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Protein Kinase C (PKC) in Cellular Signalling System: Translocation of Six Protein Kinase C Isozymes in Human Prostate Adenocarcinoma PC-3 Cell Line

Won Chul Choi* and Chang Ho Ahn**

**Department of Biology, College of Natural Sciences, Pusan National University, Pusan, Korea, and **Division of Oncology and Pulmonary Drug Product, HFD-150, CDER U.S. Food and Drug Administration, Rockvill, MD 20857, USA*

Protein kinase C isozymes in a human prostate adenocarcinoma PC-3 cell line were characterized. Immunoreactive bands and immunocytochemical stains were observed in PC-3 cells with antibodies raised against protein kinase C α, β, γ, δ, ε, and ζ types, respectively. Protein kinase C α corresponded to a immunoreactive band at a molecular weight of 80,000-dalton, whereas molecular weights of other immunoreactive isozymes of protein kinase C were detected at 68,000-dalton. Protein kinase C δ and ζ antibodies detected additional bands at 55,000-dalton and 80,000-dalton, respectively. Immunocytochemical study confirmed the results of the immunoblotting experiments qualitatively: all six protein kinase C isozymes were detected in the cytoplasm of PC-3 cells. Translocation of protein kinase C in PC-3 cells were also examined with phorbol 12-myristate 13-acetate (PMA), bryostatin 2, diolein, and 1-oleoyl-2-acetyl glycerol (OAG). Differential reactions of protein kinase C isozymes to these activators were observed. When PC-3 cells were treated with 10nM bryostatin 2, protein kinase C isozyme α was translocated into the nucleus, whereas ε type was translocated into the plasma membrane and the nucleus. Protein kinase C α and ε types were translocated into the nucleus following the treatment with 10μM diolein, whereas protein kinase C α, β, γ, and ε types were translocated into the nucleus by the treatment with 10μM OAG. Protein kinase C α and ε types were translocated into the nucleus in the presence of 100nM PMA. Protein kinase C δ type was translocated to the nuclear membrane by these activators, however, only PMA-induced translocation was inhibited by protein kinase C inhibitor, 1-(5-isoquinolinesulfonyl)-2-methylpiperazine dihydrochloride (H7). H7 inhibited translocation of protein kinase C α type induced by PMA, β type by OAG and ε type by PMA and OAG, whereas it did not affect translocations induced by bryostatin and diolein, respectively. These results suggest that there exist six isoformes of protein kinase C (α, β, γ, δ, ε, and ζ types) in PC-3 cells and that each of these isozymes distinctively reacts to bryostatin, diolein, OAG and PMA, in part due to an altered molecular size and conceivably discrete binding site(s).

KEY WORDS: PC-3 cell, Signal tranduction, Protein kinase C isozymes, Translocation, Phobol esters

Prostate cancer is the third highest cause of cancer death in men in the Unite State with over 30,000 deaths annually (Silverberg *et al.*, 1990; Boring *et al.*, 1991). Majority of patients with prostate cancer are diagnosed as having advanced disease or eventually develop metastases to lymph nodes, bones, lung and liver (Torti, 1985). Since most of cytotoxic anticancer drugs do not exert effects to prostate cancer cells, treatment of prostate cancer apply somewhat unique approach such as androgen-related therapy and retinoids or its modulators (Merk, *et al.*, 1986; Robinson, *et al.*, 1990; Sporn, *et al.*, 1991). Androgen ablation or total androgen blockade has some effect on the progression of prostate carcinoma in humans, since it stabilizes the disease, improves urinary symptoms, or reduces pain in over 50% of patients with advanced prostate cancer (Beland, *et al.*, 1990; Iversen, *et al.*, 1990). Despite of these androgen-related therapy, over 30% of prostate cancer patients ultimately become resistant to the therapy, in part due to the clonal development of resistant cells to the effect of androgen in cell growth and to oncogenic and growth factor changes leading to malignancy (Rijnders, *et al.*, 1985; Viola, *et al.*, 1986; Peehl. *et al.*, 1987; Davies, *et al.*, 1988; Isaacs, *et al.*, 1991). Changes in prostate cancer cell proliferation and oncogene expressions would accompany modified cellular signal transduction mechanisms, which might include protein kinase C-mediated pathways (Nishizuka, 1992).

PC-3 cell is a poorly differentiated adenocarcinoma isolated from a vertebral metastasis and is independent of androgen (Horoszewics, *et al.*, 1983). There are a few studies on characterization of PKC-mediated signal transduction mechanisms of androgen-dependent prostate cancer cells in relation to cell proliferation and induction of prostate-specific antigen (Goueli, 1990; Andrews, *et al.*, 1992; Mariotti, *et al.*, 1992). However, protein kinase C has not been characterized in either androgen-dependent or androgen-independent prostate cancer cells and possible role(s) of protein kinase C in androgen-independent prostate cancer cells has not been investigated. We now report that six isoforms of protein kinase C are detected immunologically in the PC-3 cells and react distinctively to protein kinase C activators such as bryostatin, diolein, 1-oleoyl-2-acetyl glycerol, and phorbol 12-myristate 13-acetate, and PKC inhibitor H7.

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*To whom correspondent should be sent.

Material and Methods

Materials

diolein, 1-oleoyl-2-acetylglycerol (OAG), phorbol 12-myristate 13-acetate (PMA), 4-α phorbol didecanoate (4-α PDD, histone type IIIs, phenylmethylsulfonyl fluoride (PMSF), and streptavidine rhodamine-conjugated anti-rabbit IgG antibody were purchased from Sigma (St. Louis, MO); protein kinase C antibodies α, β, γ, δ, ε, and ζ types were obtained from GIBCO/BRL (Gaithersburg, MD); 1-(5-isoquinolinesulfonyl)-2-methylpiperazine dihydrochloride (H7) was obtained from Seikagaku America (Rockville, MD); DEAE-Sephacel was purchased from Pharmacia (Piscataway, NJ); bryostatin 2 was a kind gift from Dr. James Pai (Schering-Plough Research, Bloomfield, NJ); other chemicals were reagent grade.

Cell culture

PC-3 cell line was obtained from America Type Cell Culture (Rockville, MD) and cells were grown in RPMI 1640 with 5% fetal bovine serum.

Protein kinase C isolation

Protein Kinase C was isolated as described with some modifications (Ahn and Kim, 1991). Briefly, subconfluent PC-3 cells (2×10^8 cells)were harvested, followed by washing with ice-cold, Ca^{2+}- and Mg^{2+}-free phosphate-buffered saline, then homogenized in buffer A (20 mM Tris-Cl, pH 7.5, 2 mM EDTA, 10 mM EGTA, 1 mM phenylmethylsulfonyl fluoride (PMSF), 2 mM dithiothreitol, 1% Triton-$\times$100, 10 μg/ml leupeptine and 10 μg/ml aprotinine) by sonication for 5 sec three times on ice. The cell lysate was

centrifuged for 20 min at 35,000 ×g and the resulting supernatant was applied to DEAE-Sephacel column (1 cm × 10 cm) previously equilibrated with buffer B (20 mM Tris-Cl, pH 7.5, 0.5 mM EGTA, 0.5 mM EDTA, 0.5 mM PMSF, 10 μg/ml leupeptine, 10 μg/ml aprotinine, and 10 mM β-mercaptoethanol). The column was washed with 10 ml of buffer B, then eluted with 2 ml of buffer B with 0.25 M NaCl.

Immunoblot analysis of protein kinase C

Immunoreactivity of protein kinase C with antibodies was carried out as previously described with some modification (Fridman, *et al.*, 1990) and the immunoreactive protein band was detected with ^{125}I-goat anti-rabbit IgG. After transblotting proteins, the nitrocellulose membrane was incubated with 5% nonfat milk and 0.5% Tween 20 in blocking buffer (20 mM Tris-Cl, pH 7.5, 2.5 mM EDTA, 150 mM NaCl). The blocked membrane was incubated for 30 min at room temperature with primary antibody, washed three times with blocking buffer, and then finally incubated with ^{125}I-goat anti-rabbit IgG. The immunoreactive bands were visualized by autoradiography.

Immunocytochemical analysis of protein kinase C

Immunofluorescent staining of PC-3 cells were stained using protein kinase C isozyme specific antibodies and visualized by streptavidin-Texas red conjugate described by Bayer and Wilchek (Bayer and Wilchek, 1979) with some modification (Choi and Ahn, 1991).

Results

A. Immunoblot analysis of protein kinase C isozymes of PC-3 cells

Protein kinase C (PKC) was partially purified from human adenocarcinoma prostate cancer PC-3 cells by DEAE-Sephacel column chromatography. To detect PKC isozymes α, β, γ, δ, ε, and ζ, the partially purified PKC was analyzed by immunoblot using isozyme-specific antibodies. Immunoreactive bands to an antibody against each protein kinase C isozyme were observed (Fig. 1). All immunoreactive protein kinase C detected corresponded to molecular weight of 68,000-dalton except protein kinase C α type of which band was detected at 80,000-dalton. Additional immunoreactive bands to protein kinase C δ and ζ type were also observed and corresponded to approximately 55,000-dalton and 80,000-dalton, respectively. Intensities of immunoreactive bands of protein kinase C ζ type were almost equal and the lower molecular weight fragment might be a degraded form of the 80,000-dalton enzyme. Although major immunoreactive band of protein kinase C ε type was detected at 68,000 dalton, very minor band at 96,000-dalton was occasionally detected (data not shown), suggesting that 68,000-dalton band might be a degraded fragment of 96,000-dalton form. Among protein kinase C isozymes, relative intensities of immunoreactive staining of protein kinase C γ and δ types were the most strong, followed by rest of the types with almost equal intensities.

B. Immunocytochemical characterization of protein kinase C isozymes in PC-3 cells

Immunoreactive protein kinase C isozymes were also characterized in the presence or absence of protein kinase C activators such as bryostatin 2, diolein, 1-oleoyl-2-acetylglycerol (OAG), and phorbol 12-myristate 13-acetate (PMA) with or

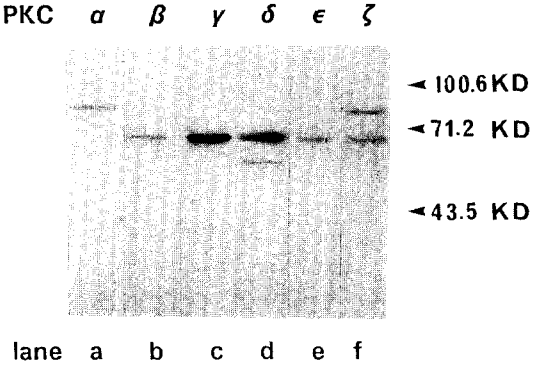

Fig. 1. Immunoblot analysis of protein kinase C isozymes in PC-3 cells. Lane a-f represent immunoreactive detection of each protein kinase C isotypes: lane a-α type; lane b-β type; lane c-γ type; lane d-δ type; lane e-ε type; lane f-ζ type.

without pretreatment with protein kinase C inhibitor, H7.

In the untreated PC-3 cells, each of immunostained protein kinase C isozymes by the respective antibodies specific to each isozymic form were detected in the cytoplasm (Fig. 2-7, a). When the cells were treated with 10 nM bryostatin 2, 10 μM diolein, 10 μM PMA, respectively, immunostained protein kinase C α type was relocated from the cytoplasm into the nucleus and to the nuclear membrane (δ type) (Fig. 2-7, b, d, f, h). Protein kinase C inhibitor H7 (30 μM) inhibited only PMA-induced translocation of protein kinase C α type (Fig. 2, c, e, g, i). Translocation of immunostained protein kinase C β and γ types from the cytoplasm to the nucleus resulted from

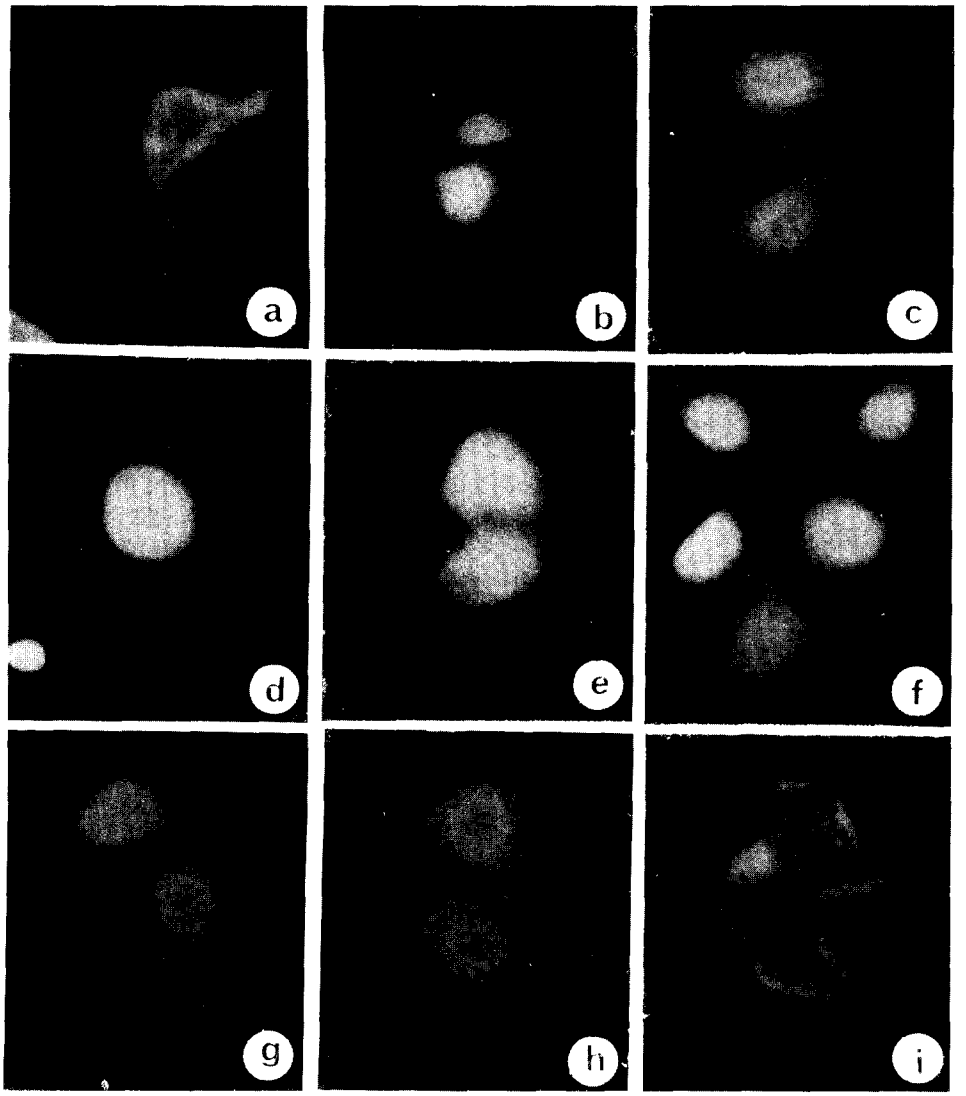

Fig. 2. Immunofluorescent detection of protein kinase C α type in PC-3 cells in the presence or absence of activators with or without H7 treatment: a. no treatment; b. 10 nM bryostatin 2; c. 10 nM bryostatin 2 with 30 μM H7 pretreatment; d. 10 μM diolein; e. 10 μM diolein with 30 μM H7 pretreatment; f. 10 μM OAG; g. 10 μM OAG with 30 μM H7 pretreatment; h. 100 nM PMA; and i. 100 nM PMA with 30 μM H7 pretreatment.

the treatment of PC-3 cells with OAG (Fig. 3 and 4, f). H7 inhibited the translocation of protein kinase C β type (Fig. 3, g), but not of protein kinase C γ type (Fig. 4, g). Protein kinase C β and γ types in PC-3 cells were not affected by the treatment with bryostatin 2, diolein, or PMA (Fig. 3 and 4, b, d, g).

Protein kinase C δ type was translocated to the nuclear membrane by these activators, however, only PMA-induced translocation was inhibited by protein kinase C inhibitor H7 (Fig. 5). Protein kinase C ε type was translocated to the nucleus by the treatment with each of these activators (Fig. 6, b, d, f, h). The translocations of protein kinase C ε type induced by OAG and PMA respectively, but not by bryostatin 2 and diolein, were inhibited by H7 (Fig. 6, c, e, g, i). Protein kinase Cζ was not affected by the treatment with any of the activators (Fig. 7).

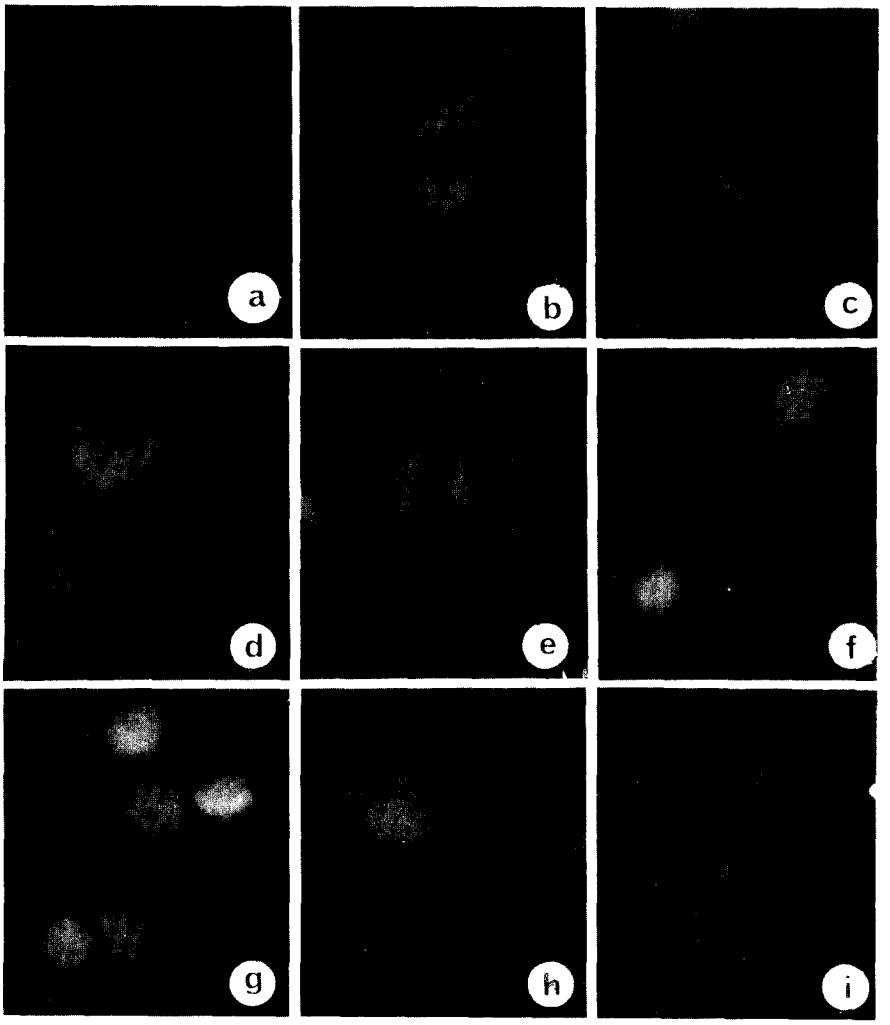

Fig. 3. Immunofluorescent detection of protein kinase C β type in PC-3 cells in the presence or absence of activators with or without H7 treatment: a. no treatment; b. 10 nM bryostatin 2; c. 10 nM bryostatin 2 with 30 μM H7 pretreatment; d. 10 μM diolein; e. 10 μM diolein with 30 μM H7 pretreatment; f. 10 μM OAG; g. 10 μM OAG with 30 μM H7 pretreatment; h. 100 nM PMA; and i. 100 nM PMA with 30 μM H7 pretreatment.

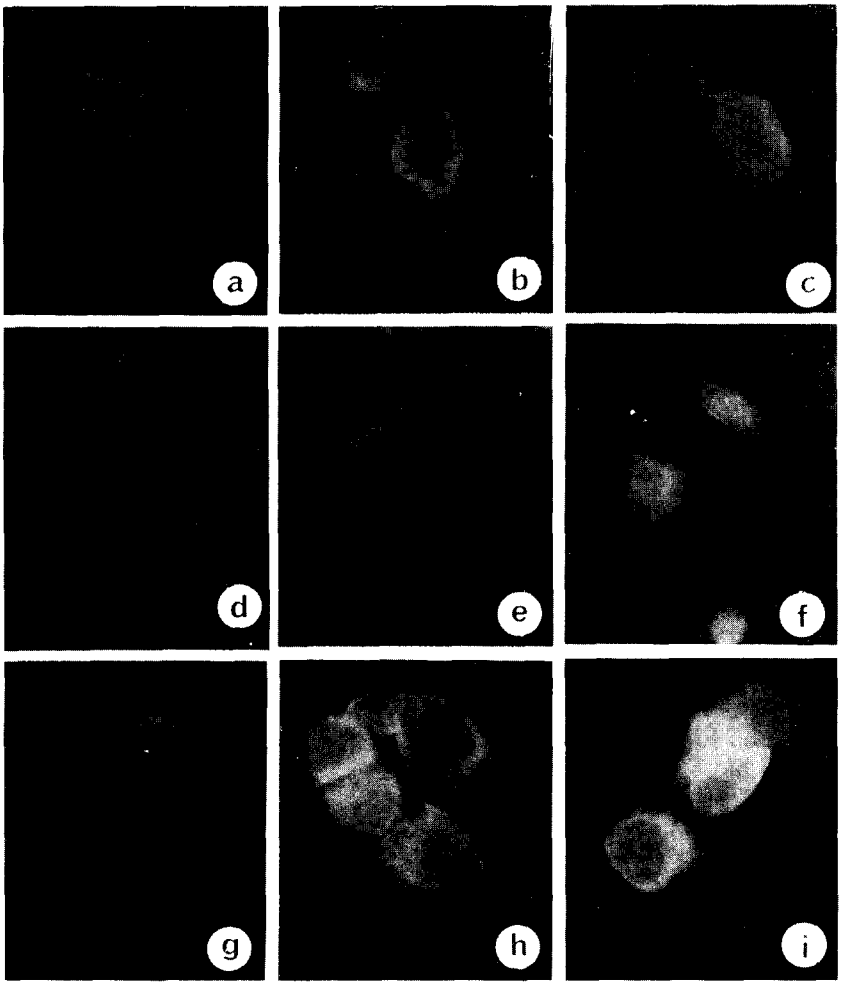

Fig. 4. Immunofluorescent detection of protein kinase C γ type in PC-3 cells in the presence or absence of activators with or without H7 treatment: a. no treatment; b. 10 nM bryostatin 2; c. 10 nM bryostatin 2 with 30 μM H7 pretreatment; d. 10 μM diolein; e. 10 μM diolein with 30 μM H7 pretreatment; f. 10 μM OAG; g. 10 μM OAG with 30 μM H7 pretreatment; h. 100 nM PMA; and i. 100 nM PMA with 30 μM H7 pretreatment.

Discussion

Our study examined localization of protein kinase C isozymes and characterized its differential reactions to bryostatin, diolein, 1-oleoyl-2-acetyl glycerol(OAG), and tumor promoting phorbol ester, phorbol 12-myristate 13-acetate (PMA) in PC-3 cells immunocytochemically or by immunoblot analysis. In addition to detection of the multiple isozymes, protein kinase C in PC-3 cell demonstrated several distinct characteristics. First, the estimated molecular sizes of protein kinase C isozymes are distinct. Calculated molecular sizes of protein kinase C ranging from 77,000- dalton (α, β, γ, and δ types) to 83,000-dalton (ε type) except ζ type (68,000-dalton) have been reported (Nishizuka, 1988; Ono, *et al.*, 1989; Bell and Burns, 1991). In PC-3 cells, molecular sizes of protein kinase C β, γ, δ, and ε types were estimated to be 68,000-dalton, respectively, except α and ζ types which were estimated to be approximately 80,000-dalton.

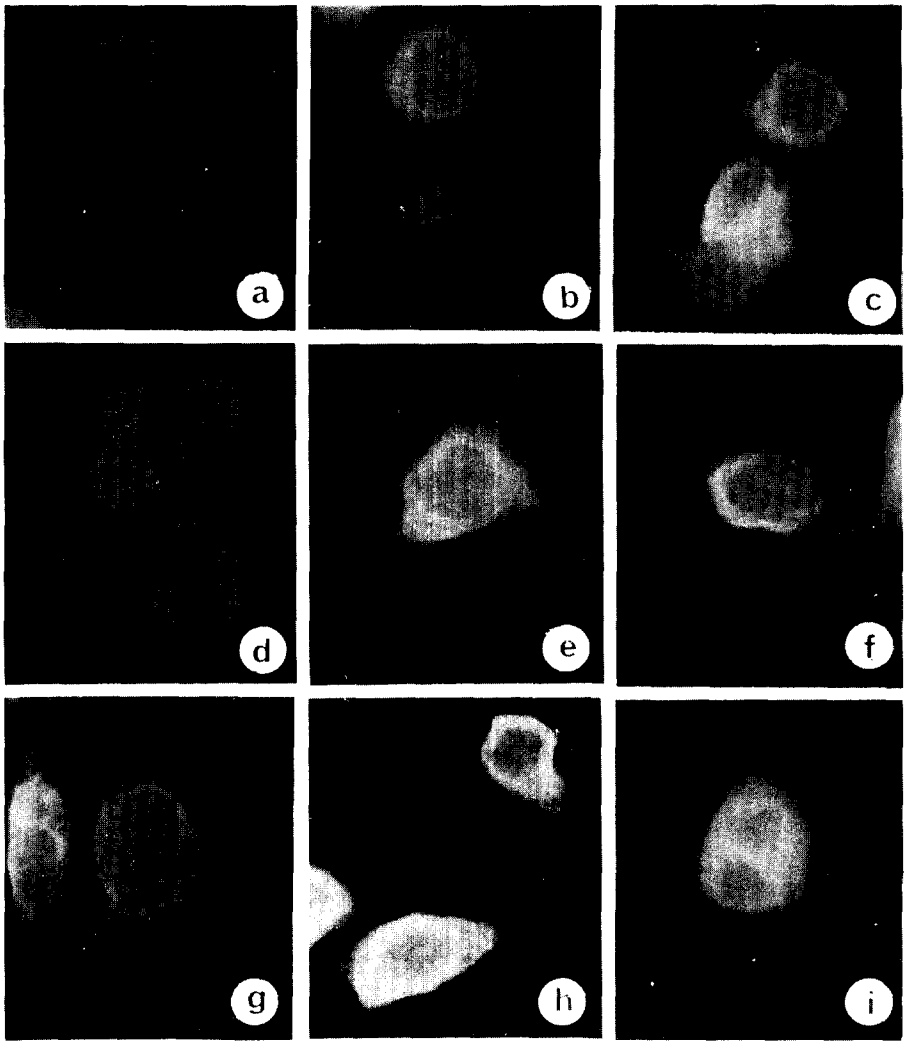

Fig. 5. Immunofluorescent detection of protein kinase C δ type in PC-3 cells in the presence or absence of activators with or without H7 treatment: a. no treatment; b. 10 nM bryostatin 2; c. 10 nM bryostatin 2 with 30 μM H7 pretreatment; d. 10 μM diolein; e. 10 μM diolein with 30 μM H7 pretreatment; f. 10 μM OAG; g. 10 μM OAG with 30 μM H7 pretreatment; h. 100 nM PMA; and i. 100 nM PMA with 30 μM H7 pretreatment.

Protein kinase C β, γ, δ, and ε types in PC-3 cells may be a either degraded form or a truncated form of respective isotypes. Since their molecular sizes were altered, binding characteristics of protein kinase C modulators to regulatory domain might be altered as well. Protein kinase C δ and ζ types showed additional immunoreactive bands, 55,000-dalton and 68,000-dalton, respectively, in that 55,000-dalton protein appeared to be a degraded fragment. Since reported molecular size of ζ type is 68,000-dalton (Ono, *et al.*, 1989), it is not clear what 80,000-dalton immunoreactive to an antibody to ζ type in PC-3 cells is. In our immunocytochemical study, however, ζ type was not affected by the presence of PMA or any other activators, suggesting that regardless of the presence of two different molecular sizes of ζ type immunoreactive proteins it still lacks binding site(s) to PMA as well as other activators. Although some results occasionally showed very minor, additional,

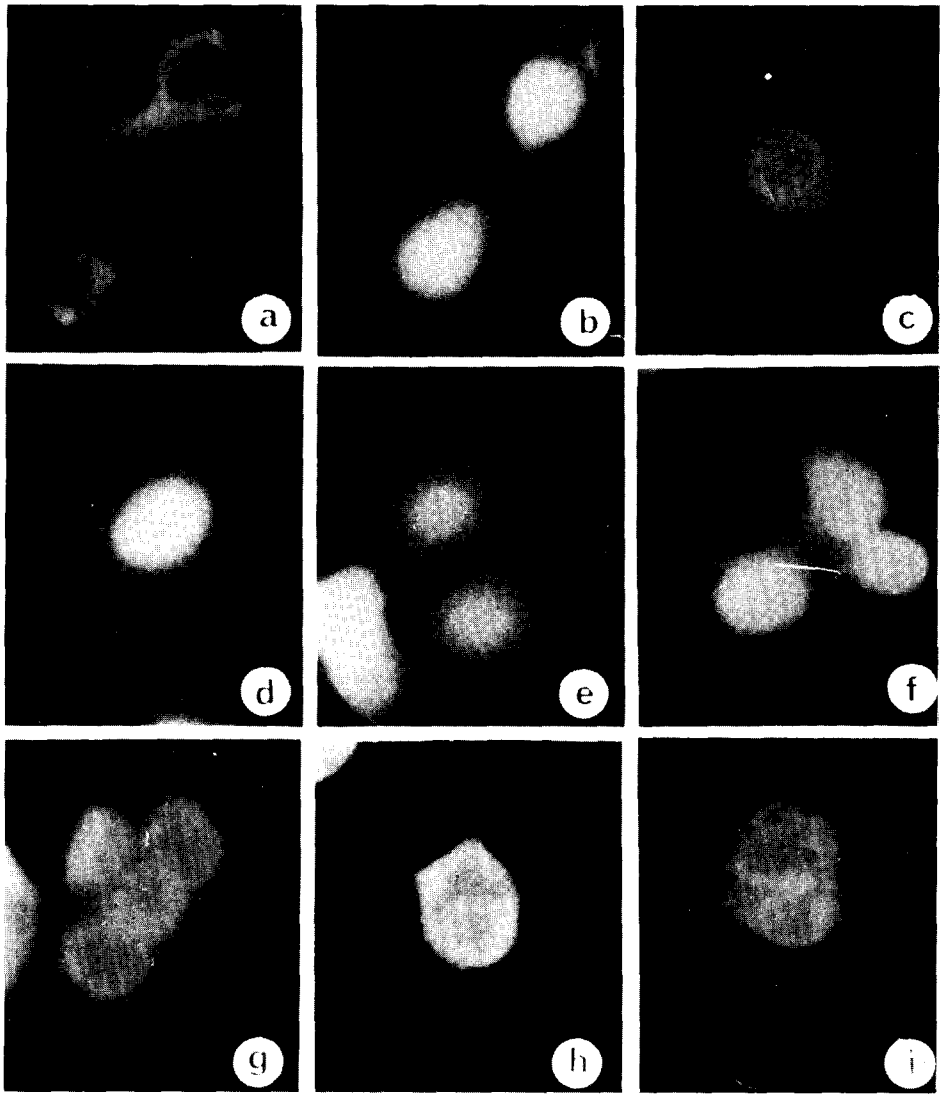

Fig. 6. Immunofluorescent detection of protein kinase C ε type in PC-3 cells in the presence or absence of activators with or without H7 treatment: a. no treatment; b. 10 nM bryostatin 2; c. 10 nM bryostatin 2 with 30 μM H7 pretreatment; d. 10 μM diolein; e. 10 μM diolein with 30 μM H7 pretreatment; f. 10 μM OAG; g. 10 μM OAG with 30 μM H7 pretreatment; h. 100 nM PMA; and i. 100 nM PMA with 30 μM H7 pretreatment.

immunoreactive band to protein kinase C ε type at 96,000-dalton as well as 68,000-dalton band, which might be a reported holoenzyme (data not shown) (Akita, *et al.*, 1990; Pears, *et al.*, 1991), 68,000-dalton band to ε type antibody was a major one in PC-3 cells and similar size of proteolytic fragment of ε type had been detected in rabbit brain (Saido, *et al.*, 1992), suggesting that this 68,000-dalton protein may be either a proteolytic fragment or a subspecies of ε type.

Although the molecular size of ε type appears to be truncated, the reactivity to PMA (Ways, *et al.*, 1992) and other activators was not changed in PC-3 cells. This suggest that the 68,000-dalton protein may be a fully functioning subspecies of ε type. Further investigation is needed to examine

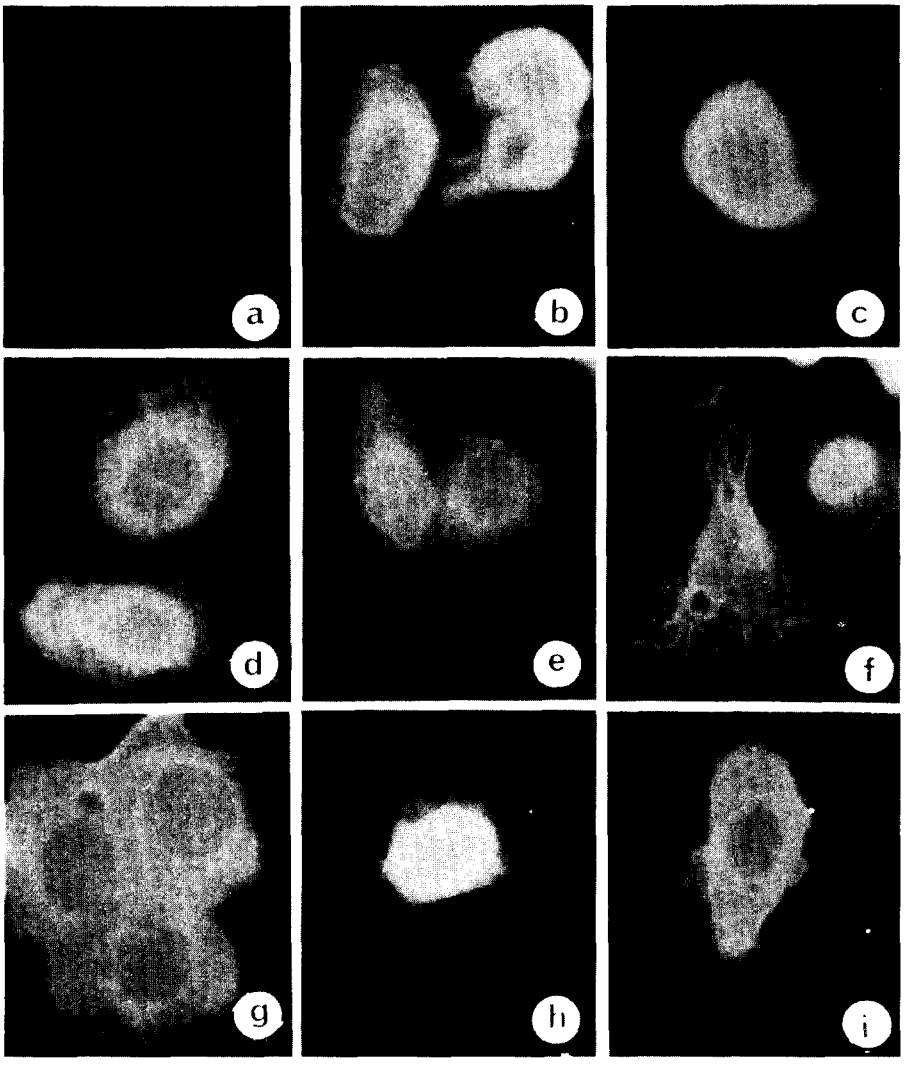

Fig. 7. Immunofluorescent detection of protein kinase C ζ type in PC-3 cells in the presence or absence of activators with or without H7 treatment: a. no treatment; b. 10 nM bryostatin 2; c. 10 nM bryostatin 2 with 30 μM H7 pretreatment; d. 10 μM diolein; e. 10 μM diolein with 30 μM H7 pretreatment; f. 10 μM OAG; g. 10 μM OAG with 30 μM H7 pretreatment; h. 100 nM PMA; and i. 100 nM PMA with 30 μM H7 pretreatment.

this possibility.

Second, the most abundant isozymic protein kinase C in PC-3 cells was γ and δ types. Since protein kinase C type has been isolated only in the brain homogenate (Huang, *et al.*, 1988) and immunologically detected in a limited cell lines as well as in the brain tissues (Huang, *et al.*, 1989; Ahn, *et al.*, 1992; Schwartz, *et al.*, 1993), the relatively high abundance of immunoreactive protein kinase C γ type found in PC-3 cells was totally unexpected. Although it may have certain specific role in a signal transduction mechanism of PC-3 cells, currently nothing is known on the function of this isozyme in prostate cancer. Unlike protein kinase C γ type, the marked increase in protein kinase C δ type was not isolated case, since it has been observed in murine myeloid tumor cells as well (Mischak, *et al.*, 1991). The

marked expression of δ type suggests that it may have a role in altered signal pathways in malignancy of prostate cancer cells, since p21, the protein product of *ras* oncogene, rises with increasing malignancy of human prostate cancer and transformed with *c-H-ras* rat fibroblasts cells displayed a marked increase in the expression of protein kinase C δ type (Viola, *et al.*, 1986; Borner, *et al.*, 1992).

Third, protein kinase C isozymes in PC-3 cell were differentially translocated from the cytoplasm into the nucleus or to the nuclear membrane (δ type) by enzyme activators: Protein kinase C α, δ, and ε types responded to bryostatin, diolein, OAG, and PMA, and protein kinase C β and tγ ypes were translocated only by OAG, while protein kinase C ζ type did not respond to any of these activators. The reaction of ζ type to these activators was expected. However, the reactions of other isozymes to PMA except α, δ, and ε types observed in PC-3 cells were rather uncommon, although the differential effects of stimulators of protein kinase C on substrate phosphorylation and functional roles in many cell lines and tissues have been well documented (Sharkey, *et al.*, 1984; Blackshear, *et al.*, 1986; Robinson, 1992). Among tumor promoting phorbol esters and activators of protein kinase C, PMA has been known to be the most potent and binds to regulatory domain of protein kinase C α, β , γ, and ε types, but not the domain of ζ type (Blumberg, *et al.*, 1984; Liyanage, *et al.*, 1992; Nishizuka, 1992). However, in PC-3 cells, OAG turned out to be the most active modulator of protein kinase C isozymes except ζ type. These altered agonistic potency of the activators may result from the altered molecular sizes of protein kinase C isozymes in PC-3 as discussed above, although the structural differences of protein kinase C isozymes may also be responsible. This assumption appears to be highly likely when considering protein kinase C α type was modulated by all activators and its molecular size was not altered in PC-3 cells, while those with truncated size (i.e., β and γ types) did not respond to PMA, but responded only to OAG. Furthermore, protein kinase C inhibitor, 1-(5-isoquinolinesulfonyl)-2-methylpiperazine dihydrochloride (H7) blocked only the translocation of protein kinase C α type by PMA but not by bryostatin, diolein or OAG, suggesting that the binding domains of these activators in the isozyme might be different following H7 binding. Translocations of protein kinase C ε type by PMA and OAG were inhibited by H7, but those by bryostatin and diolein were not. Furthermore, H7 inhibited translocation of protein kinase C β type induced by OAG. These results further indicate distinct binding domains of each of protein kinase C isozymes to OAG as well as discrete bindings of activators within a protein kinase C isozyme. Those activator binding domains (intra- and inter-isozyme binding sites) may be divided into two groups: those inhibited by H7 and those not.

In summary, immunocytochemical and immunoblot characterization of protein kinase C isozymes of PC-3 cells demonstrated the distinct molecular size and abundance of each isozymes as well as isozyme-dependent differential response to the enzyme activators and inhibitor. In PC-3 cells, it is noteworthy that among examined activators, OAG, not PMA, appears to be the most active stimulator of protein kinase C isozymes. Binding domains of protein kinase C isozymes for these activators appeared to be distinct within an isozyme and also among isozymes, in part, due to altered molecular size of the enzyme and may be divided into two groups depending upon inhibition by H7. These unique, altered isozymic size, distribution, and translocations in response to the enzyme modulators may be very important clue to the unique signal transduction mechanism in prostate cancer cells which are hard to be controlled by conventional cytotoxic chemotherapeutic agents.

References

Ahn, C.-H., W.C. Choi, J.-Y. Kong, and R.L. Fine; 1992. Detection of six immunoreactive protein kinase C isozymes in human breast MCF-7 and multidrug-resistant MCF-7/Dox cells and effects of phorbol ester treatment. *Proc. Am. Assoc. Cancer Res.* **33:** 455.

Ahn, C.-H., and J.-K. Kim; 1992 Characterization of protein kinase C in doxorubicin-sensitive and resistant human breast MCF-7 cancer cell lines. J. *Biomed.*

Res. **1:** 52-58.

Akita, Y., S. Ohno, Y. Yajima, and K. Suzuki; 1990. Expression and properties of two distinct classes of the phorbol ester receptor family, four conventional protein kinase C types, and a novel protein kinase C. *J. Biol. Chem.* **265:** 354-362.

Andrews, P.E., C.Y.-F. Young, B.T. Montgomery, and D. J. Tindall; 1992. Tumor-promoting phorbol ester down-regulates the androgen induction of prostate-specific antigen in a human prostatic adenocarcinoma cell line. *Cancer Res.* **52:** 1525-1529.

Basu, A., A. Kozikowski, and J.S. Lazo; 1992. Structural requirements of lyngbyatoxin A for activation and downregulation of protein kinase C. *Biochemistry* **31:** 3824-3830.

Bayer, E.A., and M. Wilche; 1979. The use of the avidin-biotin complex as a tool in molecular biology. *Methods Biochem. Anal.* **26:** 1-45.

Beland, G., M. Elhilali, Y. Fradet, B. Laroche, E.W. Ramsey, J. Trachtenberg, P.M. Venner, and H.D. Tewari; 1990. A controlled trial of castration with and without Nilutamide in metastatic prostatic carcinoma. *Cancer* **66:** 1074-1079

Bell, R.M., and D.J. Burns; 1991. Lipid activation of protein kinase C. *J. Biol.Chem.* **266:** 4661-4664.

Blackshear, P.J., L. Wen, B.P. Gynn, and L.A. Witters; 1986. Protein kinase C-stimulated phosphorylation in vitro of a Mr. 80,000 protein phosphorylated in response to phorbol esters and growth factors in intact fibroblasts. *J. Biol. Chem.* **261:** 1459-1469.

Blumberg, P.M., S. Jaken, B. Koenig, N. Sharkey, K.L. Leach, A.Y. Jeng, and E. Yeh; 1984. Mechanism of action of the phorbol ester tumor promoters: Specific receptors for lipophilic ligands. *Biochem. Phar.* **33:** 933-940.

Boring, C.C., T.S. Squires, and T. Tong; 1991. Cancer statistics, 1991. *CA Cancer J. Clin.* **41:** 19-36.

Borner, C., S.N. Guadagno, W.W.-L. Hsiao, D. Fabbro, M. Barr, and I.B. Weinstein; 1992. Expression of four protein kinase C isoforms in rat fibroblasts. Differential alterations in *ras*-, *src*-, and *fos*-transformed cells. *J. Biol. Chem.* **267:** 12900-12910.

Choi, W.C., and C.-H. Ahn; 1992. The phosphorylation and down-regulation of protein kinase C in myoblasts of chick embryos. *J. Biomed. Res.* **1:** 59-65.

Davies, P., C.L. Eaton, T.D. France, and M.E.A. Phillips; 1988. Growth factor receptors and oncogene expression in prostate cells. *Am. J. Clin. Oncol.* **11 (Suppl 2):** S1-S7.

Fridman, R., J.C. Lacal, R. Reich, G.R. Martin, D.R. Bonfil, And C.-H. Ahn; 1990. Differential effects of phorbol ester on the in vitro invasioness of malignant andnon-malignant human fibroblast cells. *J. Cell Physiol.* **142:** 55-60.

Goueli, S.A.; 1990. Differential regulation of prostatic protein kinase c isozymes by androgens. *FEBS Lett.* **264:** 53-55.

Horoszewicz, J.S., S.S. Leong, E. Kawinski, J.P. Karr, h. Rosenthal, T.M. Chu, E.A. Mirand, and G.P. Murphy; 1983. LNCaP model of human prostatic carcinoma. *Cancer Res.* **43:** 1809-1818.

Huang, F.L., Y. Yoshida, H. Nakabayashi, D.P. Friedman, L.G. Ungerleider, W.S. Young, and K.P. Huang; 1989. Type I protein kinase C isozyme in the visual-information processing pathway of monkey brain. *J. Cell Biochem.* **39:** 401-410.

Huang, K.P., F.L. Huang, H. Nakabayashi, and Y. Yoshida; 1988. Biochemical characterization of rat brain protein kinase C isozymes. *J. Biol. Chem.* **263:** 14839-14845.

Isaacs, W.B., B.S. Carter, and C.M. Ewing; 1991. Wild-type p53 suppresses growth of human prostate cancer cells containing mutant p53 alleles. *Cancer Res.* **51:** 4716-4720.

Iversen, Pl., M.G. Christensen, and E. Friis; 1990. A phase III trial of zoladex and flutamide versus orchiectomy in the treatment patients with advanced carcinoma of the prostate. *Cancer* **66:** 1058-1066.

Liyanage, M., D. Frity, E. Livneh, and S. Stabel; 1992. Protein kinase C group B members PKC-delta, -epsilon, -zeta and PKC-L (eta). Comparison of properties of recombinant proteins in vitro and in vivo. *Biochem. J.* **283:** 781-787.

Mariotti, A., J. Durham, and M. Mawhinney; 1992. Protein kinases and the androgen-induced proliferation of accessory sex organ smooth muscle. *Biol. Reprod.* **46:** 551-560.

Merk, F.B., M.J. Warhol, P.W. Kwan, I. Leav, J. Alroy, P. Ofner, and G.S. Pinkus; 1986. Multiple phenotypes of prostatic glandular cells in castrated dogs after individual or combined treatment with androgen and estrogen. Morphometric, ultrastructural, and cytochemical distinctions. *Lab. Invest.* **54:** 442-456.

Mischak, H., A. Bodenteich, W. Kolch, J. Goodnight, F. Hofer, and J.F. Mushinski; 1991. Mouse protein kinase C-delta, the major isoform expressed in mouse hemopoieticcells: Sequence of the cDNA, expression patterns, and characterization of the protein. *Biochemistry* **30:** 7925-7931.

Nishizuka, Y.; 1988. The mechanism heterogeneity of protein kinase C and its implications for cellular regulation. *Nature* **334:** 661-665.

Nishizuka, Y.; 1992. Intracellular signaling by hydrolysis of phospholipids and activation of protein kinase C.

Science **258:** 607-614.

Ono, Y., T. Fujii, K. Ogita, U. Kikkawa, K. Igarashi, and Y. Nishizuka; 1989. Protein kinase C zeta subspecies from rat brain: Its structur, expression, and properties. *Proc. Natl. Acad. Sci.* **86:** 3099-3103.

Pears, C., D. Schaap, and P.J. Parker; 1991. The regulatory domain of protein kinase C-epsilon restricts the catalytic-domain-specificity. *Biochem. J.* **276:** 257-260.

Pheel, D.M., N. Wehner, and T.A. Stamey; 1987. Activated kinases oncogene in human prostatic adenocarcinoma. *Prostate* **10:** 281-289.

Rijnders, A.W.M., J.A.G.M. Van der Korput, G.J. Van Steenbrugge, J.C. Romijn, and J. Trapman; 1985. Expression of cellular oncogenes in human prostatic carcinoma cell lines. *Biochem. Biosphys. Res. Comm.* **132:** 548-554.

Robinson, M.R.G., L. Denis, D.W.W. Newling, R. Sylvester, and M. De Pauw; 1990. EORTC protocol 30853: Orchidectomy versus zoladex plus flutamide in the management of metastatic carcinoma of the prostate. *Cancer* **66:** 1022-1024.

Robinson, P.J.; 1992. Potencies of protein kinase C inhibitors are dependent on the activators used to stimulate the enzyme. *Biochem. Pharmacol.* **44:** 1325-1334.

Saido, T.C., K. Misuno, Y. Konno, S.-I. Osada, S. Ohno, and K. Suzuki; 1992. Purification and characterization of protein kinase C epsilon from rabbit brain. *Biochemistry* **31:** 482-490.

Schwartz, G.K., J. Jiang, D. Kelsen, and A.P. Albino; 1993. Protein kinase C: A novel target for inhibiting gastric cancer cell invasion. *J. Natl. Cancer Inst.* **85:** 402-407.

Sharkey, N.A., K.L. Leach, and P. Blumberg; 1984. Competitive inhibition by diacylglycerol of specific phorbol ester binding. *Proc. Natl. Acad. Sci.* **81:** 607-610.

Silverberg, E., C.C. Boring, and T.S. Squires; 1990. Cancer statistics, 1990. *CA Cancer J. Clin.* **40:** 9-26.

Sporn, M.B., A.B. Roberts, L.M. Wakefield, A.B. Glick, and D. Danielpour; 1991. Growth factors and retinoids in suppression of neoplasia. *Proc. Am. Assoc.Cancer Res.* **31:** 463-464.

Torti, F.; 1988. Approaches to chemotherapy of prostate cancer. In medical oncology (Calabresi, P., S. Schein, S.A. Rosenberg, and D. MacMillan eds.) *New York, MacMillan*, pp1045.

Viola, M.V., F. Fromowitz, S. Oravez, S. Deb, G. Finkel, J. Lundy, P. Hand, A. Thor, and J.Schlom; 1986. Expression of *ras* oncogene p21 in prostate cancer. *N. Engl. J. Med.* **314:** 133-137.

Ways, D.K., B.R. Messer, T.O. Garris, W. Qin, P.P. Cook, and P.J. Parker; 1992. Modulation of protein kinase C-epsilon by phorbol esters in the monoblastoid U937 cell. *Cancer Res.* **52:** 5604-5609.

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細胞信號系에 있어서 Protein Kinase C(PKC):
사람의 前立腺 adenocarcinoma PC-3 細胞內의 여섯개의 Protein kinase C 同位酵素의 translocation

崔源哲* · 安昌浩**(*釜山大, 自然大, 生物學科, **Division of Oncology and Pulmonary Drug Product, FDA, USA)

人間의 前立腺癌細胞인 PC-3 cell line에 있어서 protein kinase C 同位酵素를 調査하였다. PC-3 細胞를 protein kinase C (PKC) α, β, γ, δ, ε과 ζ同位酵素에 대한 各各의 抗體로서 免疫反應한 band와 免疫細胞化學的인 染色을하여 觀察하였다. PKC α는 分子量 80,000에 band가 나타나는 反面에 다른 PKC 同位酵素는 分子量 68,000에 反應이 나타났다. PKC δ와 ζ는 各各 分子量 55,000과 80,000에 免疫反應이 나타났다. PKC의 同位酵素는 γ와 δ를 除外하고는 거의 同一한 染色程度를 나타내었다. 免疫細胞化學的 硏究는 immunoblotting 實驗으로 그 結果를 確認하였다. 즉 모든 여섯가지 PKC의 同位酵素가 PC-3의 細胞質에서 免疫反應을 일으켰다. 또한 PC-3 細胞에 있어서 PKC의 translocation은 phorbol 12-myristate 13-acetate(PMA), bryostatin 2, diolein, 1-oleoyl-2-acetyl glycerol(OAG)를 處理하여 調査하였다. 이들 活性劑에대해 PKC의 同位酵素는 各各 다른 反應을 나타나는것이 觀察되었다. PC-3細胞가 10 nM의 bryostatin 2에 處理했을때 PKC α同位酵素는 核內로 translocation되는 反面에 同位酵素 ε는 原形質膜과 核內로 translocation되었다. PKC α와 ε는 10 μM diolein으로 處理하면 核內로 translocation되고, PKC α, β, γ, δ, 와 ε는 OAG를 處理해야 核內로 translocation 되었다. PKC α와 ε는 PMA 100 nM에서 核內로 translocation되지만 PKC 抑制劑인 1-(5-isoqunolinesulfonyl)-2-methylpiperazine dihydrochloride (H7)에 의해서 PMA에의해 誘導된 translocation이 抑制되었다. H7이 PMA에의해서 α가 OAG에의해 β, PMA와 OAG에 의해 ε의 translocation이 抑制되었으나 bryostatin 2나 diolein에서 誘導되는 translocation은 抑制하지 못하였다. 이러한 結果들은 PKC의 여섯개 同位酵素(α, β, γ, δ, ε, ζ)가 다 存在함을 알수있고, bryostatin 2, diolein, OAG와 PMA에 反應하는 site가 各各 틀리거나 變形된 分子의 크기가 틀리기 때문에 일어나는 것임을 暗示해주는 것이다.