × 10⁵) were plated in 25-cm² flasks, and at the indicated days after plating, domes were observed in low power fields as described by Pinto et al. (28).

Transmission Electron Microscopy. Empty vector-, sense-, and antisense-transfected CaCo-2 cells (5 × 10⁴) were grown to postconfluent monolayers (12 days after plating) on Nunc Slide Flasks (Nunc, Inc., Naperville, IL). Monolayers were washed twice with PBS and then fixed for 2 h at room temperature with 2% paraformaldehyde, 2.5% glutaraldehyde, and 0.05 M sodium cacodylate buffer (pH 7.2; Polysciences Corp., Warrington, PA). Monolayers were washed twice with sym-collidine buffer and postfixed in 1% osmium tetroxide for 1 h at room temperature. Samples were then washed again twice with sym-collidine buffer. After dehydration in graded alcohols, the cell monolayers were embedded in Epon, which was polymerized overnight at 60°C. Sections were cut perpendicular to the monolayer using a diamond knife in an ultramicrotome (41). The sections were contrasted using uranyl acetate and lead citrate and examined with a Philips CM-120 electron microscope.

Alkaline Phosphatase Assay. Transfected cells grown in six-well multiwell plates were washed with PBS, removed by scraping, and collected at 4°C by centrifugation at 16,000 × g for 5 min. Pellets were snap-frozen and stored at −80°C for collective analysis. Cells were homogenized with 10 strokes of a microcentrifuge sample pestle (Bel-Art Products, Poughquagon, NY) and then sonicated two times for 15 s each at a power setting of 2 using a Model 250 Branson Sonifier (Branson Instruments, Danbury, CT) in 2.0 mm Tris-50 mM mannitol (pH 7.4) buffer. The total cell lysates were then assayed for alkaline phosphatase activity using p-nitrophenyl phosphate as a substrate as described previously (42). Proteins were determined using the method of Smith et al. (39).

Assay of Growth in Soft Agar. Tissue culture dishes (35 mm; Corning) were precoated with 5 ml of media containing 20% FBS and 0.7% Bactoagar (Difco, Detroit, MI). The agar layer was allowed to harden, and 8 ml of media containing 20% FBS, 0.35% Bactoagar, and 10² cells were overlaid on the agar-coated dishes. All dishes were incubated at 37°C in 5% CO₂/95% air with fresh media added every 3 days for 4 weeks. Colonies were stained with 1% Giemsa stain, and colonies larger than 0.2 mm were quantified as described (30).

Tumorigenicity in Nude Mice. Single-cell suspensions of each of the transfected cell lines were prepared by removing cells from flasks using 0.1% trypsin-EDTA (Sigma) in HBSS. Cells were washed three times in sterile tissue culture PBS (pH 7.2; Life Technologies, Inc., Bethesda, MD), and single-cell suspensions were confirmed using light microscopy. Cell viability was >90%, as determined by trypan blue exclusion. Transfected cells (3.5 × 10⁶) were inoculated s.c. into the flank area of 5-week-old athymic NCr-nu male mice (National Cancer Institute, Frederick, MD). After their appearance, tumors were measured with calipers two times per week to monitor their growth. Tumor volume was estimated using the following formula (30):

$$\text{Volume} = \frac{\text{length} \times \text{(width)}^2}{2}$$

Fourteen weeks after inoculation, animals were sacrificed, and tumors were excised, weighed, and fixed in buffered formalin. Tumor histology was confirmed using criteria established previously (30).

Statistical Analysis of Data. Student's unpaired t test, Fisher’s exact test for analysis of small numbers of samples, and one-way ANOVA were performed.
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formed using MINITAB statistical analysis software (Minitab, Inc., State College, PA) as appropriate (43). Values of P < 0.05 were considered significant.

RESULTS

Stable CaCo-2 Cell Transfectants. Four sense-transfected clones and five antisense-transfected clones were identified that demonstrated no significant changes in expression of the nontargeted isoforms (see below) and were studied in more detail. An empty vector clone (EVB3), which most closely matched the untransfected parental CaCo-2 cells for PKC isoform expression, together with the parental cell line served as the controls for these experiments.

mRNA Expression. To assess transcript abundance of PKC-α, total RNA was isolated from parental CaCo-2 cells, as well as empty vector-, sense-, and antisense-transfected cells, and examined by Northern blot analysis with a cDNA probe specific for PKC-α. As shown in Fig. 1, transfection of the empty vector resulted in no significant change in the transcript abundance of PKC-α compared to that seen in parental cells. Transfection of PKC-α in the sense orientation produced a ~2.5-fold increase in mRNA expression, whereas transfection in the antisense orientation caused a ~90% decrease in expression of PKC-α mRNA, compared with that present in parental CaCo-2 cells (Fig. 1). Decreased mRNA expression of PKC-α in the antisense transfectants was consistent with degradation of RNA products as described previously (44).

Protein Expression. To select transfected clones that possessed alterations only in the protein expression of PKC-α, cell lysates (20 µg) from transfected clones were separated by SDS-PAGE, electroblotted, and probed with isoform-specific antibodies for expression of PKC-α, PKC-βI, PKC-βIII, PKC-δ, and PKC-ζ. As shown in Fig. 2, the expression of PKC-α in empty vector-transfected cells was similar to that seen in the parental CaCo-2 cells. Sense-transfected cells demonstrated a ~3-fold increase in PKC-α expression, whereas antisense-transfected cells showed markedly reduced PKC-α protein abundance (~5% of empty vector-transfected cells; Fig. 2). In contrast to PKC-α, the targeted isoform, expression levels of PKC-βI, PKC-βIII, PKC-δ, and PKC-ζ exhibited minimal changes in these transfected cells compared with their expression in parental or empty vector control cell lines (Fig. 2).

Proliferation Assays of Transfected CaCo-2 Cells. To ascertain the effect of over- and underexpression of PKC-α on cellular prolif-
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Fig. 3. Effect of PKC-α expression on cellular proliferation. Cellular proliferation was assessed by counting cells collected as described in "Materials and Methods." The total cell numbers per well are reported as means of three wells for an empty vector clone (EVB3); a sense clone (PKCaB7); and an antisense clone (cPKCD7). *, P < 0.05 compared with empty vector. Bars, SE; error bars that are not visible are contained within the points. In the inset, DNA synthesis was determined by [3H]thymidine incorporation in cells plated in six-well multwell plates as described in "Materials and Methods." Data are reported as mean cpm X 10^5 number of cells of three experiments for empty vector (EVB3); sense (PKCaB7); and antisense (cPKCD7). *, P < 0.05 compared with empty vector.

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As an additional index of transformation, transfected cells were analyzed for their ability to form colonies of 0.2 mm or greater in soft agar. Transfection of the empty vector did not significantly affect the average number of colonies formed in soft agar compared with their parental CaCo-2 counterparts (data not shown). Sense transfectants demonstrated a significant decrease in the number of colonies formed in soft agar (Fig. 7). Specifically, sense clones PKCaB7, PKCa-C3, and PKCa-H6 had decreased soft-agar colony formation with only 35, 62, and 60% of that of empty vector control cells, respectively. In contrast, antisense-transfected cells exhibited a significant increase in anchorage-independent growth in soft agar (Fig. 7). Specifically, antisense clones cPKC-C5, cPKC-D2, cPKC-D7, and cPKC-I4 had 290, 195, 237, and 154% more soft-agar colonies than empty vector controls, respectively.

Assays of Transformation. To assess the possible influence of alterations in the expression of PKC-α on the transformation phenotype, transfected were analyzed for their ability to form colonies of 0.2 mm or greater in soft agar. Transfection of the empty vector did not significantly affect the average number of colonies formed in soft agar compared with their parental CaCo-2 counterparts (data not shown). Sense transfectants demonstrated a significant decrease in the number of colonies formed in soft agar (Fig. 7). Specifically, sense clones PKCaB7, PKCa-C3, and PKCa-H6 had decreased soft-agar colony formation with only 35, 62, and 60% of that of empty vector control cells, respectively. In contrast, antisense-transfected cells exhibited a significant increase in anchorage-independent growth in soft agar (Fig. 7). Specifically, antisense clones cPKC-C5, cPKC-D2, cPKC-D7, and cPKC-I4 had 290, 195, 237, and 154% more soft-agar colonies than empty vector controls, respectively.

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DISCUSSION

In the present studies, alterations in the expression of PKC-α, via transfection of full-length cDNAs in both the sense and antisense orientation, were found to influence several phenotypic characteristics of CaCo-2 cells. Specifically, sense-transfected cells in which the protein expression of PKC-α was increased ~3-fold demonstrated decreased proliferation, as assessed both by cell numbers and [3H]thymidine incorporation. In contrast, antisense-transfected cells, in which PKC-α expression was decreased by ~95%, exhibited enhanced cell growth. These findings are consistent with studies performed in HL-60 cells (45), R6 rat fibroblast cells (46), K562 human erythroleukemia cells (47), and F9 teratocarcinoma cells (48) in which...
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overexpression of PKC-α caused an inhibition of cellular proliferation compared with vector-transfected control cells. These results are similar to those observed for PKCβI, in which expression of this calcium-dependent isoform in HT29 cells, another intestinal cell line, was also associated with inhibited proliferation (21). In studies in human colonic mucosa and tumors in which immunolocalization of PKC isoforms were studied compared with that of the proliferation antigen marker, Ki67 (17), the abundance of PKC-α has been shown to be decreased in areas of active proliferation. These findings lend further support to the contention that an inverse relationship may exist between the expression of PKC-α and cell growth, at least in certain cell types, including CaCo-2 cells. Recent evidence in IEC-18 cells, a small intestinal epithelial cell line, also strongly supports PKC-α involvement in inhibiting cellular proliferation in these cells (49). Furthermore, increased PKC-α expression in several other cell types (24, 27) has been directly correlated with proliferation, indicating that the effects of this PKC isoform on the growth phenotype may be cell specific.

In the present studies, PKC-α expression also appeared to be involved in the regulation of the differentiation phenotype of CaCo-2 cells. In this regard, compared with empty vector-transfected cells, antisense-transfected cells demonstrated a decrease in alkaline phosphatase-specific activity, an absence of dome formation, and a significantly lower linear density of microvilli formed on their cell surface. In contrast, sense-transfected cells demonstrated an enhanced differentiation phenotype, as assessed by an increase in alkaline phosphatase-specific activity, the presence of dome formation, and greater density of microvilli, compared with these same control cells. The acquisition of a more differentiated phenotype in PKC-α-overexpressing CaCo-2 cells is in agreement with observations in HL-60, R6, K562, and F9 cells in which these cells with amplified PKC-α expression demonstrated an enhanced differentiation phenotype (45–48). In contrast to these findings, however, in ras-transfected Jurkat cells, a decrease in calcium-dependent PKC activity was correlated with differentiation and apoptosis, perhaps reflecting cell-specific and oncogene-related effects (50).

PKC activation, as assessed by increased membrane association, has been detected in differentiated villus cells of small intestinal epithelia relative to their proliferative crypt cell counterparts (51). Furthermore, in normal human colonic mucosa, PKC-α expression followed an increasing gradient of immunoreactivity from the crypt to the colonic luminal surface, with PKC-α antisera staining predominantly terminally differentiated cells (17). The activation of PKC-α by phorbol esters in an immature, actively proliferating intestinal crypt cell line, IEC-18, moreover, has been shown to result in cellular differentiation, as well as in growth inhibition (51).

Taken together, these findings indicate that alterations in PKC-α are involved in the regulation of cellular differentiation, as well as in proliferation, of intestinal epithelial cells, including CaCo-2 cells. The mechanisms mediating these important PKC-α-induced cellular events are undoubtedly complex. In this regard, in preliminary studies in these transfected cells (52), we have found that PKC-α stimulated
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the expression of p21Waf1, a cell cycle regulatory protein involved in both growth arrest (53) and cellular differentiation (31, 54). Further studies to elucidate the underlying mechanisms of the actions of PKC-α, including involvement of p21Waf1, are presently under way in our laboratory.

CaCo-2 cells are a transformed cell line, and transfection of the empty vector plasmid did not result in alterations of the transformed phenotype. Sense-transfected cells exhibited a partial reversal of the transformed phenotype with cells displaying a significant inhibition of growth in soft agar and forming significantly fewer tumors, which were also of lower mass, in nude mice. In contrast, antisense-transfected cells, the more actively proliferating cells, displayed a more aggressive transformed phenotype with a significant increase in the number of colonies in soft agar, as well as a tendency for increased

Table 1  Morphometric analysis of microvilli on the surface of transfected CaCo-2 cells
Results are derived from measurements of 50–75 microvilli from at least 20 different fields/group.

<table>
<thead>
<tr>
<th>Transfectant</th>
<th>Mean microvillar length</th>
<th>Mean linear microvillar density</th>
</tr>
</thead>
<tbody>
<tr>
<td>Empty vector (EVB3)</td>
<td>1.40 ± 0.06 μm</td>
<td>1.95 ± 0.17 μm</td>
</tr>
<tr>
<td>Sense (PKCaB7)</td>
<td>1.30 ± 0.06 μm</td>
<td>2.60 ± 0.23 μm†</td>
</tr>
<tr>
<td>Antisense (αCKPD7)</td>
<td>1.40 ± 0.06 μm</td>
<td>1.15 ± 0.18 μm†</td>
</tr>
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† P ≤ 0.005, compared with EVB3 using Student’s t test.
† P ≤ 0.05, compared with EVB3 using Student’s t test.

Fig. 6. Effect of over- and underexpression of PKC-α on alkaline phosphatase activity. Transfected CaCo-2 cells were plated at 1 x 10⁵ cells/ml, and at the indicated times, cells were harvested and assayed for alkaline phosphatase-specific activity as described in “Materials and Methods.” Values represent means of three separate samples; bars, SE. Empty vector (○), sense (△, PKCaB7), antisense (□, αCKPD7) transfected cells (P < 0.005) at all days for sense transfectants and at days 6–12 for antisense transfectants compared with empty vector, as assessed by Student’s t test, are shown.

Fig. 7. Assays of growth in soft agar. Empty vector (EV, EVB3), sense (PKCaB7), and antisense (αCKPD7)-transfected CaCo-2 cells were plated in 0.35% agar and were analyzed for their ability to form colonies ≥0.2 mm in soft agar as described in “Materials and Methods.” Data are means of five separate assays; bars, SE. * P = 0.001, compared with EVB3 as assessed by Student’s t test.

Fig. 5. Transmission electron micrographs of monolayers of transfected CaCo-2 cells. Transfected cells were plated in slide flasks as described in “Materials and Methods,” and at 12 days after plating, cells were fixed and stained as described in “Materials and Methods.” Representative cross sections of the apical surface of monolayers of empty vector (EVB3: A), sense (PKCaB7; B), and antisense (αCKPD7; C) are shown at x×16,000.

formation of tumors in nude mice with tumors of increased mass, although these latter parameters of tumorigenicity were not statistically different from empty vector control cells. This lack of significantly enhanced tumor formation in nude mice injected with antisense transfected cells, moreover, supports the concept of colonie tumor significantly enhanced tumor formation in nude mice injected with antisense transfection was associated with a proliferative, more differentiated phenotype, whereas reduction in the expression of PKC-α by antisense transfection was associated with a less differentiated phenotype in these malignant phenotype of CaCo-2 cells, as well as other cell types, it is clearly not the only determinant of this complex process.

In summary, these studies demonstrate a role for alterations in the expression of PKC-α in the regulation of cell growth, differentiation, and tumorigenicity of CaCo-2 cells. Higher levels of PKC-α expression in sense-transfected CaCo-2 cells were associated with a less proliferative, more differentiated phenotype, whereas reduction in the expression of PKC-α by antisense transfection was associated with a more proliferative, less differentiated phenotype when compared with empty vector-transfected controls. These differences are even more striking when the sense-transfected cell phenotypes are compared with antisense phenotypes. These findings, together with the previous results of studies that demonstrated a decrease in the expression of PKC-α in sporadic human tumors (11–13, 60) and experimentally induced colonic tumors (18, 19), indicate that a reduction in the expression of the α isoform of PKC confers a relative growth advantage, as well as a less differentiated phenotype in these malignant colonic cells.

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