Phorbol 12-Myristate 13-Acetate Induces Protein Kinase Cγ-specific Proliferative Response in Astrocytic Tumor Cells*

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Protein kinase C (PKC) activation has been implicated in cellular proliferation in neoplastic astrocytes. The roles for specific PKC isozymes in regulating this glial response, however, are not well understood. The aim of this study was to characterize the expression of PKC isozymes and the role of PKC-γ expression in regulating cellular proliferation in two well characterized astrocytic tumor cell lines (U-1242 MG and U-251 MG) with different properties of growth in cell culture. Both cell lines expressed an array of conventional (α, βI, βII, and γ) and novel (θ and ε) PKC isozymes that can be activated by phorbol myristate acetate (PMA). A novel novel PKC isozyme, PKC-η, was only expressed by U-251 MG cells. In contrast, PKC-δ was readily detected in U-1242 MG cells but was present only at low levels in U-251 MG cells. PMA (100 nM) treatment for 24 h increased cell proliferation by over 2-fold in the U-251 MG cell line; whereas, it decreased the mitogenic response in the U-1242 MG cells by over 90%. This suggested that two glioblastoma lines, with functionally distinct PKC isozymes and atypical (λ, ζ) PKC isozymes are Ca2+-independent (11). The atypical PKC isozymes are not activated by diacylglycerol, a product of receptor-mediated phospholipid hydrolysis (12). The activation of PKC in various cell types may lead to the stimulation of tyrosine phosphorylation pathways, the activation of mitogen-activated protein (MAP) kinase pathway and c-fos induction, all of which may increase cellular proliferation (13-16). However, the exact molecular mechanisms and signaling pathways involving PKC isozymes in neural cell types are not well understood.

Both human and experimental rodent gliomas express multiple classes of PKC isozymes, including PKC-α, β, γ, δ, ε, θ, η, and ζ (17–21). In cultured human and rat glioma cells, the differential expression of specific isozymes accompanies alterations in both proliferative activity and phenotype differentiation that are related to specific cell lineages. Despite the putative role(s) of PKC signaling in the invasive growth of gliomas, there are conflicting data as to how specific PKC isozyme(s) may affect this malignant growth (22). Although most studies have focused on the role of conventional PKC isozymes, particularly PKC-α, with reports of either increasing proliferation or blocking apoptosis in malignant astrocytic tumor cells, the differential expression of nonconventional PKC isozymes may account for the apparent contradictory biologic effects of phorbol ester treatment or the experimental overexpression of different PKC isozymes (23).

The relationship between the expression of a specific novel PKC isozyme and increased proliferative capacity in glioblastomas was examined in two well characterized glioblastoma cell lines with different growth phenotypes and opposite patterns of [3H]thymidine incorporation in response to PMA treatment. Stable expression of PKC-η in a glioblastoma cell line not expressing this isozyme converted the transfectants into a PMA growth-stimulated phenotype. These data suggest that the different profiles of PKC isozymes may determine the type of cell proliferation and differentiation, gene expression, hormone secretion, and membrane function (6–10). The activities of both the conventional (α, βI, βII, and γ) and novel (θ and ε) PKC isozymes are regulated by phorbol esters, diacylglycerols, and phospholipids. Conventional PKC isozymes require Ca2+ for activity, whereas novel PKC and atypical (λ, ζ) PKC isozymes are Ca2+-independent (11). The atypical PKC isozymes are not activated by diacylglycerol, a product of receptor-mediated phospholipid hydrolysis (12). The activation of PKC in various cell types may lead to the stimulation of tyrosine phosphorylation pathways, the activation of mitogen-activated protein (MAP) kinase pathway and c-fos induction, all of which may increase cellular proliferation (13–16). However, the exact molecular mechanisms and signaling pathways involving PKC isozymes in neural cell types are not well understood.

Malignant astrocytomas, common brain tumors, have a high rate of cellular proliferation and a propensity to infiltrate regional and, ultimately, brain structures. This aggressive and invasive growth is the hallmark feature that confers high morbidity and mortality of these tumors. Malignant glioma cells have higher levels of PKC1 than non-neoplastic astrocytes (1–4), suggesting that excessive PKC activity may significantly contribute to astroglial tumorigenicity. Tight regulation of PKC activity may control neoplastic glial proliferation (5).

PKC is a family of ubiquitous phospholipid-dependent enzymes involved in signal transduction pathways associated with a variety of cellular responses including cell growth and differentiation, gene expression, hormone secretion, and membrane function (6–10). The activities of both the conventional (α, βI, βII, and γ) and novel (θ and ε) PKC isozymes are regulated by phorbol esters, diacylglycerols, and phospholipids. Conventional PKC isozymes require Ca2+ for activity, whereas novel PKC and atypical (λ, ζ) PKC isozymes are Ca2+-independent (11). The atypical PKC isozymes are not activated by diacylglycerol, a product of receptor-mediated phospholipid hydrolysis (12). The activation of PKC in various cell types may lead to the stimulation of tyrosine phosphorylation pathways, the activation of mitogen-activated protein (MAP) kinase pathway and c-fos induction, all of which may increase cellular proliferation (13–16). However, the exact molecular mechanisms and signaling pathways involving PKC isozymes in neural cell types are not well understood.

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of biologic response to cytokines and other mitogenic stimuli acting via PKC signaling pathways. The differential expression of PKC-γ appears to determine the proliferative response of glioblastoma to PMA and thus may play a role in mediating the invasive growth of glioblastomas.

**EXPERIMENTAL PROCEDURES**

**Materials**—PMA and tubulin antibody (DMA1) were purchased from Sigma. A cDNA probe and polyclonal antibody specific for human PKC-γ were from the American Tissue Culture Collection (Manassas, VA) and Santa Cruz Biotechnology, Inc. (Santa Cruz, CA), respectively. The specific phospho-antibody to doubly phosphorylated extracellular signal-regulated kinase-1 and -2 (ERK1/ERK2) was obtained from New England BioLabs (Madison, WI). The specific PKC inhibitor, bisindolylmaleimide (BIM), and a mitogen-activated kinase effector kinase (MEK) inhibitor, PD 98059, are products of Calbiochem (San Diego, CA). Human PKC-γ (sense 5′-caggaattttcaaccctcG-3′) and antisense (5′-agagtaccaaccacctgC-3′) phosphorothioate oligonucleotides were synthesized by Operon Technologies, Inc. (Alameda, CA).

**Cell Cultures**—Human U-251 MG and U-1242 MG cell lines were generously supplied by Dr. D. D. Bigner (Duke University) and Dr. A. J. Yates (Ohio State University), respectively. Both cell lines were originally isolated from astrocytic tumors that were designated as glioblastomas based on morphologic and electrophoretic characteristics with U-251 MG cells (100–120% confluent) do not exhibit contact inhibition at confluence that U-251 MG cells do not exhibit (data not shown). Both lines were regularly determined to be free of mycoplasma with reagents from Gen-Probe, Inc. (San Diego, CA). Cells were grown in minimal essential medium-alpha modification (α-MEM) with 10% fetal bovine serum (HyClone, Logan, OH) and 20 μg/ml bovine insulin (25.7 IU/mg; Sigma). The cells were cultured to 100% confluence and passed every 4–5 days, from an initial concentration of 6–8 × 10^5/cm^2 in T-flasks or 6- or 24-well plates and cultured in astrocyte growth medium (AGM7, containing 5% fetal bovine serum) at 37°C in 4.5% CO_2, 90% relative humidity. Prior to assays, cultures that were 80–100% confluent were washed three times with serum-free medium before exposure to PMA (100 nM) in the presence or absence of BIM for 1–15 min. The incubation was terminated by the addition of ice-cold phosphate-buffered saline (137 mM NaCl, 8.1 mM Na_2HPO_4, 2.7 mM KCl, 1.5 mM KH_2PO_4, at pH 7.4) containing 0.2 mM sodium orthovanadate to the culture. Phosphate-buffered saline was subsequently aspirated, and cells were solubilized with 1% Nonidet P-40, 50 mM HEPE, 100 mM NaCl, 2 mM EDTA, 1 μg/ml leupeptin, 2 μg/ml aprotinin, 0.4 mg/ml sodium fluoride, 5 mg/ml dithiothreitol, and 0.2 mM sodium orthovanadate. The Nonidet P-40 extract was centrifuged at 14,000 × g for 15 min, the supernatant was immunoprecipitated as described above with a polyclonal antibody specific for phosphorylated ERK1/ERK2 (1:10,000). Densitometer and Image-Quant software (Molecular Dynamics) were used to quantitate the protein bands.

**Northern Blot Analysis**—Total RNA was isolated from adherent cells using Trizol (Life Technologies, Inc.) as a monophosphorylation solution of phenol and guanidine isothiocyanate, as modified from the single-step RNA isolation method developed by Chomczynski and Sacchi (28). All total RNA samples were quantified by UV spectrophotometry (200–25 μg/l × 10^3 cells; the 260/280 ratio ranged between 1.6 and 1.75) and stored at −70°C before use. Total RNA (20 μg) from control and treated glioblastoma cells was electrophoresed on 1% (w/v) agarose gels and electroblotted onto Zeta probe nylon membranes (Bio-Rad). A cDNA probe specific for human PKC-γ was cut with XbaI and separated on low melt Seaplaque agarose (FMC BioProducts, Rockland, ME). The probe (25 ng) was labeled with [α-32P]dCTP (50 μCi) using a random primed DNA labeling kit (Life Technologies, Inc.). Electrobotted RNA on nylon membranes was hybridized with the labeled probe for 4 h, washed twice with stringent wash buffer for leading, membranes also were hybridized with labeled human specific phosphoglyceraldehyde dehydrogenase cDNA. PhosphorImager (Molecular Dynamics) analysis was used to quantitate the radioactive bands.

**RESULTS**

**Expression and Regulation of PKC Isozymes in Astroglial Tumor Cells**—The basal expression of eight PKC isozymes was determined by Western blot analysis in the U-251 MG and U-1242 MG cells (Fig. 1). Both astrocytic cell lines expressed multiple conventional (α, β, and βII) as well as novel (ε and θ) PKC isozymes. The βI antibody appeared to cross react with βII, the lower band in βI blots. A doublet of PKC-γ-reactive band(s) was detected only in U-251 MG cells and not in U-1242 MG cells or cultured fetal human astrocytes (data not shown). PKC-δ was conspicuously present in U-1242 MG cells and only at trace levels in U-251 MG cells. Treatment with PMA (100 nM) for 48 h significantly decreased the basal expression of PKC-α, -β, -ε, and -θ in both lines and PKC-δ in the U-1242 MG cells, a doublet (Fig. 1). PMA treatment increased PKC-γ and had no significant effect on PKC-βI in the U-251 MG cells. Following PMA treatment, PKC-γ was still not detectable in either U-1242 MG cells or cultured fetal human astrocytes (data not shown).

The time course for PMA regulation of the PKC isozymes is shown in Fig. 2. PMA transiently increased the expression of most isozymes before causing the decrease that was observed at 24 h. The most intense and rapid responses to PMA were associated with the conventional isozymes (after approximately 2 h) compared with the later and more moderate responses of the novel isozymes (6–16 h). In U-251 MG cells, the expression of PKC-βI and the two PKC-γ bands were greatly increased with concomitant decrease the expression of PKC-βII. In U-1242 MG cells, PMA treatment of U-251 MG cells increased the lower and upper PKC-γ bands by 1.7- and 4.3-fold, respectively. In U-1242 MG cells, PKC-α and -δ were more significantly up-regulated.

**Effect of PMA on [3H]Thymidine Incorporation in U-251 MG and U-1242 MG Cells**—As shown in Fig. 3, treatment with PMA had opposite effects on [3H]thymidine uptake in the two glioblastoma cell lines that expressed different profiles of the
PKC isozymes. PMA increased [3H]thymidine uptake in U-251 MG cells (PKC-α expressing) by 2.2-fold (Fig. 3A) and decreased [3H]thymidine uptake in U-1242 MG cells by 0.9-fold (PKC-α deficient, Fig. 3B). Pretreatment of the cells with the PKC inhibitor BIM at 0.5 and 10 μM abrogated both types of PMA effect on [3H]thymidine uptake, i.e. the stimulation in U-251 MG cells or the inhibition in U-1242 MG cells (Fig. 3).

EGF (25 ng/ml) increased [3H]thymidine incorporation by 1.7- and 1.9-fold in U-251 MG and U-1242 MG cells, respectively.

Stable Overexpression of PKC-α Isozyme in U-1242 MG Cells—To determine whether the 2.2-fold mitogenic response of U-251 MG cells to the PMA treatment was specifically mediated by PKC-α, the PKC-α-deficient U-1242 MG cells were transfected with a 2.2-kilobase human PKC-α cDNA fragment containing a complete coding sequence inserted into a pCI expression vector with a CMV promoter and SV40-neo resistance gene cassette. U-1242 MG cells were transfected with PKC-α or an empty vector and selected with G418 for 4 weeks. Cultures of cells transfected with PKC-α cDNA demonstrated detectable basal levels of PKC-α, as determined by Western and Northern blot analyses, whereas cells that were only transfected with empty vector (PCI) did not. Single cell cloning was performed by limiting dilution, and several clones of U-1242 MG that expressed PKC-α (U-1242-PKC-α) were selected for further studies.

Representative Western and Northern blots of a stable clone of U-1242 MG that expressed PKC-α are shown in Fig. 4. The PKC-α antibody used in this study recognized a doublet of bands in U-251 MG cells, but only the lower molecular weight species was detected in the U-1242-PKC-α cells. As shown in Fig. 4A, both PMA and EGF increased the expression of PKC-α in the U-1242-PKC-α clones and in U-251 MG cells. Treatment of the U-1242-PKC-α clone with PMA (100 nM) also increased the PKC-α mRNA (2.2 kilobases) (Fig. 4B). The stable transfection of PKC-α in U-1242 MG cells did not alter the expression and PMA-induced regulation of βII and γ (Fig. 4C).

Effect of PMA on [3H]Thymidine Uptake in U-1242-PKC-α MG Cells—Stable expression of PKC-α in U-1242 MG cells dramatically reversed the inhibitory effects of PMA treatment on [3H]thymidine uptake in the U-1242 MG cells (PKC-α deficient). PMA increased the [3H]thymidine uptake in U-1242 PKC-α overexpressing cells 2.2-fold, analogous to the PMA effect on wild type glioblastoma cells (U-251 MG) that expressed PKC-α. Pretreatment of U-1242-PKC-α cells with BIM (0.5 μM) completely abolished the stimulatory effect of PMA (Fig. 5), similar to the effect on U-251 MG cells (Fig. 3). In the clone that was transfected with an empty vector (without PKC-α insert), treatment with PMA still decreased [3H]thymidine uptake.

Transient Knockdown of PKC-α Expression in Wild Type U-251 MG Cells—We synthesized the human version of the mouse PKC-α antisense oligonucleotide that was successfully used to knock down the expression of PKC-α mRNA in mouse Bend cells by Cooper et al. (29). To determine whether PKC-α is necessary for the PMA-induced increases in cell proliferation in U-251 MG cells, we transiently knocked down expression of the isozyme with phosphothioate oligonucleotide (ODN) antisense and assessed its effect on [3H]thymidine incorporation. Fig. 6 shows the effect of treating U-251 MG cells for 24 h with sense and antisense ODNs. The control sense ODN (1 μM) had
mediated via PKC-phosphorylation of ERK1/ERK2 by PMA was not exclusively the cells to PMA (Fig. 6 over 80% (Fig. 6 of the antisense ODN reduced the expression of the isozyme by increase in cell proliferation, whereas the same concentration [3H]thymidine uptake was determined after 20 h. The MEK cultures were then pulsed with [3H]thymidine (2 mCi/ml) for 2 h and assayed for radioactivity.

no apparent effect on PKC-γ expression or on the PMA-induced increase in cell proliferation, whereas the same concentration of the antisense ODN reduced the expression of the isozyme by over 80% (Fig. 6A) and abrogated the proliferative response of the cells to PMA (Fig. 6B). Other PKC isozymes (α, βII and γ) were not affected by the PKC-γ ODN antisense.

PMA Treatment, MAP Kinase Phosphorylation, and [3H]Thymidine Uptake—Activation of MAP kinase (ERK1/ERK2) is often an important step in the signaling cascade that leads to cellular proliferation. Because stimulation of PKC activity has been shown to increase cell proliferation by both MAP kinase-dependent and -independent pathways, we assessed MAP kinase phosphorylation and the effect of an inhibitor, PD 98509, of the upstream kinase, MEK, on the astrocyte mitogenic response to PMA. Both ERK1 and ERK2 were phosphorylated in response to PMA in all of these glioblastoma cells (U-251 MG, U-1242 MG, empty vector control and U-1242 PKC-γ overexpressors), and this phosphorylation was blocked by BIM (Fig. 7A) and PD 98509 (Fig. 7B). These data indicated that the phosphorylation of ERK1/ERK2 by PMA was not exclusively mediated via PKC-γ, because the ERK1 and ERK2 were comparably phosphorylated in the PKC-γ-deficient cells (wild type U-1242 MG and pCI-CMV empty vector transfected U-1242).

To assess the role(s) of MAP kinase activation in PKC-γ associated stimulation of [3H]thymidine uptake, both PKC-γ-expressing and -deficient cells were pretreated with PD 98509 (50 µM) for 10 min before the addition of PMA (100 nM), and [3H]thymidine uptake was determined after 20 h. The MEK inhibitor, PD 98509, significantly reduced the basal mitogenic response in all cell lines (U-251 MG, pCI-CMV U-1242 empty vector, and U-1242 PKC-γ overexpressors) but to different degrees. In both PKC-γ-expressing lines (U-251 MG and U-1242 PKC-γ overexpressors), basal levels were only reduced to 70–80% compared with the PKC-γ-deficient (pCI-CMV U-1242 empty vector) line, which was reduced to 41% of basal levels. In the U-1242 MG cells that overexpress PKC-γ, the stimulatory effect of PMA on [3H]thymidine uptake appears to be totally mediated via MAP kinase activation (Fig. 8A), because inhibition with PD 98509 abrogates the response. In contrast, the MAP kinase inhibitor was less effective in counteracting the mitogenic response in wt U-1242 MG cells. In both cases, the PMA actions on proliferation, either stimulation in the presence of PKC-γ or inhibition in the absence of PKC-γ, may be modified by the presence of other PKC isoform(s) in the two cell lines.

**DISCUSSION**

Although previous studies have strongly implicated PKC in regulating the growth of glioblastomas (5, 30), there are conflicting data as to how it regulates the malignant phenotype(s) of a high proliferative rate and cellular invasiveness (22, 31, 32). The differential expression of PKC isoforms, each mediating diverse biologic functions, could account for the contrasting effects of phorbol esters on specific biologic activities in astrocytic tumors (33–35). Likewise, this PKC isozyme diversity also could account for the apparently different cellular responses following the expression or overexpression of specific PKC.
isozymes (18, 17, 36).

The responses of glioblastomas to therapy with relatively nonspecific PKC inhibitors in several clinical trials have been ambiguous. These outcomes suggested that the in situ growth of only certain, as yet undefined, tumor subgroups may be sensitive to the disruption of PKC signaling (23, 37). Previous in vitro studies with both human glioma and experimental rodent tumor cell lines have demonstrated that specific PKC isozymes may selectively affect astrocytic tumor growth. PKC-\(\alpha\) has been implicated in proliferation or suppression of apoptosis (19). A role for PKC-\(\gamma\) in augmenting anchorage-independent growth in cell culture has also been suggested (18).

Regulation of human astrocyte proliferation or apoptosis by the novel isozymes has not been clearly defined. PKC-\(\epsilon\) appeared to affect the initial exit from the G0/G1 phase in human 132–1N1 astrocytoma cells (38), but PKC-\(\alpha\) played the major determinant role for proliferation. PKC\(\delta\) overexpression decreased anchorage-independent growth in U-251 MG but did not affect basal or growth factor-stimulated proliferation (18).

We have found that both U-251 MG and U-1242 MG cells expressed PKC-\(\alpha\), -\(\beta\), -\(\beta\II, -\delta\), and -\(\epsilon\) but that PKC-\(\gamma\) was expressed only in the invasive U-251 MG cells (Fig. 1). Treatment of the cells with PMA for 24–48 h caused a decrease in expression of all isozymes, except that PKC-\(\beta\II\) and PKC-\(\gamma\) were up-regulated in the U-251 cells (Figs. 1 and 2). Prior to the decrease in isozyme expression detectable by Western blotting, some increase in expression was observed for most of the isozymes. The peak in expression tended to occur earlier (~2 h after PMA stimulation) for the classical, Ca\(^{2+}\)-dependent isozymes and later (~6–16 h) for the novel, Ca\(^{2+}\)-independent isozymes.

There is also a trend for PMA-induced up-regulation of PKC-\(\delta\) or for the absence of down-regulation in other cell types. Unique up-regulation of PKC-\(\gamma\) has been noted previously in interleukin 2-producing EL4 mouse thymoma cells (12), in murine keratinocytes (39), and in breast cancer lines (40), and the lack of down-regulation was observed also in A431 human epidermal carcinoma cells (41). Basu (40) observed down-regulation of PKC-\(\delta\) after treatment of MCF-7 cells with BIM, but we did not see a BIM-induced down-regulation of PKC-\(\gamma\) expression in the astrocytic cell lines (data not shown). In the astrocytic tumor cells studied here, it is notable that each cell line appears to up-regulate one classical isozyme and one novel isozyme significantly; but the predominant up-regulated isozymes differ: PKC-\(\beta\II\) and PKC-\(\gamma\) in U-251 MG cells and PKC-\(\alpha\) and PKC-\(\delta\) in U-1242 MG cells. This may indicate involvement of different classes of isozymes in different phases of a biologic response and may suggest that other members of a class may mediate analogous early or late functions.

The biologic responses of U-251 MG and U-1242 MG cells to...
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PMA differ. U-251 MG cells are growth-stimulated by PMA, whereas U-1242 MG cells are growth-inhibited (Fig. 3). The general PKC inhibitor BIM blocked the PMA-induced growth stimulation or growth-inhibition (Fig. 3), suggesting that PKC is responsible for both effects. The opposing effects may be attributable to the different isozymes expressed. Stable expression of PKC-\(\eta\) in the U-1242 MG cells (Fig. 4) converted the PMA response from growth inhibition to growth stimulation like that of the U-251 cells (Fig. 5). The PMA-induced proliferative response in wild type U-251 MG cells was also completely blocked by PKC-\(\eta\)-reactive band phosphorothioate oligonucleotide antisense (Fig. 6). These results further argue for involvement of PKC-\(\eta\) in the growth stimulatory response of the malignant astrocytic tumor cells to PMA. Although expression of PKC-\(\eta\) in the growth stimulatory response of the malignant astrocytic tumor cells, these isoform-specific trends are not present in different cell types. In EL4 thymoma cells, PKC-\(\theta\) has been associated with growth stimulatory responses (12, 42), whereas PKC-\(\eta\) has been implicated in morphological changes of EL4 cells (42) and NIH3T3 fibroblasts (44). There are no significant morphological differences between U-1242 MG cells and the U-1242-PKC-\(\eta\) clone, but the U-251 MG and U-1242 MG cells are morphologically different. U-1242 MG monolayer cultures show contact inhibition at confluence, whereas U-251 MG cells do not.

A doublet of PKC-\(\eta\)-reactive bands was present in the U-251 MG cells, whereas only the lower band was present in the U-1242 MG cells that were transfected with the PKC-\(\eta\) expression construct. Similar differences have been described previously in other cell lines. A doublet of PKC-\(\eta\)-reactive bands was present in EL4 thymoma cells that were responsive to phorbol esters (12, 43), whereas only the lower band of PKC-\(\eta\) was observed in phorbol ester-resistant EL4 cells that had been infected with a Sinbis viral construct expressing PKC-\(\eta\). The relationship between the doublet bands is not clear. These may represent differences in phosphorylation, alternatively spliced forms, or even closely related isozymes. Correlation of the growth response of U-1242 MG cells with expression of the lower molecular weight PKC-\(\eta\)-reactive band suggests that the protein of lower molecular mass is responsible for the mitogenic effects of PMA in these glioblastoma cells.

The signaling pathways by which PKC-\(\eta\) may up-regulate cell proliferation in human glioblastomas is not yet clear. In many cell types, the MAP kinase pathway has been implicated in the PMA-induced growth stimulatory response (45). The MAP kinase pathway is stimulated by PMA in the astrocytic tumor cells as indicated by phosphorylation of ERK1/ERK2. The relationship between the doublet bands is not clear. These may represent differences in phosphorylation, alternatively spliced forms, or even closely related isozymes. Correlation of the growth response of U-1242 MG cells with expression of the lower molecular weight PKC-\(\eta\)-reactive band suggests that the protein of lower molecular mass is responsible for the mitogenic effects of PMA in these glioblastoma cells.
sponse of MAP kinase activation, unless PKC-η is co-expressed. The latter is consistent with the results of Mishima et al. (18), in which overexpression of PKC-δ decreased anchorage-independent growth (46).

EGF is another potent mitogen for astrocytoma cells and induces proliferation of both U-1242 MG and U-251 MG cells. EGF can activate PKC, but the proliferative response of U-1242 MG cells argues against the involvement of PKC-η in the EGF effect. The effectiveness of EGF but not phorbol esters to induce a mitogenic response in U-1242 MG cells may suggest involvement of non-PKC pathways, such as tyrosine kinase activation, in EGF-induced mitogenesis and/or possibly atypical PKCs that are not activated by phorbol ester.

The functional characterization of PKC isozymes in human astrocytic tumors (17, 18, 21, 33, 37, 46, 47) has been impeded by the relative nonspecificity of PKC inhibitors and by the complex, differential expression of multiple isozymes. Animal cell lines may also have limited utility in the study of specific isozyme-associated biologic responses in human cells because these responses appear to differ between human and rodent cells (48). In the present studies, the overexpression of PKC-η combined with the use of inhibitors and oligonucleotide antisense has implicated this novel PKC isozyme in PMA-induced proliferative activity. PKC-η has a more limited tissue distribution than do many of the isozymes that have been previously implicated in up-regulating glial proliferation, with minimal expression in normal brain (49, 50). These distinctions make PKC-η an excellent potential therapeutic target to control invasive tumor growth in human glioblastomas.

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