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OPPOSITE EFFECTS OF THE OVEREXPRESSION OF PROTEIN KINASE Cγ AND δ ON THE GROWTH PROPERTIES OF HUMAN GLIOMA CELL LINE U251 MG*

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SUMMARY: In order to address the question of whether protein kinase C (PKC) is involved in the growth regulation of human glioma cells, we introduced PKC cDNA expression vectors into a human glioma cell line, U-251 MG, and established sets of stable cell clones that overexpress PKCγ or δ . Cell clones obtained by the transfection of PKCγ cDNA express 3.6 to 5 times more PKC activity than parental cells that express predominantly endogenous PKCα. These PKCγ overexpressing cell clones show an increased rate of growth in monolayer culture, increased colony-forming efficiency on soft agarose, and increased DNA synthesis in response to epidermal growth factor and basic fibroblast growth factor. Cell clones obtained by transfection with PKCδ cDNA express 2 to 10 times more PKC than that produced endogenously. PKCδ overexpressing cells show a decreased rate of growth and decreased colony-forming efficiency. However, these PKCδ cell clones show no significant changes in responsiveness to the growth factors described above. These results clearly indicate that different PKC family members have distinct regulatory functions in cell growth and that PKC is involved in several aspects of the growth regulation of human glioma cells.

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The abbreviations used are: PKC, protein kinase C; cPKC, conventional PKC; nPKC, novel PKC; aPKC, atypical PKC; TPA, 12-o-tetradecanoyl phorbol 13-acetate; EGTA, [ethylenebis(oxyethylenenitriol)] tetraacetic acid.

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Protein kinase C (PKC) has been implicated in a variety of cell functions such as the regulation of cell growth and differentiation, secretion of hormones, modulation of receptor functions, and modulation of transcription(1, 2), although the molecular mechanisms of these functions remain unclear. Molecular cloning and biochemical experiments have established that there are at least 10 PKC family members that can be divided into three distinct classes based on differences in structure and biochemical properties (3, 4, 5). cPKC members (α, βI, βII, and γ) show so-called PKC activity regulated by phospholipids, Ca^{2+}, and diacylglycerols as well as tumor promoting phorbol esters (6, 7). nPKC members (δ, ε, η , and θ) show Ca^{2+}-independent PKC activity (8, 9, 10, 11). On the other hand, aPKC (ζ and λ) show atypical PKC activity in that the kinase activity is not regulated by diacylglycerol or phorbol ester (12, 13). Tumor promoting phorbol esters that activate PKC have been shown to stimulate or inhibit cell proliferation depending on the cell line used. The growth properties of human glioma cell lines treated with phorbol esters also depend on the cell line used, making the role of PKC in cell growth regulation rather complicated (14, 15, 16). The presence of a family of PKC (cPKC and nPKC), all of which have been shown to be cellular receptors for phorbol esters, raises the intriguing question of whether the various members share distinct functions. Since phorbol esters activate all cPKC and nPKC family members (3) and since the available inhibitors inhibit both cPKC and nPKC (17), one promising approach to this issue is to overexpress each PKC member and examine the effects on various cell functions. Recent experiments based on this approach have provided pieces of information as to the function of each PKC member in cellular processes such as cell growth and differentiation. The overexpression of PKCβI in rat fibroblasts resulted in the modulation of several growth properties, such as an increase in growth rate and saturation density (18). On the other hand, the overexpression of the same PKC type in HT29 human colon cancer cells resulted in inhibition of cell growth and the suppression of tumorigenicity (19). These reports indicate that certain PKC types can act in different fashions depending on the cell type examined. There appeared reports examining the effects of the overexpression of different PKC types in a single cell line (20, 21) .

The present paper describes the establishment and characterization of stable cell clones of human glioma cell line U-251 MG that overexpress exogenously introduced cPKCγ or nPKCδ. A comparison of several growth properties of these cell clones clearly indicates that PKCγ and PKCδ display apparently opposite functions when introduced into human glioma cells, and that the type and expression level of PKC determines the growth properties of the glioma cell line.

EXPERIMENTAL PROCEDURES

RNA isolation and Northern blot analysis----Poly(A)$^+$RNA preparation and Northern blotting were performed as described(22). The DNA probes used included human cPKCα, nucleotide numbers -197 to 2016; human cPKC β, 22 to 1968 of PKC βII; mouse cPKCγ , 345 to 2091; mouse nPKC δ, -13 to 2524; mouse nPKCε , -22 to 2169; mouse aPKCζ, -3 to 2619; and mouse nPKCη, -59 to 2117 (5).

Cell cultures, transfection and establishment of cell clones----U-251 MG and SK-MG 15 cell lines were cultured in DMEM with 10% fetal calf serum (FCS) at 37°C in a humidified atmosphere of 5% CO_2. Expression plasmids for cPKCγ and nPKCδ cDNA (23) and pSV2neo plasmid DNA were co-transfected into U-251 MG cells as described (22). Ten to 20 G418-resistant clones for each

PKC type were selected, and several clones were identified as expressing PKC by Western blot analysis. Cells transfected with pSVneo and pcDL-SRα296 were used as control cell lines.

Cell fractionation, Western blotting, and PKC activity measurement----Cell fractionation and Western blotting were carried out as described (11). For measurement of PKC activity, the cytosol fraction was applied onto a 0.5 ml DEAE-Sephacel column to obtain PKC fractions and the protein kinase activities were determined using EGFR peptide (VRKRTLRRL) as a substrate as previously described (11).

Growth property measurements----Cells were seeded at a density of $1x10^4$ cells/35 mm plate in 3ml DMEM containing 10% FCS. Twenty-four hours later, cells in triplicate plates were trypsinized and counted. This point was designated day 0. The remaining cultures were then grown with fresh medium changes twice per week. On selected days, cells were trypsinized and counted. Agarose plates were prepared in 35 mm plastic dishes by pouring 1 ml of a base layer of 0.7% agarose in DMEM with 5% FCS. One ml of a top layer of 0.35% agarose in DMEM with 5% FCS, containing $1x10^3$ PKC overexpressing cells or control cells was added to the base layer. The cell cultures were incubated at 37°C for 3 weeks. The number of colonies with diameters greater than 0.05 mm was counted microscopically. Growth factor-induced DNA synthesis was measured as follows. Cells were seeded onto 96 microtiter plates ($3x10^4$ cells in 100 ml of 0.5% serum-supplemented DMEM/well), and grown for 24 hours. The cells were then incubated for 24 hours with 10 ng/ml EGF or bFGF in DMEM with 0.1% bovine serum albumin (BSA). Control cells were incubated without exogenous mitogens in DMEM with BSA. After 16 hours, the cells were pulse-labeled with 5 kBq/ well of [^{125}I]-iododeoxyuridine (NEN) for 16 hours, and the incorporation of radioactivity into the acid-insoluble material was determined.

RESULTS

Northern blot analysis of PKC family members in human glioma cell lines and glioma tissues

Although there have been several reports on the effects of phorbol esters on the growth of human glioma cell lines (14, 15, 16), there have been few reports describing the molecular types of PKC expressed in glioma cells, especially nPKC members (24). Thus, we initially examined the expression of mRNAs for 7 PKC members, including cPKC, nPKC, and aPKC, in human glioma cell lines and compared the results with expression in glioblastoma samples obtained from several independent patients. Figure 1 shows an example indicating that human glioma cell lines express several PKC members. Since the cDNA probes for PKCγ to ζ are mouse sequence, the intensities of the hybridized bands are expected to be weeker than those obtained with human probes. Thus, the relative abundance of these mRNA species is underestimated. The predominant PKC type is PKCα in all cases examined. The next most dominant mRNA species in glioma cell lines were those for nPKCs η (U-251 MG) and δ (SK-MG 15). In addition to these PKC mRNA species, both glioma cell lines express almost all PKC members except PKCγ , which is also not detected in any of the 7 glioblastoma samples examined (Figure 1B). It should be pointed out that PKCβ mRNA is relatively abundant in all the examined glioblastoma samples, although its level is minimal in the two glioma cell lines examined. These results clearly indicate that a single glioma cell expresses several types of PKC, although the respective roles of each member remain to be clarified.

Establishment of stable human glioma cell clones overexpressing PKCγ or PKCδ

In order to examine the role of each PKC member on the growth properties of human glioma cell lines, we established stable cell clones of U-251 MG which express cPKCγ or nPKCδ. We did not select PKCα because this PKC type is expressed in large amounts in the parental cells that seemed to be sufficient for signaling events specific for PKCα. We selected PKCγ , because it is not

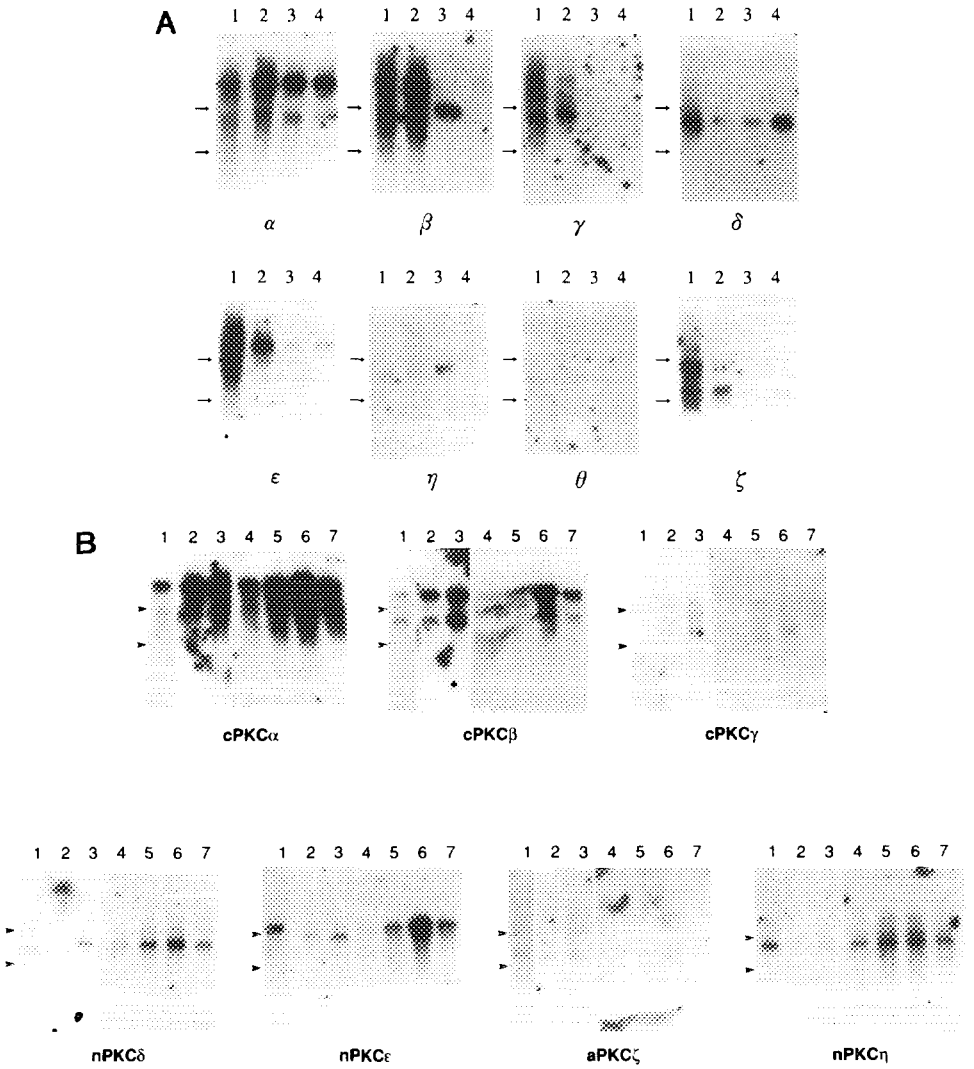

Figure 1.
Northern blot analysis of PKC isotypes in glioma cell lines (U-251 MG and SK-KG 15) (A) and human glioblastoma samples (B). Poly $(A)^+$RNA (1.5 μg) was applied to each lane. The position of ribosomal RNAs (18S and 28S) are indicated. Probing the blots with actin cDNA probe demonstrated that equivalent amounts of RNA were loaded and transferred (not shown). (A) Lanes: 1, mouse brain; 2, human brain; 3, U251-MG; 4, SK-MG15. (B) Lanes: 1 - 7, human glioblastoma samples.

expressed in glioma cells and also PKCδ because it is expressed in all glioblastoma tsamplees and cell lines in rather small amounts.

U251-MG cells were transfected with the respective PKC cDNA expression plasmid with pSV2neo as a selection marker, and G-418 resistant cell clones were isolated. These cell clones were then screened for the expression of the respective PKC members by Western blotting. Among cell clones that overexpressed PKCγ, two cell clones, G3 and G15, were selected for further

characterization; additionally, vector transfected V1 and V3 were chosen as control cell clones. Figure 2B shows that 80 kDa PKCγ is expressed only in G3 and G15 clones and not in control cell lines. Northern blot analysis of the mRNAs prepared from the cell lines also showed that G3 and G15 expressed PKC mRNA of the expected size (2.5 kb) while there was no expression in the control cells (Figure 2A). To estimate the amount of PKCγ expressed in these cell lines, we prepared cell extracts and measured PKC activity using an EGF receptor peptide as a substrate under conditions optimized for cPKC. The parental U-251 cell line expresses PKCα as well as small amounts of PKCβ and Figure 3 shows that G3 and G15 cells express 5-fold and 3.6-fold more PKCγ, respectively, than endogenous PKCα in the control cell clones.

Stable cell clones overexpressing PKC δ were also selected for their expression of 78 kDa PKCδ by Western blotting. Figure 2C shows that D18 , D21, and D30 cell lines expressed, respectively, approximately 5-fold, 2-fold, and 10-fold more PKCδ than the amount of endogenous PKCδ expressed in parental cells or vector control cell lines when estimated by densitometric measurements of the Western bands. Since the measurement of nPKC kinase activity in crude cell extracts containing large amounts of cPKC has never been performed successfully, Western blotting is the most reliable method for measuring the relative expression level of exogenous PKCδ. The cell clones described above were also examined for their expression of PKC α, β , ε, ζ, and η, by Western blotting, and only the expression of PKCα, ε, ζ, and η could be verified (data not shown). Further, there was no significant change in the level of expression of these endogenous PKC members by overexpression of PKCγ or PKCδ (data not shown).

Growth properties of the PKC overproducers

We did not detect any significant change in the morphology of any of the PKC overproducers including G-series and D-series cell clones. However, there were several clear differences between them and the parental control cells in their growth properties. When cultured in monolayer with 10% fetal calf serum, PKC γ overproducers showed somewhat increased growth rates, while PKCδ overproducers showed decreased growth rates relative to the expression of PKCδ (Figure 4) .

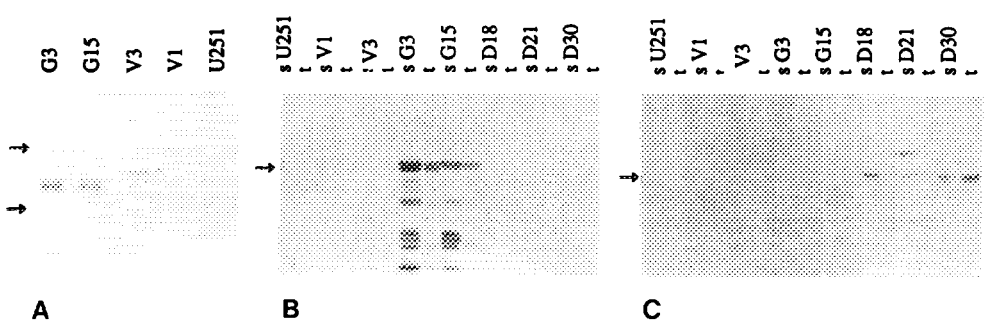

Figure 2.

(A) Northern blot analysis of PKCγ overexpressing cell lines and control transfected cell lines. Five μg of total RNA was applied to each lane. The positions of 28S and 18S RNA are indicated.

(B) and (C) Western blot analysis of U251 stable clones. Three μg of the cytosolic or particulate fractions were loaded onto all lanes. Immunoblots were probed with PKCγ-specific antibodies (B) or PKCδ-specific antibodies (C). s, supernatant cytosol fraction; t, Triton-soluble fraction.

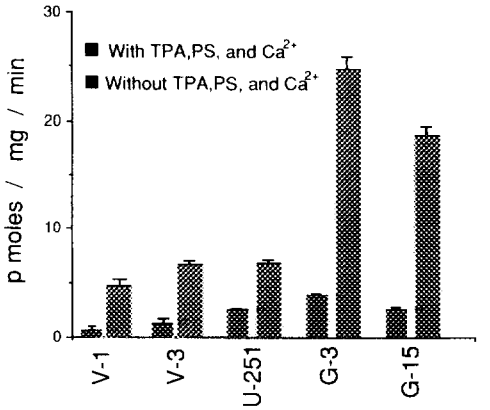

Figure 3.
Protein kinase activity of PKCγ expressing cell lines and control transfected cell lines. Total PKC activity was partially purified for each of the cell lines and assayed in Experimental Procedures. Data are presented as the means ± S.D. of two experiments each done in duplicate. Hatched bars indicate PKC activity in the presence of cofactors phosphatidyl serine, TPA, and Ca^{2+}. Filled bars indicate PKC activity in the absence of these cofactors.

Furthermore, PKCγ overproducers showed increased colony-forming efficiency on soft agarose, while PKC δ overproducers showed decreased colony-forming efficiency in a similar assay (Table 1).

These results indicate that the overexpression of PKCγ slightly stimulates the growth rate of U-251 MG glioma cells in monolayer culture and significantly stimulates the anchorage independent

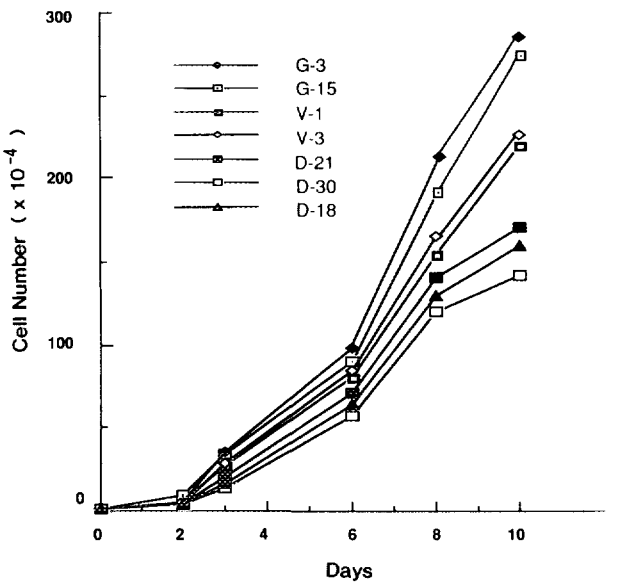

Figure 4.
Growth rates of PKC overproducers and control clones in monolayer cultures. Culture dishes (35mm) were seeded with $1x10^4$ cells in growth medium (DMEM with10% FCS). Cells were trypsinized and counted on the days indicated. Points represent means of triplicate determinations which differed by less than 5%.

Table 1. Colony formation in soft agarose of PKC overproducers and control clones. Transfected cell clones and parental U-251 MG cells were seeded in 0.35% soft agar in DMEM containing 5% fetal calf serum. Three weeks after seeding, colonies equal to or greater than 0.05 mm in diameter were counted The data are presented as the mean ± S.D. of triplicate determinations.

Cell line	Colony-forming Efficiency (%)
U-251	10.5 ± 0.3
V1	11.2 ± 1.6
G-3	18.8 ± 3.3
G-15	17.5 ± 2.3
D-18	7.2 ± 2.3
D-30	4.3 ± 1.1

growth in soft agarose. This suggests that the overexpression of PKCγ results in the increase in a certain growth factor such as TGFβ that stimulates anchorage independent growth in soft agarose. On the other hand, the overexpression of PKCδ inhibits the growth of these cells under the same culture conditions.

Responsiveness to growth factors examined in terms of stimulation of DNA synthesis

To analyze the nature of the opposite effects caused by the overexpression of the two distinct PKC members γ and δ, we next examined the responsiveness to growth factors (EGF and bFGF identified as glioma mitogens) in terms of the stimulation of DNA synthesis. Figure 5 shows that PKCγ overproducers show increased responsiveness to both growth factors inducing DNA

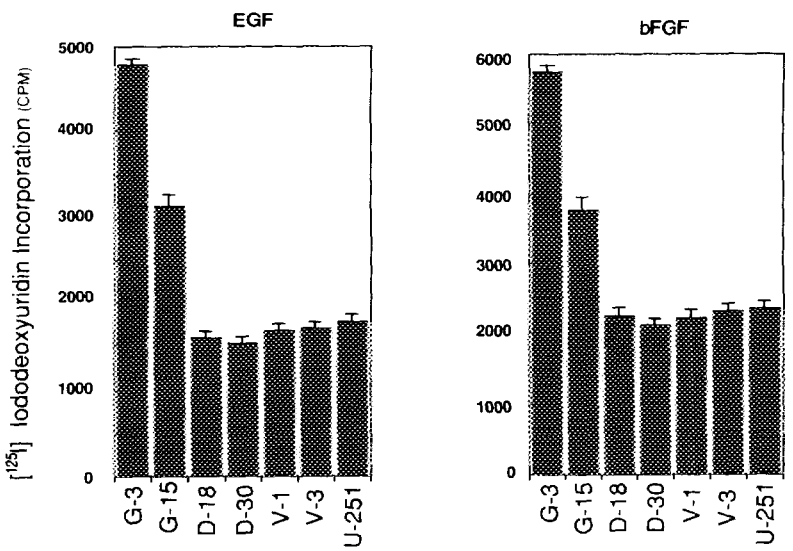

Figure 5.
Effect of EGF and bFGF on DNA synthesis in PKC overproducers and control cell lines. The [^{125}I] radioactivity incorporated into DNA was determined as described in Experimental Procedures. Baseline DNA synthesis in the absence of exogenous mitogens was subtracted from the total radioactivity . Each bar is the average ± S.D. of two determinations in a single experiment. Three additional experiments gave similar results.

synthesis. Other growth factors, such as PDGF and IGF-I showed no significant effects on the cell lines examined (data not shown). In contrast to the results on PKCγ , PKCδ overproducers did not show any significant change in responsiveness to EGF or bFGF under similar conditions. Thus, the overexpression of PKCγ increases the responsiveness of U-251 MG cells to exogenously added growth factors, while the overexpression of PKCδ results in no significant change in the responsiveness to growth factors.

Many cancer cells endogenously produce growth factors in an autocrine fashion and this is believed to explain at least partially, the changes in some growth properties of cancer cells. Thus, we next examined whether endogenous growth factors synthesized in PKC transfectants and control cells were changed or not. Cell extracts were prepared and examined for activity stimulating DNA synthesis in mouse BALB 3T3 fibroblasts. However, we could not detect any significant changes in the activities of the endogenous growth factors in the PKC overproducers (data not shown).

DISCUSSION

We presented evidence indicating that the overexpression of PKC γ or PKC δ causes opposite effects on the growth properties of human glioma U-251 MG cells. Overexpression of PKC γ results in an enhancement in the growth rate in monolayer culture and also on soft agarose. Furthermore, PKCγ overproducers show increased responsiveness to EGF and bFGF to induce DNA synthesis. On the other hand, the overexpression of PKCδ caused a decrease in the growth rate in monolayer and also on soft agarose, while the responsiveness to growth factors did not change significantly. It should be pointed out that these changes in growth properties caused by the overexpression of PKC were observed in the absence of TPA. Although Northern experiments showed that PKCγ is not expressed in parental U-251 MG cells as well as in all the glioblastoma samples (Figure 1), the results indicate that different PKC types have distinct functions in controlling the growth of glioma cells. Furthermore, our results present the first direct evidence that PKC is involved in regulating the growth of human glioma cell lines.

There have been several reports describing the changes in growth properties caused by the overexpression of several PKC members(18, 19, 20, 21, 25, 26, 27). As for PKC γ, there is a report that its overexpression in normal NIH3T3 cells results in the stimulation of several growth properties including growth rate, saturation density, colony formation on soft agarose, and tumorigenicity, although there is no report confirming these observations(25). The overexpression of PKCγ in T cells leads to immortalization (26). These results are consistent with our observation that PKCγ acts as a positive regulator of cell growth. We have also shown that PKCγ overproducers are highly responsive to extra cellular growth factors such as EGF and bFGF. This is quite interesting because there are reports showing that PKC phosphorylates the EGF receptor resulting in the desensitization or down-regulation of receptor function (28, 29). It is also reported that PKC acts on the EGF receptor to decrease the number of receptors. This is directly confirmed by an experiment in which PKCα was overexpressed in Swiss 3T3 cells (30). We found no significant differences in the dose response curve for these growth factors between PKCγ overproducers and parental control cells (data not shown). Therefore, the increase in responsiveness to these growth factors may not be the consequence of changes in the receptor, but of changes on

other target(s). This result suggests the interesting possibility that the enhanced growth properties of PKCγ overproducers may be caused by an increase in the synthesis of or responsiveness to some autocrine growth factors in these cells. In fact, we observed that parental U-251 MG cells contain intracellular growth factors that might act in an autocrine mechanism (data not shown). However, there were no significant changes in the growth promoting activities of endogenous growth factors between PKCγ overproducing cells and parental control cells when assayed on BALB 3T3 cells (data not shown). One possible mechanism to explain the increase in growth factor responsiveness observed in the PKCγ overproducers may be that PKCγ acts on some signaling pathway downstream of the receptor common to both EGF and bFGF. This target might be the one which acts in the context where PKC activators promote cell growth.

The overexpression of PKCδ in malignant Chinese hamster cell line CHO cells results in the inhibition of cell growth (27). Interestingly, in this case, TPA causes the arrest of the cell cycle at the G2/M boundary, although there is no evidence showing that PKC acts at the G2/M boundary. Recently, Mischak et al. reported that the overexpression of PKCδ or PKCε results, respectively, in the inhibition or stimulation of the growth of NIH3T3 cells (20). They showed that the overexpression of PKCε causes a stimulation of growth rates, saturation density, and colony formation in NIH3T3 cells, while the overexpression of PKC δ causes an inhibition of cell growth. These observations are consistent with ours in that PKCδ also acts as a negative regulator for cell growth in malignant cells. We also examined the responsiveness to growth factors of our PKCδ overproducers, but could detect no significant changes. Furthermore, we could not detect any significant changes in the amount of intracellular autocrine growth factor activity in these PKCδ overproducers. Thus, the target of PKCδ in the inhibition of the cell growth in the cell lines examined remains to be clarified. However, it is tempting to speculate that PKC may act as a negative regulator for cell growth in a context where PKC activators cause inhibition of cell growth.

There are contradictory reports on the effect of PKC activators such as TPA on the growth of human glioma cells. TPA acts as a growth promoter on certain cell lines such as A172 and U-87 MG under serum-free conditions, while it acts as growth inhibitor on the same cell lines in the presence of serum (14, 15). In other cell lines, such as U-373 MG and rat C6 glioma, TPA acts as a growth inhibitor even under serum-free conditions (16). This suggests that PKC may act at least two distinct points in growth regulation , one stimulation and the other inhibition. Taken together our results suggest that the PKC types expressed in a cell may at least partially determine the effect of PKC activators such as TPA on growth control, and that the promotion and inhibition of cell growth by PKC may be caused at least partially, by different PKC types.

REFERENCES

1. Nishizuka, Y. (1984) Nature 308, 693-698
2. Nishizuka, Y. (1988) Nature 334, 661-665
3. Nishizuka Y. (1992) Science 258, 607-614
4. Ohno, S., Akita, Y., Hata, A., Osada, S., Kubo, K., Konno, Y., Akimoto, K., Mizuno, K., Saido, T., Kuroki, T., and Suzuki, K. (1991) Advances in Enzyme Regulation 31, 287-303
5. Osada, S., Mizuno, K., Saido, T.C., Suzuki, K., Kuroki, T., Ohno, S. (1992) Mol. Cell. Biol. 12, 3930-3938
6. Huang, K.-P., Huang, F.L., Nakabayashi, H., Yoshida, Y. (1988) J. Biol. Chem. 263, 14839-14845

7. Sekiguchi, K., Tsukuda, M., Ase, K., Kikkawa, U., Nishizuka, Y. (1988) J. Biochem. (Tokyo) 103, 759-765
8. Ohno, S., Akita, Y., Konno, Y., Imajoh, S., Suzuki, K (1988) Cell 53, 731-741
9. Akita, Y., Ohno, S., Konno, Y., Yano, A., Suzuki, K.(1990) J. Biol. Chem. 265, 354-362
10. Konno, Y., Ohno, S., Akita, Y., Kawasaki, H., Suzuki, K. (1989) J. Biochem. 106, 673-678
11. Mizuno, K., Kubo, K., Saido, T.C., Akita, Y., Osada, S., Kuroki, T., Ohno, S., Suzuki, K. (1991) Eur. J. Biochem, 202, 931-940
12. Ono, Y. , Fujii, T., Ogita, K., Kikkawa, U., Igarashi, K., and Nishizuka, Y. (1989) Proc. Natl. Acad. Sci. U.S.A. 86, 3099-3103
13. Nakabayashi, H. and Exton, J. (1992) J. Biol. Chem. 267, 16347-16354
14. Pollack I.F., Randall M.S., Kristofik M.P., Kelly R.H., Selker R.G., Vertosick F.T. (1990) J. Neurosurg. 73, 98-105
15. Couldwell W.T., Antel J.P., Apuzzo M.L.J., Yong V.W. (1990) J. Neurosurg. 73, 594-600
16. Couldwell W.T., Antel J.P., Young V.W.Y. (1992) Neurosurg. 31, 717-724
17. Mizuno, K., Saido, T.C., Ohno, S., Tamaoki, T., and Suzuki, K. (1993) FEBS Lett. 330, 114-116
18. Housey, GM., Johnson, M.D., Hsiao, W.L.W., O' Brian, C.A., Murphy, J. P., Kirschmeier , P., and Weinstein, I.B. (1988) Cell 52, 343-354.
19. Choi,P.M., Tchou-Wong,K.M., and Weinstein, I.B. (1990) Mol. Cell. Biol. 10, 4650-4657
20. Mischak, H., Goodnight, J., Kolch, W., Martiny-Baron, G., Schaechtle, C., Kazanietz, M.G., Blumberg, P.M., Pierce, J.H., Mushinski, J.F.(1993) J. Biol. Chem. 268, 6090-6096
21. Cacace, A.M., Guadagno, S.N., Krauss, R.S., Fabbro, D., and Weinstein, I.B. (1993) Oncogene 8, 2095-2104
22. Maniatis, T., Fritsch, E.F., and Sambrook, J. (1982) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
23. Hata, A., Akita, Y., Suzuki, K., and Ohno, S. (1993) J. Biol. Chem. 268, 9122-9129
24. Todo, T., Shitara, N., Nakamura, H., Takakura, K., Ikeda, K. (1991) Neurosurgery 29, 399-404
25. Persons, D.A., Wilkinson, W.O., Bell,R.M., and Finn, O.J. (1988) Cell 52, 447-458
26. Finn, O.J., Persons, D.A., Bendt, K.M., Pirami, L., and Ricciardi, P. (1991) J. Immun. 146, 1099-1103
27. Watanabe, T., Ono, Y., Taniyama, Y., Hazama, K., Igarashi, K., Ogita, K., Kikkawa, U., Nishizuka, Y.(1992) Proc. Natl. Acad. Sci. U. S. A. 89, 10159-10163
28. Cochet, C., Gill, G.N., Meisenhelder, J., Cooper, J.A., Hunter, T.(1984) J. Biol. Chem. 259, 2553-2558
29. Iwashita, S. Fox, C.F. (1984) J. Biol. Chem. 259, 2559-2558
30. Eldar, H., Zisman, Y., Ullrich, A., Livneh, E. (1990) J. Biol. Chem. 265, 13290-13296